

Contribution of Reserves to Kernel Weight and Grain Yield Determination in Maize: Phenotypic and Genotypic Variation

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ABSTRACT

Maize (*Zea mays* L.) kernel weight (KW) and grain yield depend on plant growth during active grain filling and reserves use. The objective of our study was to analyze the phenotypic and genotypic variation in these traits in a family of recombinant inbred lines (RIL). In two field experiments we measured plant grain yield (PGY) and its components (KW and kernel number per plant, KNP), biomass production per plant and per kernel during active grain filling, and apparent reserves use (ARU) per plant (ARU_p, difference between PGY and plant biomass production during active grain filling) and per kernel (ARU_k, difference between KW and plant biomass production per kernel during active grain filling). Heritability (h^2) and phenotypic plasticity were computed for all traits. Large differences were always evident among genotypes, but phenotypic plasticity was (i) low for KW and plant biomass at R2 and physiological maturity; (ii) intermediate for KNP and PGY; and (iii) high for plant growth, plant growth per kernel after R2, and ARUs. Traits with highest h^2 were KW (0.70), KNP (0.61), and ARU_p (0.59). Final KW was related to plant growth per kernel ($r^2 = 0.64$; $P < 0.001$) but not to ARU_k, and ARU_p was driven ($r^2 \geq 0.49$; $P < 0.001$) by KNP. Because of its positive relationship with KNP (main determinant of PGY), high h^2 and high phenotypic plasticity, breeding must consider the increase in ARU_p for improving grain yield, an objective that needs to be coupled with large reserves accumulation before silking to avoid the risk of lodging.

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Abbreviations: ARU, apparent reserves use; ARU_k, ARU per kernel; ARU_p, ARU per plant; E, environment; ENSO, El Niño Southern Oscillation; G, genotype; h^2 , heritability; KNP, kernel number per plant; KW, kernel weight; PGY, plant grain yield; RIL, recombinant inbred lines; SWSC, stem water-soluble carbohydrate; Tmax, mean daily maximum temperatures.

BIO MASS ACCUMULATION in maize kernels has two origins: plant growth during active grain filling (i.e., after the R2 stage; Ritchie et al., 1992) and reserves stored as stem water-soluble carbohydrates (SWSC). The relative contribution of each source has traditionally been estimated by comparing individual KW and the plant biomass growth per kernel during the grain-filling period (Cirilo and Andrade, 1996; Borrás and Otegui, 2001). When KW is similar to the plant growth per kernel, it is assumed that stored reserves were not used for grain filling nor accumulated in other organs. Because this assumption is not based on direct SWSC assessments, the use of reserves is described as being indirect or apparent. Apparent reserves use per kernel increases when KW is larger than the plant growth per kernel during grain filling, whereas reserves accumulation takes place when KW is smaller than it. This approach is commonly accepted because of the strong positive association between SWSC and stem biomass and the fact that the largest portion of reserves mobilized

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during active grain filling accounts for SWSC mobilization (Ehdaie et al., 2008; Rattalino Edreira et al., 2014).

Early studies on reserves use for maize grain filling (Swank et al., 1982; Kiniry et al., 1992) indicated that both stem dry weight and SWSC increase up to the stages of R2–R3 then begin to decline. There are, however, large genotypic differences in the maximum assimilates level reached and the magnitude of assimilates decline. Reserves use during active grain filling can vary widely in response to growing conditions, which modify reserves initial level (R2–R3) as well as their subsequent demand (Rattalino Edreira et al., 2014).

Final KW of commercial maize crops aiming to high yield levels is known to decrease drastically in response to growth constraints imposed during active grain filling (Borrás et al., 2004). In most cropping situations, reserves are part of the assimilates needed to fulfill the growing demand set by the ear (Gambín et al., 2007). However, maize KW is a trait with minimum variation across environments, a response usually attributed to a predominant genetic control (D'Andrea et al., 2013) and probably linked to the critical buffering effect of stem reserves (Ouattar et al., 1987; Rattalino Edreira et al., 2014). On the contrary, yield, KNP, and plant growth traits show large plasticity as a result of their strong sensitivity to environmental influence (D'Andrea et al., 2013).

Although it is known that reserves are relevant for grain yield determination, there are no direct measurements describing their importance in large breeding populations. Similarly, variability and relevance of ARU_K or ARU_P have not been analyzed. As a result, it is not known whether or not increases in grain yield that are linked to improved kernel numbers and postflowering biomass production (Luque et al., 2006; Lee and Tollenaar, 2007) are limited by reserves availability and use during grain filling. Echarte et al. (2006) suggested that increased ear demand produced by enhanced kernel number and potential KW of modern hybrids have caused a reduction in KW stability. The larger sink demand may represent an enhanced demand on reserves, but authors gave no evidence about ARU_K and ARU_P . Provided grain yield improvement represents an increase in reserves demand, future breeding efforts should focus on reserves use as much as on actual photosynthesis during active grain filling. Knowledge on available genotypic variability for ARU_K and ARU_P , and their h^2 values, is relevant for this purpose.

Information on ARU_P and ARU_K is not available for maize inbreds. The only study addressing the effects of plant growth during grain filling and KW using inbreds (Severini et al., 2011) highlighted the difference between a few popcorn and dented genotypes. Authors, however, did not report on ARU_P or AUR_K of control plots, and consequently it is not possible to infer if observed genotypic differences in KW sensitivity to stress during grain

filling were a result of intrinsic differences in reserves use between groups (e.g., dented > popcorn).

In summary, current knowledge on ARU_P and ARU_K during maize grain filling is not conclusive about the relevance of these traits for KW and yield determination and on available genotypic variation. The objective of our study was to quantify and analyze ARUs. For this purpose we used a family of RIL derived from the cross of two inbreds with contrasting genetic background.

MATERIALS AND METHODS

Plant Material

The plant material used in our research was a population of 167 RIL and their two parental inbreds (B100 and LP2). Parental inbreds differ in canopy size, grain yield, and grain yield components (D'Andrea et al., 2006). They also differ in the heterotic group of origin: B100 is a US semident germplasm and LP2 was derived from Caribbean × flint-Argentine germplasm (Munaro et al., 2011).

Crop Husbandry and Experimental Design

Field experiments were conducted at the Pergamino Experimental Station of the National Institute of Agricultural Technology, Argentina (33°56' S, 60°34' W) on a Typic Argiudol soil, during the 2009–2010 (Exp. 1) and 2011–2012 (Exp. 2) growing seasons. Explored weather and soil nitrogen (N) conditions were very contrasting between experiments, yielding two different environments. Each growing season corresponded to an extreme phase of the El Niño Southern Oscillation (ENSO) phenomenon, being El Niño for Exp. 1 and La Niña for Exp. 2 (Climate Prediction Center; www.cpc.ncep.noaa.gov). Regarding topsoil conditions (0–40 cm), Exp. 1 had a good soil N availability at sowing (72.6 kg N-NO₃ ha⁻¹). Experiment 2 was sown after a catch crop of wheat (*Triticum aestivum* L.) that was killed by means of glyphosate application (5 L ha⁻¹ at 48% a.i.) immediately before sowing. Consequently, initial soil N availability was very low (11 kg N-NO₃ ha⁻¹) in Exp. 2. Organic matter level was stable across experiments (2.06–2.08%). No N fertilizer was ever added, and phosphorus level was always high (>30 mg kg⁻¹). Water deficit was always prevented by means of sprinkler irrigation used to keep the uppermost 1 m of soil near field capacity throughout the growing season. Experiments were kept free of pests, weeds, and diseases.

All inbreds were distributed in a complete randomized block design with two replicates. Each plot had three rows of 5.5-m length and 0.7-m between the rows. Stand density was always 7 plants m⁻². Sowing date took place on 21 Oct. 2009 in Exp. 1, but not on a single date in Exp. 2. Because of the expected hot, midsummer weather associated with the La Niña phase of the ENSO in the Pampas region of Argentina during the 2011–2012 season, sowing was delayed for flowering to start at the end of January when solar radiation and temperature start to decline (Otegui et al., 1996). Additionally, inbreds were grouped in three categories (early, intermediate, and late) based on anthesis dates registered in Exp. 1 and were sown on 14 (late), 18 (intermediate), and 23 Nov. 2011 (early) in Exp. 2. This strategy helped

synchronize the flowering event of the whole experiment (Liu et al., 2011) and, in this way, minimized possible confounded effects of differences in weather conditions during grain filling between early and late inbreds associated to a late sowing date (Otegui et al., 1996) when solar radiation is already declining markedly (Otegui and Bonhomme, 1998; Borrás et al., 2004). In summary, we evaluated genotypes in two contrasting environments: (i) a potential condition in Exp. 1, associated with an early sowing date (Otegui et al., 1996) and high initial soil N and (ii) a nonpotential condition in Exp. 2 associated with a delayed sowing date and low initial soil N.

All plots were hand planted at a rate of three seeds per site and thinned to the desired stand density of 7 plants m⁻² at V3.

Measurements

Weather conditions were monitored at the experimental site (Campbell Scientific Inc.), and daily records obtained for mean and maximum (Tmax) air temperatures (°C), photosynthetically active radiation (MJ d⁻¹), rainfall (mm), and potential evapotranspiration (mm). Mean daily air temperature was calculated as the average of hourly air temperature records.

Five consecutive plants were tagged early in their growth cycle (at approximately V5) in the center row of each plot. They were used for the nondestructive assessment of (i) anthesis (at least one anther visible in the tassel) and silking (at least one silk visible in the apical ear) dates of each plant and (ii) shoot biomass production per plant at R2. They were also used for the destructive assessment of PGY (g plant⁻¹), KNP, mean individual KW (mg), and final shoot plant biomass (g plant⁻¹) at physiological maturity (black layer visible in kernels at the middle of the ear). Biomass estimation at R2 was made by means of allometric models described in previous studies (Borrás and Otegui, 2001; D'Andrea et al., 2006, 2009), which included (i) stem volume, based on plant height to the uppermost collar and mean stem diameter at the base of the stalk (average of maximum and minimum values), for the estimation of vegetative biomass (i.e., excluding the ear shoot) and (ii) maximum ear diameter, for the estimation of ear-shoot biomass. These measurements were performed on all tagged plants, at R2 (silking + 15 d) of each plant, and on at least 16 extra plants of very different size that were collected for building the models (vegetative and ear shoot) of each inbred (all models had $r^2 \geq 0.65$, and 96% of them had $r^2 \geq 0.8$; $P \leq 0.001$). For each tagged plant we computed post-R2 shoot biomass accumulation per plant (g) as the difference between observed biomass at physiological maturity and estimated biomass at R2, plant growth per kernel during the effective grain-filling period (mg kernel⁻¹) as the quotient between cumulative post-R2 shoot biomass production and KNP, and ARU per kernel (Eq. [1]) and per plant (Eq. [2])

$$\text{ARU}_k = \text{KW} - \text{post-R2 plant growth per kernel} \quad [1]$$

$$\text{ARU}_p = \text{PGY} - \text{post-R2 shoot plant growth} \quad [2]$$

being $\text{ARU}_k = \text{ARU}_p / \text{KNP}$

Statistical Analyses

A tester inbred was used to evaluate spatial heterogeneity (Gilmour et al., 2006), and no trend was detected. Frequency distributions were computed for each trait to evaluate the range and type of variation produced by genotypes (Kolmogorov–Smirnov normality test). Subsequently, traits were normalized for a common comparison of phenotypic plasticity (Sadras and Slafer, 2012) across genotypes (G) and environments (E). For this purpose, for each trait within each genotype ($n = 20$) we obtained the values representative of the 10th, 50th (i.e., the median), and 90th percentile, as in D'Andrea et al. (2013). These values were averaged across genotypes ($n = 169$), the median value was set to 1, and the 10th and 90th percentiles were expressed as ratios with the median of each attribute. Because all traits were measured on a per-plant basis, we used individual plant data across experiments for the described computation of phenotypic plasticity, which was expressed as the difference between the 10th and 90th percentiles.

Each attribute was evaluated fitting a linear mixed model (Eq. [3]). The phenotypic observation of measured trait Y_{ijk} on genotype i in replicate k of environment j was modeled as follows (D'Andrea et al., 2008):

$$Y_{ijk} = \mu + G_i + E_j + (R/E)_{jk} + (GE)_{ij} + \varepsilon_{ijk} \quad [3]$$

where μ is the general mean, G is the effect of the i th genotype, E is the effect of the j th experiment (environment), R is the effect of the k th replicate nested in the environment, GE is the $G \times E$ interaction effect, and ε is the residual error. Genotype and $G \times E$ interaction effects were treated as random, while E and R effects were treated as fixed (Alvarez Prado et al., 2013). Variance components were estimated for each random term while a standard test of fixed effects was performed for E and R . When necessary (normality test $P < 0.05$), traits were transformed to reach normality. Heritability on a family-mean basis (Holland et al., 2003) was estimated from the components of variance as in Eq. [4].

$$h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 / N_E + \sigma_e^2 / N_E N_R) \quad [4]$$

where σ_G^2 is the G variance, σ_{GE}^2 is $G \times E$ interaction variance, σ_e^2 is the error variance, N_E is the number of environments, and N_R is the number of replicates. These analyses were performed with the MIXED procedure of SAS v. 8.2 (SAS Institute, 1999).

Best linear unbiased predictors of G effects were estimated using the described mixed model (Alvarez Prado et al., 2013), and linear regression analysis was applied to the relationships between variables. Regression analysis was based on the best linear unbiased predictors of actually measured variables (i.e., KNP, PGY, and plant biomass at R2 and at physiological maturity), which were also used for the computation of estimated variables (i.e., KW, post-R2 shoot biomass production, plant growth per kernel during active grain filling, and ARUs). The existence of spurious correlations was evaluated by means of a Monte Carlo simulation approach (Brett, 2004), as described in Rotundo et al. (2014). Monte Carlo simulations were performed by means of R (R Development Core Team, 2011). The procedure was repeated 10,000 times for each correlation, and the r -values obtained with real data were assumed as spurious when they fell within the 10th and 90th randomly generated intervals.

Table 1. Descriptive statistics, heritability (h^2), and ANOVA of measured traits of each inbred.

Trait	Exp. 1			Exp. 2			h^2	ANOVA†		
	Range‡	Median	NT§	Range	Median	NT		G	E	G × E
Kernel number per plant	49–558	280	ns	36–416	260	*	0.61	5.37***	34***	4.04***
Kernel weight, mg	106–296	206	ns	102–237	170	ns	0.70	6.28***	199***	4.74***
Plant grain yield, g	11–130	56	ns	6.0–70	43	*	0.38	3.14***	130***	4.19***
Biomass at R2, g plant ⁻¹	66–215	114	ns	65–142	99	ns	0.38	3.13***	88***	3.79***
Biomass at physiological maturity, g plant ⁻¹	85–287	147	*	73–168	119	ns	0.31	2.49**	183***	3.66***
Plant growth during active grain filling, g plant ⁻¹	-14–81¶	36	ns	-2.6–58.7	20	ns	0.35	2.87**	164***	2.51**
Plant growth per kernel during active grain filling, mg kernel ⁻¹	-88–350	130	ns	6.4–372	82	***	0.43	3.55***	34***	3.26***
Apparent reserves use per plant, g	-15–59	21	ns	-9.9–58	22	ns	0.59	5.14***	0.05ns	1.91*
Apparent reserves use per kernel, mg	-99–279	78	**	-136–176	87	ns	0.28	2.24*	1.51ns	2.87**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† *F* values for the fixed effect of environment (E), and *Z* values for the random effects of genotype (G) and G × E.

‡ Range is from minimum to maximum values.

§ NT, Kolmogorov–Smirnov normality test; ns, not significant.

¶ Negative values indicate a decrease in total shoot biomass between R2 and physiological maturity, which yields negative values of plant growth per kernel.

RESULTS

Weather Conditions

Mean air temperature along the growing season was similar between experiments (~22.0°C), but mean Tmax (average of maximum daily values) was higher in Exp. 2 (29.8°C) than in Exp. 1 (28.2°C). Moreover, there was only 1 d with Tmax ≥ 35°C in Exp. 1, and 20 d with records above this threshold in Exp. 2. These conditions, however, took place before flowering of Exp. 2. As expected from the ENSO forecast, total rainfall during the cycle was markedly larger during Exp. 1 (~900 mm) than in Exp. 2 (~563 mm), which caused the opposite trend in potential evapotranspiration (mean of 4.8 and 5.8 mm d⁻¹, respectively). Nevertheless, rainfall during the flowering period did not differ markedly between experiments (~240 mm), and delayed flowering of Exp. 2 (mean silking date of 6 January for Exp. 1 and 3 February for Exp. 2) exposed the crop to already declining levels of incident photosynthetically active radiation and consequently lower mean potential evapotranspiration values (4.7 mm d⁻¹) than those registered in Exp. 1 (5.7 mm d⁻¹).

Genotypes and Phenotypic Plasticity

Overall differences in weather and initial soil N conditions between experiments caused a pronounced E effect ($P < 0.0001$) on all measured traits except ARU_p and ARU_k (Table 1). On a mean basis (data not shown), improved conditions of Exp. 1 were evident in larger values of all production traits. Experiment 1 showed 26% more plant biomass at physiological maturity, 32% more PGY, and 43% more plant growth per kernel during grain filling when compared with Exp. 1.

The G and G × E effects were highly significant ($P < 0.01$) for most traits, except the G effect for ARU_k and the G × E effect for ARU_p ($P > 0.01$). A large range of variation was registered for each trait in both experiments (Table 1). This variation was (i) smallest for KW, and biomass at R2 and at physiological maturity (less than twofold variation between minimum and maximum values), (ii) intermediate for KNP and PGY (11-fold to 12-fold variation), and (iii) maximum for plant growth during active grain filling (total and per kernel) and ARUs (62-fold to 439-fold variation). As a proportion of final KW, mean ARU_k was 36% in Exp. 1 and 51% in Exp. 2. The same proportions corresponded to mean ARU_p with respect to PGY.

Except for a few cases, most traits were normally distributed across genotypes at both environments (Table 1). Minor specific cases where some data departed from normal distribution were evident, like biomass at physiological maturity and ARU_k in Exp. 1, and KNP, PGY and the plant growth per kernel during active grain filling in Exp. 2 (Table 1).

Despite the observed environmental differences between experiments, and G × E effects registered for all traits (Table 1), relatively high h^2 levels were evident for many traits (Table 1). High h^2 values ($h^2 \geq 0.59$) were computed for both grain yield components (KNP and KW) and ARU_p. The other traits showed lower h^2 estimates ($0.43 \geq h^2 \geq 0.28$).

Traits differed markedly in their phenotypic plasticity across G and E (Fig. 1), from a minimum value of 0.58 registered for KW to a maximum of 25 for ARU_k. For KW, the maximum variation with respect to the median corresponded to the 10th percentile and was 31% (for the 90th percentile of this trait it was 27%). For ARU_k it was 1486% and also corresponded to the 10th percentile (i.e., the largest negative

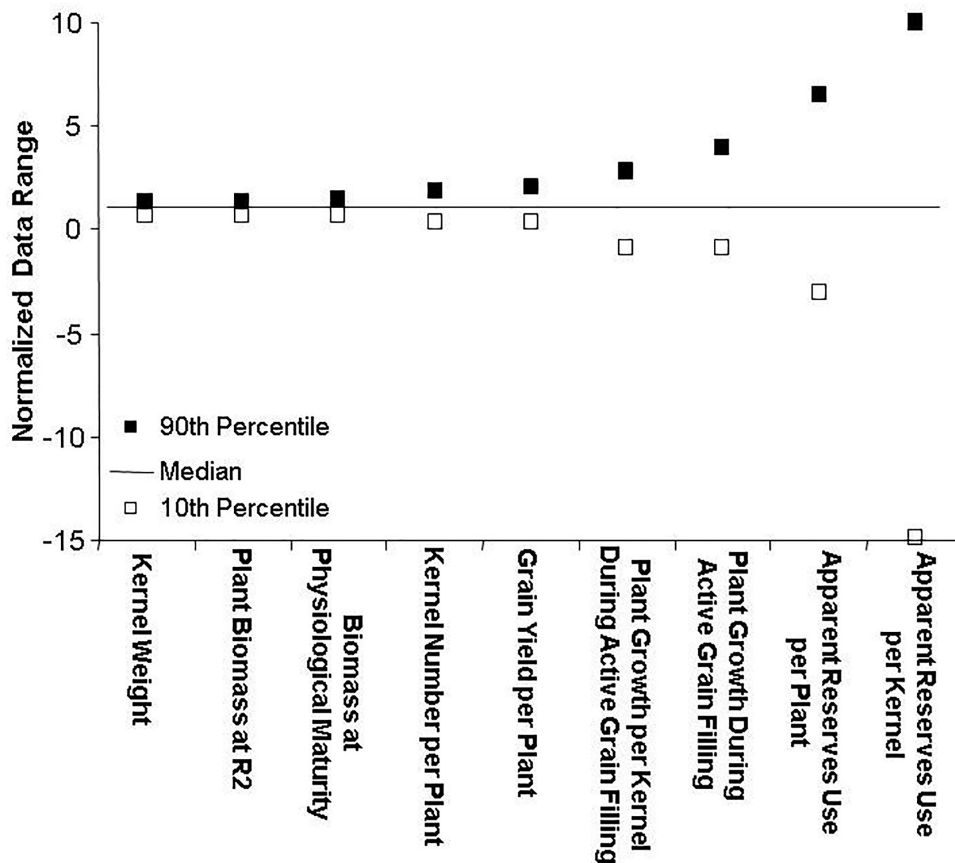


Figure 1. Normalized data range of evaluated variables. The distance between the 10th and 90th percentiles represents the phenotypic plasticity. Computation is based on individual plant data of each inbred \times experiment combination ($n = 20$) and then averaged across inbreds ($n = 169$, including parents).

values, representative of reserves accumulation during grain filling). Other traits with low phenotypic plasticity (<1) were biomass at R2 (0.62) and at physiological maturity (0.77). All other traits had phenotypic plasticity >1 , particularly those linked to biomass production and use during active grain filling (post-R2 biomass production, plant growth per kernel during grain filling, ARU_p , and ARU_K) that had phenotypic plasticity >3.6 . For most traits, data in the 90th percentile corresponded predominantly to Exp. 1 (superior environment based on mean grain yield) and data in the 10th percentile to Exp. 2 (inferior environment based on the same criterion). The only exceptions to this trend were ARU_p and ARU_K , for which data of both experiments were found in similar proportion in the extreme percentiles, in agreement with the lack of an E effect mentioned above. For ARUs, large phenotypic plasticity was mainly driven by the genotypic variation. No clear relationship could be established between phenotypic plasticity and h^2 .

Kernel Weight, Grain Yield, and Assimilates Sources

A bilinear model with plateau gave a significant fit ($r^2 = 0.64$; $P < 0.001$) to the response of KW to the plant growth per kernel during active grain filling (Fig. 2A), and no difference was detected between experiments.

Except for one genotype in Exp. 1, final KW always depended on some degree of reserves use, but no significant correlation was evident between KW and ARU_K . A plant growth per kernel of 233 mg was estimated as the threshold above which no ARU_K was necessary for fulfilling kernel demand (intersection between fitted model and the 1:1 ratio in Fig. 1). Similarly, the variation observed in PGY was explained by the variation registered in plant growth during active grain filling ($r^2 = 0.62$; $P < 0.001$; Fig. 2B), and no difference was detected between experiments in the general trend. As for KW, almost all data fell above the 1:1 relationship, indicative of a positive ARU_p .

The increase in ARU_p was strongly driven by enhanced sink demand represented by increased kernel numbers ($0.55 \geq r^2 \geq 0.49$; $P < 0.001$; Fig. 3A). The number of kernels set per plant was also the main determinant of PGY ($0.67 \geq r^2 \geq 0.64$; $P < 0.001$; Fig. 3B), because the response of PGY to KW was poor ($r^2 \leq 0.03$; $P > 0.025$). In both cases of Fig. 3, the ordinate of fitted models differed between experiments ($P < 0.001$). This trend indicated that improved KNP (Table 1; Fig. 3B) registered during Exp. 1 did not cause an increase dependence on reserves used per each kernel set (Table 1; Fig. 3A), because enhanced sink demand was accompanied by environmental conditions that allowed high plant growth

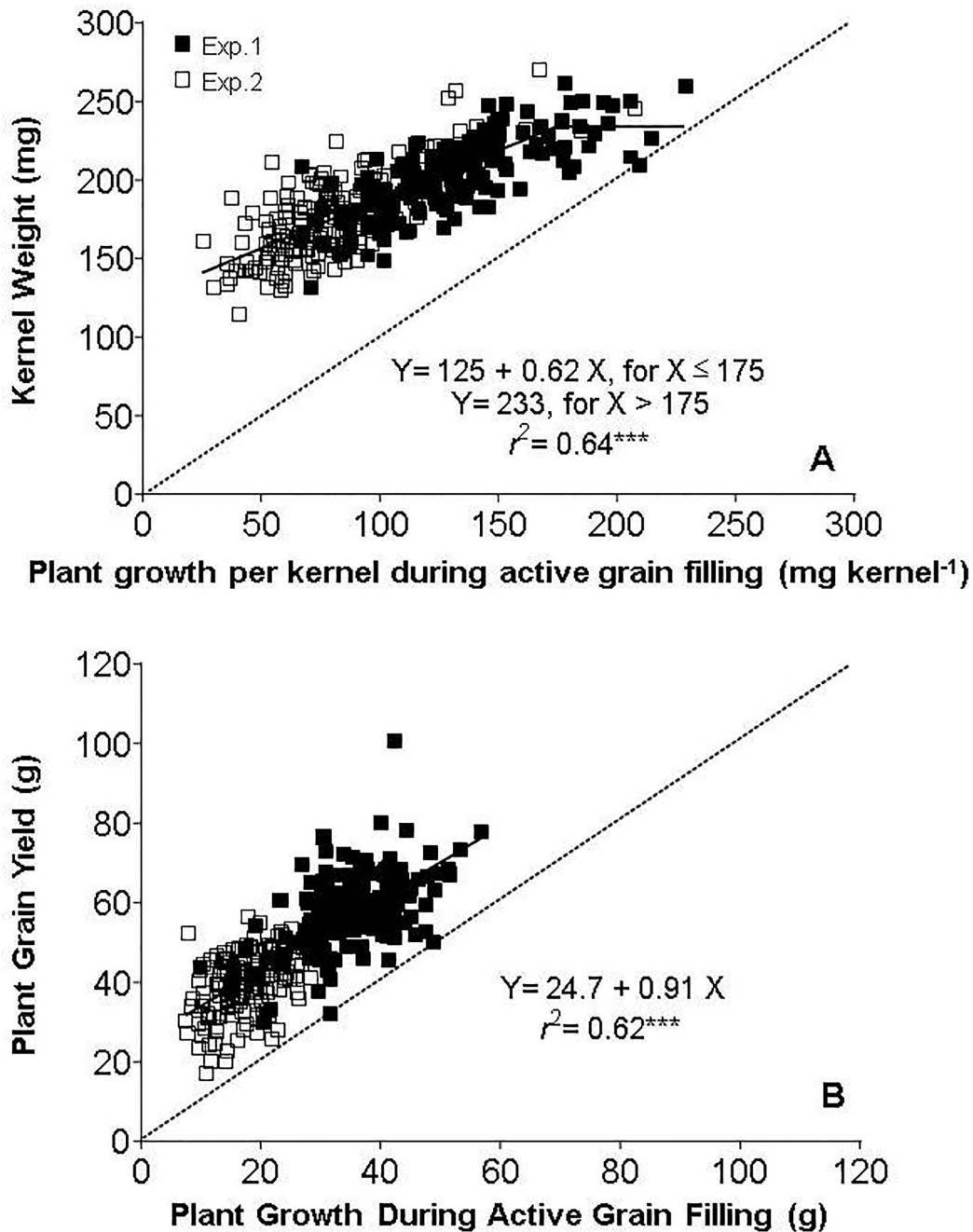


Figure 2. Response of best linear unbiased predictors of (A) individual kernel weight to plant growth per kernel during active grain filling and (B) plant grain yield to plant growth during active grain filling. Two growing seasons were evaluated (Exp. 1 and Exp. 2), and each data point represents an inbred. A single bilinear with plateau model (A) or linear model (B) fitted the whole data set in each case. The dotted line represents the 1:1 ratio.

and consequently high KW. The opposite was verified during Exp. 2. All described relationships were genuine (Supplemental Material S1).

DISCUSSION

The evaluated germplasm and environments produced a wide range of variation in all traits under analysis. However, the degree of variation differed among traits when expressed as phenotypic plasticity. As expected (Sadras and Slafer, 2012; D'Andrea et al., 2013), KW was among the traits with low plasticity, together with total shoot biomass

at the start of active grain filling (R2) and at physiological maturity, whereas KNP and PGY were among those with large plasticity. Nevertheless, estimated plasticity for all mentioned traits was always larger in this study than in a previous survey that included a reduced number of inbreds (six) and hybrids (12) grown under two contrasting N levels in three growing seasons (D'Andrea et al., 2013). Evidently, the genotypic variation evaluated here was broader and enhanced the breadth of this type of analysis.

Important new insights in phenotypic plasticity for this species came with traits describing plant growth after

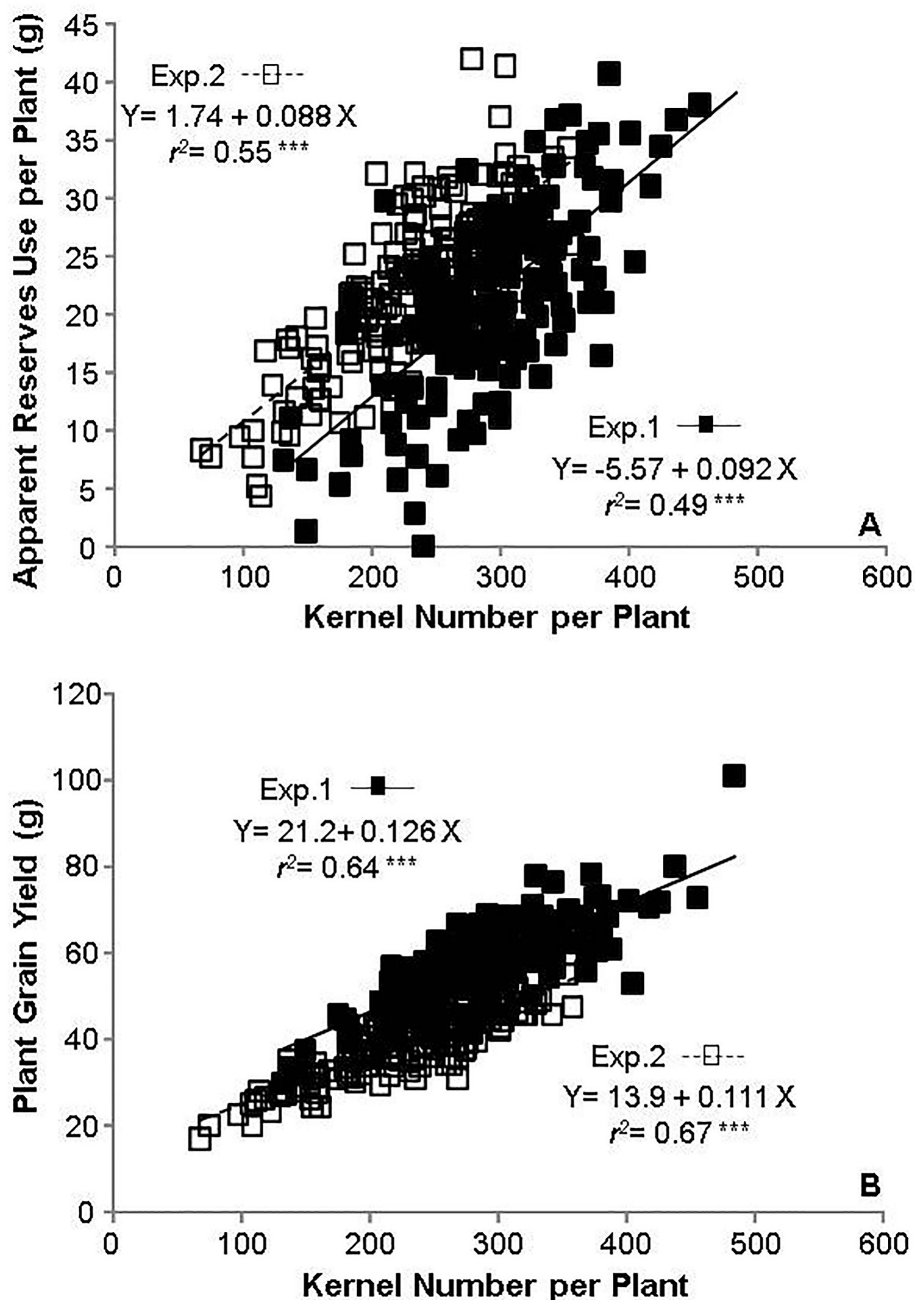


Figure 3. Response of best linear unbiased predictors of (A) apparent reserves use per plant and (B) plant grain yield to kernel number per plant. Symbols as in Fig. 2.

silking, as plant growth per kernel (i.e., a source–sink relationship) and apparent reserves fate, for which there was no previous evidence. All these traits had a very large plasticity level (>3), never reported for maize. Many traits with very high plasticity in our study (post-R2 biomass, plant growth per kernel, and ARU_K) are the result of computations that combine traits with comparatively low plasticity, but no one of this group exhibits the high h^2 observed in ARU_p . For this trait, phenotypic plasticity was predominantly linked to genotypic variation and not to the environment. On one hand, this trend confirms the finding of a large plasticity obtained for the anthesis–silking interval in maize (largest of all evaluated traits by D’Andrea

et al., 2013) in spite of its computation based on phenological traits with very low plasticity and high h^2 (anthesis and silking dates). On the other hand, it does not support the hypothesis proposed by Sadras and Slafer (2012) of a decrease in plasticity and an increase in h^2 with ontogeny, which is valid for yield components but not for biomass related traits. We found several exceptions to this rule in our study and could not establish a negative relationship between phenotypic plasticity and h^2 , as already reported for other traits in maize (D’Andrea et al., 2013).

It is well known that maize final KW depends on kernel growth during an early phase, during which the potential KW is set, and a phase of active biomass accumulation in the

kernels, during which actual KW is set. There is some overlapping between both phases, because the former spans along the lag phase (when the number of endosperm cells is defined) and the early stage of the active grain-filling period (when maximum water content is reached). In previous research (Borrás et al., 2003; Gambín et al. 2006, 2007) we focused on this early phase of grain filling. Nevertheless, the achievement of maize potential KW can be pronouncedly affected by growing conditions during the subsequent phase (Borrás et al., 2004). For this reason, in current research, the strength of the analysis was on the second phase, and particularly on reserves use along it, because depletion of reserves can limit final KW and there were evidences of important genotypic differences for this response (Rattalino Edreira et al., 2014). Reserves are the direct complement of current assimilates production for grain filling, and their use is tightly related to the established source–sink relationship (e.g., plant growth per kernel during active grain filling). The response of KW to this relationship had the typical linear with plateau shape (Cirilo and Andrade, 1996; Borrás and Otegui, 2001). The threshold plant growth per kernel during active grain filling that canceled ARU_K matched the analysis made for two commercial maize hybrids by Uhart and Andrade (1995). They evaluated the variation in SWSC contribution to final KW in response to the same estimator of the source–sink relationship used in current work and detected a threshold between 241 and 291 mg of plant growth per kernel during active grain filling for having no kernel growth dependence on reserves. This range is slightly larger than our finding (233 mg kernel⁻¹), a trend that is supported by computations based on data sets published by Borrás and Otegui (2001) using two different hybrids (258–279 mg kernel⁻¹) and Cirilo and Andrade (1996) using one hybrid (421 mg kernel⁻¹). The threshold per se depends on the plateau of the model (i.e., potential KW). A larger threshold may represent an enhanced dependence of grain filling on reserves use, and available evidence suggests that this dependence was more critical among previously evaluated hybrids than among current inbreds. Complete depletion of reserves before the expected physiological maturity date defined by kernel desiccation level (Borrás et al., 2004; Gambín et al., 2007) leads to anticipated grain-filling arrest (Jones and Simmons, 1983; Uhart and Andrade, 1991, 1995; Rattalino Edreira et al., 2014).

Maize KW dependence on reserves cannot be attributed directly to a different balance between kernel numbers and the level of current assimilates during grain filling because hybrids evaluated in several studies (Uhart and Andrade, 1995; Cirilo and Andrade, 1996) did not have a smaller plant growth per kernel (mean values between 153 and 349 mg kernel⁻¹) than inbreds included in our study (mean values between 75 and 127 mg kernel⁻¹). However, hybrids used in previous studies had larger kernels (mean KW values between 271 and 283 mg among their hybrids)

than inbreds used in current analysis (between 175 and 201 mg), which, combined with the large KNP of the former, may have represented an enhanced instant assimilates demand (Echarte et al., 2006) and consequently an enhanced dependence on reserves. In agreement with this contention, the variation registered in ARU_P was strongly linked to KNP, the main determinant of PGY. These findings may explain differences between hybrids and inbreds in reserves use because the former usually double the latter in KNP (D'Andrea et al., 2009). They also support the contention of a reduced KW stability among modern maize hybrids as a result of their enhanced number of kernels (Echarte et al., 2006). Nevertheless, this response will be substantially affected by growth conditions during active grain filling, which modify current plant growth and consequently the actual dependence of KW on reserves use (Cirilo and Andrade, 1996; Borrás et al., 2004) particularly in high latitude environments that may affect reserves mobilization as a result of low temperatures (Ruguet, 1993; Kiniry and Otegui, 2000).

Collectively, our findings highlighted the importance of breeding for an increased photosynthetic activity during grain filling, but this effort should not be limited to the postsilking period (Tollenaar, 1989; Tollenaar and Aguilera, 1992; Rajcan and Tollenaar, 1999; Luque et al., 2006) and genetically controlled delayed leaf senescence (He et al., 2005). This has been the predominant idea at many studies. High-yielding maize crops depend primarily on increasing kernel numbers, which enhance the demand set on reserves (Fig. 3A). Consequently, high photosynthetic activity during late stem elongation and pollination is essential for granting adequate assimilate provision to satisfy ear demand and kernel set (Schussler and Westgate, 1994) as well as high accumulation of SWSC for granting adequate grain filling and reducing lodging risk. High accumulation of SWSC may be particularly important in environments prone to terminal stress (Ouattar et al., 1987; Rattalino Edreira et al., 2014) as already demonstrated for wheat crops (Ehdaie et al., 2008; Dreccer et al., 2009).

CONCLUSIONS

We evaluated the phenotypic and genotypic variation in KW and different biomass sources for supporting grain filling in a RIL family. A high h^2 was detected for grain yield components (KNP and KW) and ARU_P , but the latter was the only trait with a large phenotypic plasticity linked almost exclusively to *G* effects. This characteristic, together with its positive relationship with kernel number and grain yield, as well as its relatively fast, easy and inexpensive measurement, makes ARU_P a promising trait in future studies for maize breeding.

Supplemental Information Available

Supplemental information is available with this article.

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