RESEARCH

Correlations Between Parental Inbred Lines and Derived Hybrid Performance for Grain Filling Traits in Maize

Santiago Alvarez Prado, Brenda L. Gambín, A. Daniel Novoa, David Foster, M. Lynn Senior, Christopher Zinselmeier, María E. Otegui, and Lucas Borrás*

ABSTRACT

Individual kernel weight (KW) is largely genetically determined, and its variability is achieved through different combinations of rate and duration of kernel growth. Genetic variability for grain-filling patterns has been observed among inbred lines and commercial hybrids, and there is current interest on dissecting its genetic basis. However, suitable grain filling phenotyping protocols are still to be determined, such as the value to study traits at the inbred or hybrid levels. The objective of our study was to evaluate the correlation between parental inbred line and derived hybrid performance for several grain-filling traits in maize (*Zea mays* L.). We hypothesized that there would be high correlations due to the relative high heritability of grain-filling traits. Three trials were conducted (two in Argentina and one in the United States) with commercial relevant germplasm (totaling 25 parental inbreds and 31 single-cross hybrids). Traits were KW, kernel growth rate (KGR), grain-filling duration (GFD), maximum water content (MWC), moisture concentration at physiological maturity (MCPM), and kernel desiccation rate (KDR) during the effective grain filling. Both heterosis and correlations between midparental value and hybrid performance were significant ($p < 0.05$) for all traits (r values of 0.63, 0.71, 0.81, 0.83, 0.61, and 0.71 for KW, KGR, GFD, MWC, KDR, and MCPM, respectively). Our results confirm that studying inbred lines for grain-filling traits generates valuable information for derived hybrid performance.

S. Alvarez Prado, B.L. Gambín, and L. Borrás, Departamento de Producción Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental Villarino S/N, (S2125ZAA) Zavalla, Prov. de Santa Fe, Argentina; A.D. Novoa, Nidera S.A., Ruta 8 Km 376,5, Venado Tuerto (2600), Prov. de Santa Fe, Argentina; M.L. Senior, Syngenta Biotechnology, Inc., 3054 East Cornwallis Rd., RTP, NC 27709; D. Foster and C. Zinselmeier, Syngenta Seeds, 2369 330th Street, PO Box 500, Slater, IA 50244; M.E. Otegui, Fac. de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, 1426, CABA, Argentina. Received 16 Jan. 2013. *Corresponding author (lborras@unr.edu.ar)

Abbreviations: DHM, derived hybrid mean; GCA, general combining ability; GFD, grain-filling duration; KDR, kernel desiccation rate; KGR, kernel growth rate; KW, kernel weight; MC, moisture concentration; MCPM, moisture concentration at physiological maturity; MP, midparental value; MPH, midparental heterosis; MWC, maximum water content; PVP, Plant Variety Protection; SCA, specific combining ability.

Grain yield is considered a complex trait. A common way to simplify this complexity is to study individually the physiological mechanisms related to the determination of both main yield components, kernel number per unit land area and individual kernel weight (KW). Although kernel number is usually the component explaining most yield variations, both components affect final yield (Borrás and Gambín, 2010). Individual KW varies markedly among genotypes of the same species grown under no environmental constraint. It is largely a genetically determined trait (Reddy and Daynard, 1983), and its variability is achieved through different combinations of kernel growth rate (KGR) and grain-filling duration (GFD).

Published in Crop Sci. 53:1–10 (2013).
doi: 10.2135/cropsci2013.01.0035

Genetic variability for grain-filling patterns has been observed among maize elite and exotic inbred lines and among commercial hybrids from different production regions (Cross, 1975; Wang et al., 1999; Gambín et al., 2007; Borrás et al., 2009). Gambín et al. (2007) characterized 12 commercial Argentine hybrids and showed that KW differences were a consequence of different KGR and GFD combinations. Similar results were shown by Borrás et al. (2009) when characterizing 60 inbred lines adapted to the central United States. These studies were mainly focused on dissecting the genetic basis of variability for grain filling. What still needs to be determined is the value of studying grain-filling traits at the inbred or hybrid levels. Recent studies on the genetic basis of grain-filling traits have been conducted at the inbred level (Liu et al., 2011; Li et al., 2012; Alvarez Prado et al., 2013) without considering the value these results have for hybrid performance.

For breeding purposes, trait evaluation at the inbred level has little value if the parental inbred performance is not correlated to the derived hybrid performance (Hallauer and Miranda, 1988). Any information on parental inbred lines that is indicative of derived hybrid performance is highly desirable. It helps eliminate the need for doing testcrosses and conducting extensive trials. Studies are well documented for traits such as grain yield, plant height, and prolificacy where results have shown correlations between inbreds and hybrids to be generally low (see Hallauer and Miranda, 1988). Results could be explained by the high phenotypic plasticity and significant environmental modulation of several of these traits, which is reflected in their low heritability (Sadras and Slafer, 2012). In contrast, KW and its component traits (KGR and GFD) are known to show intermediate to high heritability values (Sadras, 2007; Gambín and Borrás, 2011). At present, correlations between parental inbred line and derived hybrid performance for maize grain-filling traits are basically unknown.

Kernel weight determination is generally described in terms of dry matter and water content accumulation (Schnyder and Baum, 1992; Borrás et al., 2003; Rondanini et al., 2007; Bingham et al., 2007; Borrás and Gambín, 2010). Kernel development is usually divided into three phases: the lag phase, the effective grain-filling period, and the maturation drying phase (Bewley and Black, 1985). The lag phase begins at pollination and is a period of active cell division. It is characterized by water content increases with almost no dry matter accumulation. The effective grain-filling period is characterized by rapid dry matter accumulation resulting from the deposition of reserves. During this period, at about mid grain filling, maize kernels reach their maximum water content (MWC) (Borrás et al., 2003). After MWC is attained, water is gradually replaced by dry matter deposition. Moisture concentration (MC) within kernels is reduced throughout grain filling. At a particular critical MC biomass deposition is stopped. This moment is known as physiological maturity and is defined by the occurrence of maximum KW (Shaw and Loomis, 1950). The maturation phase begins at physiological maturity, when kernels continue loosing water and enter a quiescent state (Bewley and Black, 1985).

Little information involving testcross performance for KW and its physiological determinants is available. Cross (1975) and Wang et al. (1999) conducted diallel crosses for analyzing maize GFD and KGR and reported additive effects for both traits. Comparing three to four lines and derived hybrids in reciprocal combinations, Poneleit and Egli (1979, 1983) also reported additive effects together with heterosis effects commonly observed in hybrid performance. Selection techniques that take advantage of additive variation could be used to alter GFD and KGR. However, information on performance correlations between midparental inbred lines and derived hybrids for relevant grain-filling traits is lacking. The objective of our study was to evaluate the correlation between parental inbred line performance and derived single-cross hybrid performance for several grain-filling traits in maize.

MATERIALS AND METHODS

Plant Materials

Three experiments containing different sets of genotypes were evaluated. In Exp. 1, 10 proprietary Syngenta Seeds Inc. inbred lines, subsequently coded as L1, L2, L3, L4, L5, L6, L7, L8, L9, and T1, and nine single-cross derived hybrids were evaluated. Crosses were made by mating the inbred tester (T1) as pollinator to the other nine inbred lines (L1, L2, L3, L4, L5, L6, L7, L8, and L9).

Experiment 2 evaluated nine expired PVP (Plant Variety Protection) inbred lines (USDA-ARS National Genetic Resources Program, 2005), two proprietary Nidera S.A. inbred lines, and 18 single-cross derived hybrids. Expired PVP inbred lines PHM10, PHH93, LH150, PHK29, PHN29, PHP02, PHK42, ML606, and WIL901 were crossed to the two proprietary Nidera S.A. inbred lines (coded as T2 and T3) for developing the 18 single-cross hybrids.

Experiment 3 consisted in evaluating four inbred lines and four derived hybrids. Parental inbred lines were all proprietary inbred lines from Nidera S.A. Two inbred lines were used as females (coded as L10 and L11) and two as males (T4 and T5), and the four possible single-cross hybrid combinations were tested (T4 × L10 is Ax889Mg, T4 × L11 is Ax882Mg, T5 × L10 is Ax675Mg, and T5 × L11 is Ax820Mg). Except Ax675Mg, all other derived hybrids have been commercially available genotypes in Argentina.

Field Experiments

Experiment 1 was conducted at the experimental field of Syngenta Seeds Inc. at Slater, IA, during 2008 growing season. The experimental design was a randomized complete block with three replications. Sowing date was 6 May 2008. A stand density of 8 plants m^2 was used. Plots were oversown and thinned between V2 and V3 and consisted of three and four rows for inbred lines and hybrids, respectively. Plots were 6 m long with 0.76 m of interrow spacing.

Experiment 2 was conducted during the 2011/2012 growing season at the Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, in Zavalla, Provincia de Santa Fe, Argentina. The experimental design was a randomized complete block with three replications. Sowing was 17 Oct. 2011. A stand density of 7 plants m^{-2} was used. Plots were oversown and thinned between V2 and V3 and consisted of three rows 6 m long and 0.52 m apart.

Experiment 3 was conducted at the experimental field of Nidera S.A. in Venado Tuerto, Provincia de Santa Fe, Argentina, during the 2003/2004 growing season. The experimental design was a randomized complete block with three replications. Sowing was 30 Oct. 2003. A stand density of 9 plants m^{-2} was used. Plots were oversown and thinned between V2 and V3 and consisted of five rows 0.52 m apart and 6 m long.

Experiments were conducted without N and water limitations, and no visible signs of water stress were evident. In Exp. 2, 150 mm of water were applied with a sprinkler irrigation system during flowering and early grain filling. In Exp. 3, a total of 200 mm of water were applied with a sprinkler irrigation system throughout the growing season. In all experiments pests, weeds, and diseases were controlled by spraying commercially recommended maize fungicides, herbicides, and insecticides. Weeds were also periodically removed by hand whenever necessary.

Phenotypic Measurements

In each experiment, kernel dry matter and water content were measured throughout kernel development beginning 15 d after each plot reached anthesis. Sampling continued until harvest maturity (15% kernel MC) following procedures described in Borrás et al. (2003). Briefly, one plant per plot was sampled every 4 to 5 d between 0700 and 1000 h. The entire ear with surrounding husks was enclosed in an airtight plastic bag at the field and transported to the laboratory. Kernels were removed from the ear at floret positions 8 to 15 from the bottom of the rachis within a humidified box. Ten kernels per ear were sampled. Fresh weight was measured immediately after sampling, and kernel dry weight was determined after drying samples at 70°C for at least 96 h. Fresh and dry weight were used to calculate kernel water content (mg of water per kernel) and kernel MC (g water per g of fresh weight).

Kernel growth rate and GFD were determined for each genotype × replication combination by fitting a bilinear model (Eq. [1] and [2]) as in Borrás et al. (2009):

$$KW = a + bTT \text{ for } TT \le c \text{ and} \quad [1]$$

$$KW = a + bc \text{ for } TT > c, \quad [2]$$

in which TT is the number of heat units after pollination (°C day), a is the y-intercept (°C day), b is the KGR during the effective grain-filling period (mg °C d^{-1}), and c is the GFD (°C day). Figure 1 has a graphical representation of KGR and GFD together with the other grain-filling traits of interest. Heat units were calculated using 0°C as base temperature (Muchow, 1990; Borrás et al., 2009). Mean daily air temperature was registered at all sites using a weather station located 50 to 200 m from the experimental plots.

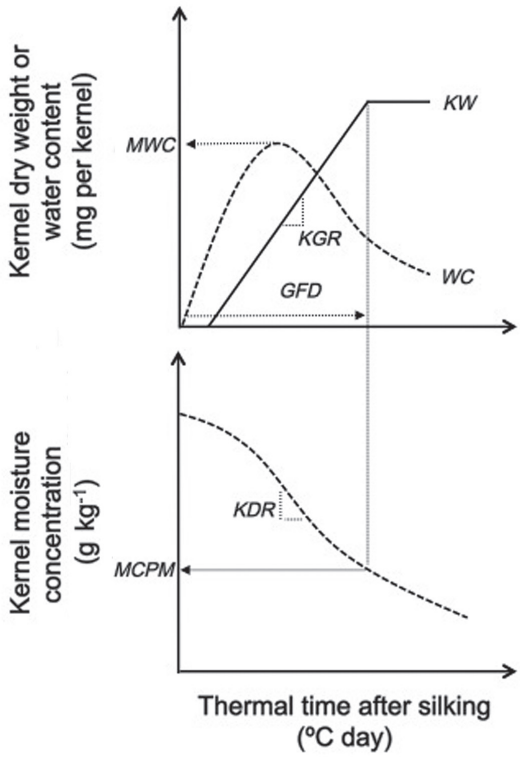

Figure 1. Schematic figure describing phenotypic grain-filling traits of interest: kernel weight (KW), kernel growth rate (KGR), grain-filling duration (GFD), maximum water content (MWC) moisture concentration at physiological maturity (MCPM), and kernel desiccation rate (KDR) during the effective grain-filling period. See Materials and Methods for details regarding the measurement of each specific trait. WC, water content.

Kernel MWC was determined for each genotype × replication combination by fitting a curvilinear model (Eq. [3]) as in Borrás et al. (2009):

$$WC = d + eTT + fTT^{1.5} + gTT^2, \quad [3]$$

in which WC is kernel water content and d, e, f, and g are model parameters.

Kernel moisture concentration at physiological maturity (MCPM) was determined using a bilinear model relating kernel dry weight and kernel MC data (Eq. [4] and [5]) following Borrás et al. (2009):

$$KW = h - iMC \text{ for } MC \ge j \text{ and} \quad [4]$$

$$KW = h - ij \text{ for } MC < j, \quad [5]$$

in which MC is moisture concentration (%), h is the y-intercept (mg), i is the rate of kernel MC decline during grain filling (mg per percent relative humidity), and j is the MCPM (%) (Fig. 1B).

Kernel desiccation rate (KDR) was determined using a linear regression model fitted for each genotype × replication combination relating kernel moisture concentration and thermal time from MC values from 80 to 35%:

$$MC = k + lTT,$$

in which MC is kernel moisture concentration (g kg^{-1}), k is the y-intercept (g kg^{-1}), and l is the KDR (g kg^{-1} °C d^{-1}) (Fig. 1B).

All curves were fitted using the GraphPad Prism version 5.0 (Raduschev, 2007) iterative optimization technique for each genotype × replication combination. This gave us three estimates of each grain-filling trait at each experiment and the possibility to analyze trait variability.

However, maternal influence on kernel growth has always shown to be more important than nonmaternal or xenia effects (Poneleit and Egli, 1983; Jones et al., 1996). Therefore, sampling was done in the central plot rows to minimize cross-pollination.

Statistical Analysis

Each experiment was analyzed separately. An ANOVA was performed for each trait using PROC GLM of SAS (SAS Institute, 1999). Inbred lines and testcrosses from every experiment were evaluated jointly by using the following linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k(\beta_j) + \varepsilon_{ij},$$

in which Y_{ijk} is the observed trait value of the kth genotype from the jth type in the ith block, μ is the overall mean, α_i is the block effect, β_j is the type (inbred line or derived hybrid) effect, $\gamma_k(\beta_j)$ is the effect of the kth genotype nested within the jth type, and ε_{ij} is the residual effect. For all genotypes, corrected means were calculated.

For testcrosses from Exp. 2 and 3, the hybrid effect was partitioned into different sources of variation. The linear model for statistical analysis was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk} + \varepsilon_{ijk},$$

in which Y_{ijk} is the observed trait value of the jth female line with the kth tester in the ith block, μ is the overall mean, α_i is the block effect, β_j is the female line effect or general combining ability (GCA) of the line, γ_k is the male tester effect or GCA of the tester, $\beta\gamma_{jk}$ is the interaction between line and tester or the specific combining ability (SCA) effect for the jkth cross (SCA), and ε_{ij} is the residual effect.

Midparental heterosis (MPH) was calculated as the superiority of the derived hybrid compared to its midparental mean (Munaro et al., 2011):

$$MPH = [(DHM - MP)/MP] \times 100,$$

in which DHM is the derived hybrid mean and MP is the midparental value. Statistical significance of heterosis values for each trait in each experiment and in all three experiments jointly was determined by a t test:

$$t = (X_{DHM} - X_{MP})/SE(X_{DHM} - X_{MP}),$$

in which X_{DHM} is the average of all derived hybrid means, X_{MP} is the average of all mean parental values, and SE the standard error of the difference between averages.

Correlation analyses were done by comparing the average of the two parental inbred lines and their specific testcross for each trait. The Pearson correlation coefficient r was used for establishing the association degree between the midparental inbred line performance and derived hybrid performance for each trait.

For each experiment, broad-sense heritability of each trait was calculated on a mean basis following Holland et al. (1998, 2003). Because of the small sample size (only 8 genotypes), heritability was not estimated in Exp. 3. In Exp. 1 and 2, variance components were estimated by the restricted maximum likelihood method using the MIXED procedure from SAS (SAS Institute, 1999) where block and genotype effects were considered random factors with independent normal distribution and mean zero and there was an unstructured variance–covariance matrix. Type effect (mean parental line or derived hybrid) was considered as a fixed effect according to Möhring et al. (2011). Approximate standard errors of heritability estimates were obtained by means of the delta method (Lynch and Walsh, 1997).

RESULTS

Phenotypic Variability

Experiment 1

Variation within each type (i.e., inbred lines or hybrids) was significant for all traits ($p < 0.05$; Table 1). Kernel weight was significantly ($p < 0.01$) lower for the parental inbred lines than for the derived hybrids, ranging from 192 to 294 mg per kernel among inbred lines and from 258 to 317 mg per kernel among derived hybrids. The inbred line used as common tester (T1) showed a slightly higher than average KW of 263 mg per kernel (Table 1) when compared to the other inbreds.

The two KW component traits, KGR and GFD, showed significant differences within each type (Table 1). Kernel growth rate was not significantly higher in hybrids when compared to inbreds ($p > 0.05$) and ranged from 0.295 to 0.444 mg °C d^{-1} across the entire set of genotypes. The tester line (T1) showed a KGR close to average (0.333 mg °C d^{-1}) when compared to the other inbred lines. For GFD large differences among inbred lines and across hybrids were observed (Table 1), with hybrids showing a significantly longer grain filling than inbreds ($p < 0.05$). The GFD observed at the hybrid level was probably related to the large GFD showed by tester line T1. This inbred line T1 shown the largest GFD among all phenotyped inbred lines (Table 1).

When analyzing kernel water related traits, inbred lines showed smaller MWC ($p < 0.01$), higher KDR ($p < 0.01$), and similar MCPM ($p > 0.05$) when compared to hybrids. Maximum water content ranged from 148 to 230 mg per kernel at the inbred level and 197 to 255 mg per kernel at the hybrid one. The tester line showed slightly higher than average MWC when compared to the

Table 1. Kernel weight, kernel growth rate, grain-filling duration, maximum water content, kernel desiccation rate, and kernel moisture concentration at physiological maturity of 10 inbred lines and nine testcrosses (Exp. 1).

Type	Genotype	Kernel weight	Kernel growth rate	Grain-filling duration	Maximum water content	Kernel desiccation rate	Moisture concentration at physiological maturity
		mg per kernel	mg °C d^{-1}	°C day	mg per kernel	g kg^{-1} °C d^{-1}	%
Inbred line	L1	244	0.368	936	180	0.640	36.9
	L2	294	0.444	915	230	0.677	39.6
	L3	205	0.316	874	170	0.592	36.0
	L4	234	0.359	909	178	0.675	36.3
	L5	219	0.333	878	193	0.673	41.5
	L6	192	0.295	879	148	0.671	38.9
	L7	246	0.328	960	200	0.669	37.0
	L8	252	0.334	1003	152	0.597	32.2
	L9	273	0.346	1023	228	0.555	38.4
	T1	263	0.333	1047	203	0.535	36.2
Hybrid	L1 × T1	258	0.358	974	228	0.567	42.4
	L2 × T1	317	0.404	1040	237	0.625	37.8
	L3 × T1	272	0.345	1030	202	0.623	37.7
	L4 × T1	270	0.370	977	232	0.579	36.1
	L5 × T1	278	0.373	990	230	0.515	34.7
	L6 × T1	260	0.314	1047	197	0.562	36.3
	L7 × T1	287	0.385	1000	211	0.557	34.0
	L8 × T1	275	0.330	1068	204	0.549	35.7
	L9 × T1	316	0.326	1167	255	0.492	33.2
	Inbred line mean	240	0.347	931	187	0.639	37.4
	Hybrid mean	281	0.356	1033	222	0.563	36.4
	Type	*** (18)†	ns‡	*** (99)	*** (11)	*** (0.078)	ns
	Genotype (type)	*** (11)	*** (0.029)	** (58)	*** (6)	** (0.046)	*** (2)

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†The data in parentheses represents LSD values for $p \leq 0.05$.

‡ns, not significant, $p > 0.05$

other inbred lines. Kernel desiccation rate ranged from 0.535 to 0.677 g kg^{-1} °C d^{-1} among the inbred lines and from 0.492 to 0.625 g kg^{-1} °C d^{-1} among the hybrids. The lower KDR among the derived hybrids is in accordance with their longer GFD. Also, the inbred tester (T1) showed the smallest KDR among inbred lines, in accordance to its longer GFD.

Moisture concentration at physiological maturity was not different when inbreds and hybrids were compared, but significant ($p < 0.001$) differences across genotypes were observed within types (Table 1). Values ranged from 32 to 42% (Table 1). The inbred tester (T1) showed an average MCPM value (36.2%).

Experiment 2

Results from Exp. 2 showed large differences across genotypes within each type for all evaluated grain-filling traits, as observed in Exp. 1. In Exp. 2, KW was significantly higher in hybrids than inbred lines ($p < 0.01$) and ranged from 229 to 314 mg per kernel across all genotypes (Table 2).

Both KW component traits, KGR and GFD, showed significant differences among lines and hybrids ($p < 0.01$; Table 2). Inbred KGR was similar to that of the hybrids and ranged from 0.249 to 0.364 mg °C d^{-1} across all evaluated genotypes. Both tester lines (T2 and T3) showed intermediate KGR values when compared to the other inbreds. In contrast, hybrids showed longer GFDs when compared to their parental inbred lines ($p < 0.01$) and ranged from 1086 to 1322°C day for the hybrids and 928 to 1277°C day for the inbreds (Table 2). For GFD, both testers (T2 and T3) showed larger values when compared to the inbred line average (Table 2).

When kernel water related traits were analyzed, inbred lines showed lower MWC ($p < 0.01$), higher KDRs ($p < 0.01$), and higher MCPM values ($p < 0.01$) compared with hybrids. Significant differences in the kernel MWC attained at mid grain filling were evident, ranging from 141 to 213 mg per kernel for inbreds and from 157 to 224 mg per kernel for hybrids. Testers showed slightly lower than average MWC values when compared to the other inbreds (Table 2). Kernel desiccation rate varied from 0.445 to 0.611 g kg^{-1} °C d^{-1} for the hybrids and from 0.511 to 0.762 g kg^{-1} °C d^{-1} for the inbreds. The lower KDR observed at the hybrid level was associated with

Table 2. Kernel weight, kernel growth rate, grain-filling duration, maximum water content, kernel desiccation rate, and kernel moisture concentration at physiological maturity of 11 inbred lines and 18 testcrosses (Exp. 2).

Type	Genotype	Kernel weight	Kernel growth rate	Grain-filling duration	Maximum water content	Kernel desiccation rate	Moisture concentration at physiological maturity
		mg per kernel	mg °C d^{-1}	°C day	mg per kernel	g kg^{-1} °C d^{-1}	%
Inbred line	PHM10	235	0.356	945	191	0.752	37.5
	PHH93	239	0.332	981	195	0.675	38.2
	LH150	306	0.314	1277	195	0.571	32.2
	PHK29	241	0.327	1060	199	0.579	35.9
	PHN29	251	0.340	1045	200	0.671	37.7
	PHP02	231	0.364	928	197	0.762	40.0
	PHK42	229	0.328	962	188	0.758	37.3
	ML606	238	0.270	1170	157	0.588	28.6
	WIL901	244	0.363	998	213	0.655	43.0
	T2	251	0.249	1224	174	0.541	31.7
	T3	262	0.301	1146	141	0.511	29.1
Hybrid	PHM10 × T2	286	0.347	1107	209	0.608	32.8
	PHM10 × T3	285	0.310	1194	179	0.583	26.2
	PHH93 × T2	301	0.330	1197	214	0.595	32.5
	PHH93 × T3	279	0.344	1086	179	0.637	30.8
	LH150 × T2	301	0.322	1235	224	0.511	32.7
	LH150 × T3	286	0.275	1322	159	0.445	27.2
	PHK29 × T2	276	0.310	1197	198	0.577	30.7
	PHK29 × T3	281	0.318	1178	173	0.526	30.8
	PHN29 × T2	300	0.348	1169	221	0.611	34.6
	PHN29 × T3	297	0.348	1152	183	0.583	28.9
	PHP02 × T2	293	0.356	1136	221	0.608	36.1
	PHP02 × T3	314	0.335	1200	205	0.572	29.2
	PHK42 × T2	301	0.348	1156	220	0.575	33.8
	PHK42 × T3	292	0.355	1088	195	0.582	32.3
	ML606 × T2	292	0.304	1244	186	0.527	28.9
	ML606 × T3	262	0.290	1171	157	0.467	19.7
	WIL901 × T2	298	0.296	1304	189	0.523	29.3
	WIL901 × T3	308	0.340	1199	189	0.551	32.2
	Inbred line mean	246	0.333	1041	193	0.668	36.7
	Hybrid mean	292	0.326	1185	195	0.560	30.5
	Type	*** (24)†	ns‡	*** (116)	** (15)	*** (0.070)	*** (6)
	Genotype (type)	*** (14)	*** (0.024)	*** (67)	*** (9)	*** (0.039)	*** (3)
	Line§	*** (22)	*** (0.037)	** (88)	*** (14)	*** (0.056)	** (4)
	Tester	ns	ns	ns	*** (27)	* (0.108)	*** (10)
	Line × tester	* (5)	ns	ns	*** (14)	ns	ns

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†The data in parentheses represents LSD values for $p \leq 0.05$.

‡ns, not significant, $p > 0.05$

§Line, tester, and line × tester effects represent general combining ability (GCA) for lines, GCA for tester, and specific combining ability, respectively. See Materials and Methods for further details.

the longer GFD these genotypes showed. The two testers (T2 and T3) showed slightly lower KDRs, in accordance to their longer than average GFD when compared to the other inbred lines (Table 2).

Moisture concentration at physiological maturity was higher for the parental inbred lines when compared to their derived hybrids, also in accordance to their shorter GFD (Table 2). Testers showed slightly lower than average values for this trait. Parental inbred lines ranged from 29 to 43% and hybrids from 26 to 36% (Table 2).

Because in Exp. 2 we used two testers we were able to partition the different sources of variation (line, tester, and line × tester) over the hybrid performance. The contribution of the inbred line over the derived hybrid performance

was detected with the additive (GCA) portion of the genetic variance. The line × tester interaction was detected by calculating the nonadditive (SCA) portion of the genetic variance. A significant line effect was detected for all evaluated traits ($p < 0.01$; Table 2) where several lines contributed to heavier KW through different KGR or GFD combinations. A tester effect was detected for all kernel water related traits, such as MWC, MCPM, and KDR ($p < 0.05$; Table 2), showing genes with additive effects were provided by both inbred lines and testers. Tester T3 contributed to increasing these traits for hybrid performance while tester T2 decreased average values for the three traits. Within the nonadditive portion of the genetic effects (SCA), significant differences among line × tester combinations were detected for KW ($p < 0.05$) and MWC ($p < 0.001$). These results indicate the presence of genes with both additive and nonadditive effects responsible for the genetic variability among inbred lines × tester crosses for KW and MWC. For KGR, GFD, MCPM, and KDR, genetic variability was only related to genes with additive effects.

Experiment 3

For Exp. 3 only dry matter accumulation traits were phenotyped, and results also showed large differences across genotypes ($p < 0.01$), except for GFD (Table 3).

Parental inbred lines showed significantly lower KWs than their derived hybrids ($p < 0.01$; Table 3). This KW difference was related to a longer GFD observed for the hybrids, as KGR was similar among parental inbred lines and derived hybrids (Table 3).

Similar to Exp. 2, the hybrids performance was dissected in relation to the parental inbred lines (GCA) portion of the variance and the line × tester interaction calculating the nonadditive (SCA) portion of the genetic variance. Traits showed no significant SCA ($p > 0.05$), and line effects were significant for KW ($p < 0.05$) and KGR ($p < 0.001$; Table 3). This suggests that genes with additive effects were responsible for the genetic variability of the traits.

Heterosis

Kernel weight was significantly higher in derived hybrids than in inbred lines regardless of the experiment (Table 4), showing a MPH of 11.9, 16.4, and 21.8% for Exp. 1, 2, and 3, respectively. When dissecting KW into its physiological components, Exp. 2 showed that the higher KW of hybrids relative to their parental inbred lines was associated with heterosis for all evaluated traits (Table 4). In Exp. 1 higher KW in hybrids when compared to inbreds was associated with heterosis for MWC ($p < 0.01$), and in Exp. 3 KW MPH was related to heterosis for GFD ($p < 0.01$; Table 4).

When analyzing the three experiments together all evaluated traits showed significant MPH ($p < 0.01$; Table 4), with hybrids showing higher KW, KGR, GFD, and MWC values and lower KDR and MCPM values when compared to their midparental inbred line values (Table 4).

Table 3. Kernel weight, kernel growth rate, and grain-filling duration were measured in four parental inbred lines and four derived hybrids (Exp. 3).

Type	Genotype	Kernel weight	Kernel growth rate	Grain-filling duration
		mg per kernel	mg °C d^{-1}	°C day
Inbred line	L10	300	0.410	1101
	L11	308	0.373	1128
	T4	259	0.355	1046
Hybrid	L10 × T4 (Ax889Mg)	352	0.412	1160
	L10 × T5 (Ax675Mg)	381	0.412	1235
	L11 × T4 (Ax882Mg)	308	0.358	1158
	L11 × T5 (Ax820Mg)	331	0.347	1247
	Type	*** (70)†	ns‡	** (228)
	Genotype (type)	** (21)	*** (0.019)	ns
	Line§	* (78)	*** (0.040)	ns
	Tester	ns	ns	ns
	Line × tester	ns	ns	ns

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†The data in parentheses represents LSD values for $p \leq 0.05$.

‡ns, not significant, $p > 0.05$

§Line, tester, and line × tester effects represent general combining ability (GCA) for lines, GCA for tester, and specific combining ability, respectively. See Materials and Methods for further details.

Table 4. Midparental heterosis (MPH) based on midparent values for kernel weight (KW), kernel growth rate (KGR), grain-filling duration (GFD), maximum water content (MWC), kernel desiccation rate (KDR), and moisture concentration at physiological maturity (MCPM) across experiments.

Trait	Average MPH	Range	Exp. 1 MPH	Exp. 2 MPH	Exp. 3 MPH
			%		
KW	15.8***	0.7 to 36.1	11.9**	16.4***	21.8*
KGR	6.4*	–10.6 to 20.6	4.8 ns†	7.7**	3.9 ns
GFD	6.4**	–1.8 to 17.4	4.4 ns	6.6**	9.8**
MWC	11.2***	–5.3 to 21.8	13.9**	11.0**	–
KDR	–4.8**	–17.7 to 10.5	–4.0 ns	–6.2*	–
MCPM	–6.3*	–31.8 to 16.0	–0.9 ns	–9.3**	–

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†ns, not significant, $p > 0.05$

Correlation Analysis

Experiments (Exp. 1, 2, and 3) showed significant ($p < 0.01$) differences for most grain-filling traits at the inbred level, which generated significant ($p < 0.01$) differences at the derived hybrid level for the same traits when crossed to common testers. These data provided the necessary information for testing how the different parental inbred

Table 5. Pearson correlation coefficients for midparental inbred line performance and derived hybrid performance and heritability values for kernel weight (KW), kernel growth rate (KGR), grain-filling duration (GFD), maximum water content (MWC), kernel desiccation rate (KDR), and moisture concentration at physiological maturity (MCPM). Midparental inbred line performance was calculated as the average of the female and male tester performance for the trait of interest. Heritability values for Exp. 1 and 2 are shown ± their SE.

Trait	Pearson correlation	*n*	Significance	Average H^2†	Exp. 1 H^2	Exp. 2 H^2
KW	0.63	31	$p < 0.001$	0.73	0.90 ± 0.03	0.57 ± 0.07
KGR	0.71	31	$p < 0.001$	0.70	0.74 ± 0.10	0.67 ± 0.07
GFD	0.81	31	$p < 0.001$	0.63	0.60 ± 0.09	0.67 ± 0.06
MWC	0.83	27	$p < 0.001$	0.94	0.96 ± 0.01	0.92 ± 0.02
KDR	0.61	27	$p < 0.001$	0.73	0.79 ± 0.07	0.68 ± 0.06
MCPM	0.71	27	$p < 0.001$	0.78	0.72 ± 0.08	0.84 ± 0.03

†H^2, broad-sense heritability.

line performance for the grain-filling traits of interest correlated to the observed performance of derived hybrids. For this correlation analysis we calculated the midparental inbred line performance: the average between the female and male tester.

When pooling the information from Exp. 1, 2, and 3 the derived hybrid performance was significantly correlated to their midparental inbred line performance for all evaluated grain-filling traits ($p < 0.001$; Table 5). Correlations were 0.63 for KW, 0.71 for KGR, 0.81 for GFD, 0.83 for MWC, 0.61 for KDR, and 0.71 for MCPM (Table 5). The hybrids showing the heaviest KWs were derived from parental inbred lines having the heaviest KWs, and the same was also true for KGR, GFD, MWC, KDR, and MCPM.

Heritability estimates for each trait at each trial are presented in Table 5. The traits with the highest values across experiments were MWC (0.94) and MCPM (0.78). All other traits had lower and quite similar values (0.73 for KW, 0.73 for KDR, 0.70 for KGR, and 0.63 for GFD).

DISCUSSION

In this study three experiments were performed at different environments using different germplasm to analyze the correlation between parental inbred and derived hybrid performance for several grain-filling traits. We are aware that several experimental limitations, such as the small sample size (few testers and/or few hybrids) and the lack of replication of each experiment in different locations, could induce possible biased GCA and SCA estimations and inaccurate heritability estimates. Despite this, results were consistent across experiments, and the correlation analysis in which all traits were pooled together showed significant results. Correlation studies between midparental inbred line performance and their derived hybrids represent useful breeding information. They help determine phenotyping protocols for the traits of interest and the need for making crosses to conduct extensive single-cross hybrid trials if there is a need for evaluating at the hybrid level (Hallauer and Miranda, 1988). Positive correlations found between midparental inbred lines and hybrid performance for our grain-filling traits add relevant information as phenotyping these grain-filling traits with inbred lines is indicative of their derived hybrid performance. Therefore, selection of inbred lines showing high performance of desirable grain-filling traits or conducting quantitative trait loci detection studies for these traits at the inbred level (Liu et al., 2011; Li et al., 2012; Alvarez Prado et al., 2013) are relevant for maize breeding.

From a genetic point of view, the positive correlation of lines and hybrids for grain-filling characters may indicate that a large amount of additive gene action is affecting testcross performance (Rojas and Sprague, 1952). Additive effects are the predictable portion of the genetic effect and are therefore useful to plant breeders. In this sense, from the grain-filling traits evaluated in this study, KW and KGR showed the highest additive gene action affecting derived hybrid performance. Delucchi et al. (2012) showed that genetic KW variability was associated with genetic additive effects, and their results support early findings (Cross, 1975; Poneleit and Egli, 1983; Wang et al., 1999). These studies highlight the importance of the additive contribution of genes underlying GFD and KGR on hybrid performance. Additionally, our results add relevant information to these evidences reporting on the correlation between midparental inbred lines and hybrids for these characters.

It is accepted that additive gene action has played a major role in the improved performance of present day hybrids (Duvick et al., 2004). Still, the relation between performance of inbred lines per se and their respective testcrosses is still challenging and difficult to predict (Hallauer and Miranda, 1988). For example, Gama and Hallauer (1977) evaluated eight plant and ear traits for 160 lines and 320 hybrids. The authors concluded that traits measured in inbred lines do not predict hybrid performance because of their low correlation coefficients ($r = 0.09–0.39$). These low correlations could be related to the high environmental modulation of the evaluated traits and their low heritability. We showed that KW and most grain-filling traits show relative high heritability values (Table 4), very similar to recently estimated ones for sorghum [*Sorghum bicolor* (L.)

Moench] grain-filling traits (Gambín and Borrás, 2011). However, our heritability estimates should be taken with caution because experiments had a small sample size and were not replicated in different environments. Heritability estimates on KW determination are higher than values related to kernel number determination and plant growth traits (Sadras and Slafer, 2012). At flowering, plants adjust the number of kernels set to the growth environment, minimizing kernel size variability (Sadras, 2007; Gambín and Borrás, 2010). As such, it is not surprising that grain-filling traits show high correlation values between parental lines and derived hybrids.

Heterosis, which is the difference between hybrid performance and average parental performance, is also an important component of the genetic effect. Our data showed heterosis for KW, which is in accordance with other studies (Poneleit and Egli, 1979, 1983; Munaro et al., 2011). When analyzing kernel growth traits, larger KW in hybrids compared to inbred lines was mainly related to a larger GFD, which was in turn associated with a slower KDR. Heterosis for GFD has been previously reported (Daynard et al., 1971; Poneleit and Egli, 1979), and authors have suggested that a more desirable gene combination could maintain the physiological processes supporting longer GFD. Mechanisms underlying the control of GFD are not entirely clear, but a higher capacity of maintaining the source activity relative to the sink growth, a lower KDR, and a lower MCPM might explain the longer GFD of hybrids compared to lines.

CONCLUSIONS

Three independent sets of commercially relevant breeding materials from different growth environments (Argentina and the United States) and tested at different sites were used to explore as much genetic and environmental diversity as possible. Correlations between midparental inbred line performance and single-cross hybrid performance for several grain-filling traits were significant, with medium to high correlation values.

At present several research groups interested in dissecting the genetic basis of grain filling traits are using inbred lines as their genetic material. Our results confirm these studies are producing valuable information for derived hybrid performance.

Acknowledgments

Experiments were partially funded by Syngenta Seeds Inc. and Nidera S.A. S Alvarez Prado held a graduate scholarship from CONICET, and L Borrás, BL Gambín and ME Otegui are members of CONICET, the Argentina National Research Council.

References

Alvarez Prado, S., C.G. López, B.L. Gambín, V.J. Abertondo, and L. Borrás. 2013. Dissecting the genetic basis of physiological processes determining maize kernel weight using the IBM (B73 × Mo17) Syn 4 population. Field Crops Res. 145:33–43. doi:10.1016/j.fcr.2013.02.002

Bewley, J.D., and M. Black. 1985. Seeds: Physiology of development and germination. Plenum, New York, NY.

Bingham, I.J., J. Blake, M.J. Foulkes, and J. Spink. 2007. Is barley yield in the UK sink limited? II. Factors affecting potential grain size. Field Crops Res. 101:212–220. doi:10.1016/j.fcr.2006.11.004

Borrás, L., and B.L. Gambín. 2010. Trait dissection of maize kernel weight: Towards integrating hierarchical scales using a plant growth approach. Field Crops Res. 118:1–12. doi:10.1016/j.fcr.2010.04.010

Borrás, L., C. Zinselmeier, M.L. Senior, M.E. Westgate, and M.G. Muszynski. 2009. Characterization of grain-filling patterns in diverse maize germplasm. Crop Sci. 49:999–1009. doi:10.2135/cropsci2008.08.0475

Borrás, L., M.E. Westgate, and M.E. Otegui. 2003. Control of kernel weight and kernel water relations by post-flowering source-sink ratio in maize. Ann. Bot. (London) 91:857–867. doi:10.1093/aob/mcg090

Cross, H.Z. 1975. Diallel analysis of duration and rate of grain filling of seven inbred lines of corn. Crop Sci. 15:532–535. doi:10.2135/cropsci1975.0011183X001500040023x

Daynard, T.B., J.W. Tanner, and W.G. Duncan. 1971. Duration of the grain filling period and its relation to grain yield in corn, *Zea mays* L. Crop Sci. 11:45–48. doi:10.2135/cropsci1971.0011183X001100010015x

Delucchi, C., G.H. Eyhérabide, R.D. Lorea, D.A. Presello, M.E. Otegui, and C.G. López. 2012. Classification of argentine maize landraces in heterotic groups. Maydica 57:26–33.

Duvick, N.D., J.S.C. Smith, and M. Cooper. 2004. Long-term selection in a commercial hybrid maize breeding program. Plant Breed. Rev. 24:109–151.

Gambín, B.L., and L. Borrás. 2011. Genotypic diversity in sorghum inbred lines for grain-filling patterns and other related agronomic traits. Crop Pasture Sci. 62:1026–1036. doi:10.1071/CP11051

Gambín, B.L., and L. Borrás. 2010. Resource distribution and the trade-off between seed number and seed weight: A comparison across crop species. Ann. Appl. Biol. 156:91–102. doi:10.1111/j.1744-7348.2009.00367.x

Gambín, B.L., L. Borrás, and M.E. Otegui. 2007. Kernel water relations and duration of grain filling in maize temperate hybrids. Field Crops Res. 101:1–9. doi:10.1016/j.fcr.2006.09.001

Gama, E.E.G., and A.R. Hallauer. 1977. Relation between inbred and hybrids traits in maize. Crop Sci. 17:703–706. doi:10.2135/cropsci1977.0011183X001700050007x

Hallauer, A.R., and J.B. Miranda. 1988. Quantitative genetics in maize breeding, 2nd ed. The Iowa State Univ. Press, Ames, IA.

Holland, J.B. 2013. Heritability based on regression of $F_{2:3}$ progeny means on individual F_2 parents and based on variance among $F_{2:3}$ families evaluated in an unbalanced, multi-environment trial. USDA-ARS Maize Breeding and Genetics at NCSU. http://www4.ncsu.edu/~jholland/homepage.htm (verified 15 Mar. 2013).

Holland, J.B., W.E. Nyquist, and C.T. Cervantez-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. In: J. Janick, editor, Plant breeding. Rev. vol. 22. John Wiley & Sons, Hoboken, NJ. p. 9–112.

Holland, J.B., D.V. Uhr, D. Jeffers, and M.M. Goodman. 1998. Inheritance of resistance to southern corn rust in

tropical-by-corn-belt maize populations. Theor. Appl. Genet. 96:232–241.

Jones, R.J., B.M.N. Schreiber, and J.A. Roessler. 1996. Kernel sink capacity in maize: Genotypic and maternal regulation. Crop Sci. 36:301–306. doi:10.2135/cropsci1996.0011183X003600020015x

Li, Y., M. Yang, Y. Dong, Q. Wang, Y. Zhou, Q. Zhou, B. Shen, F. Zhang, and X. Liang. 2012. Three main genetic regions for grain development revealed through QTL detection and meta-analysis in maize. Mol. Breed. 30:195–211. doi:10.1007/s11032-011-9610-x

Liu, Z.H., H.Q. Ji, T.T. Cui, X. Wu, L.J. Duan, X.X. Feng, and J.H. Tang. 2011. QTL detected for grain-filling rate in maize using a RIL population. Mol. Breed. 27:25–36. doi:10.1007/s11032-010-9410-8

Lynch, M., and B. Walsh. 1997. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Möhring, J., A.E. Melchinger, and H.P. Piepho. 2011. REML-based diallel analysis. Crop Sci. 51:470–478. doi:10.2135/cropsci2010.05.0272

Muchow, R.C. 1990. Effect of high temperature on grain-growth in field grown maize. Field Crops Res. 23:145–158. doi:10.1016/0378-4290(90)90109-O

Munaro, E.M., K.E. D'Andrea, M.E. Otegui, A.G. Cirilo, and G.H. Eyhérabide. 2011. Heterotic response for grain yield and ecophysiological related traits to nitrogen availability in maize. Crop Sci. 51:1172–1187. doi:10.2135/cropsci2010.08.0461

Poneleit, C.G., and D.B. Egli. 1979. Kernel growth rate and duration in maize as affected by plant density and genotype. Crop Sci. 19:385–388. doi:10.2135/cropsci1979.0011183X001900030027x

Poneleit, C.G., and D.B. Egli. 1983. Differences between reciprocal crosses of maize for kernel growth characteristics. Crop Sci. 23:871–875. doi:10.2135/cropsci1983.0011183X002300050013x

Raduschev, D. 2007. Graph Pad Prism version 5.0. Graph Pad Software, Inc., San Diego, CA.

Reddy, V.M., and T.B. Daynard. 1983. Endosperm characteristics associated with rate of grain filling and kernel size in corn. Maydica 28:339–355.

Rojas, B.A., and G.F. Sprague. 1952. A comparison of variance components in corn yield trials: III. General and Specific combining ability and their interaction with locations and years. Agron. J. 44:462–466. doi:10.2134/agronj1952.00021962004400090002x

Rondanini, D.P., R. Savin, and A.J. Hall. 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. Eur. J. Agron. 26:295–309. doi:10.1016/j.eja.2006.11.001

SAS Institute. 1999. The SAS online doc version 8. SAS Institute, Inc., Cary, NC.

Sadras, V.O., and G.A. Slafer. 2012. Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. Field Crops Res. 127:215–224. doi:10.1016/j.fcr.2011.11.014

Sadras, V.O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. Field Crops Res. 100:125–138. doi:10.1016/j.fcr.2006.07.004

Schnyder, H., and U. Baum. 1992. Growth of the grain of wheat (*Triticum aestivum* L.). The relationship between water content and dry matter accumulation. Eur. J. Agron. 1:51–57.

Shaw, R.H., and W.E. Loomis. 1950. Bases for the prediction of corn yields. Plant Physiol. 25:225–244. doi:10.1104/pp.25.2.225

USDA-ARS National Genetic Resources Program. 2005. Germplasm Resources Information Network (GRIN) database. National Germplasm Resources Laboratory, Beltsville, MD. http://www.ars-grin.gov/npgs/acc/acc_queries.html (accessed 18 Jan. 2012).

Wang, G., M.S. Kang, and O. Moreno. 1999. Genetic analyses of grain-filling rate and duration in maize. Field Crops Res. 61:211–222. doi:10.1016/S0378-4290(98)00163-4