RESEARCH

# Exploiting Grain-Filling Rate and Effective Grain-Filling Duration to Improve Grain Yield of Early-Maturing Maize

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## ABSTRACT

Early-maturing maize (*Zea mays* L.) genotypes yield 15 to 30% less than late-maturing genotypes. One strategy for improving grain yield in the early-maturing group involves assessment of grain-filling traits as secondary traits for selection for high grain yield. In this study, we investigated the possibility of using grain-filling rate and duration for improving grain yield in early-maturing tropical maize. Forty-four hybrids generated using North Carolina design II were evaluated at CIMMYT-Zimbabwe during the 2011/2012 season under irrigated and nonirrigated environments. Although grain-filling rate and effective grain-filling duration were negatively correlated, several hybrids were distinctly above the trend line. The earliest-maturing hybrid took 127 d to reach physiological maturity and produced grain yields comparable to those of the medium-maturing genotypes (7 t ha$^{-1}$). It had a high grain-filling rate of 2.40 g per plant d$^{-1}$ (18% higher than those of the low-yielding hybrids) and a relatively longer effective grain-filling duration. Grain-filling rate and effective grain-filling duration had high coefficients of genetic determination, positive correlations with grain yield, low error terms, and low genotype × environment interactions, making them appropriate selection traits for improved grain yield. The study shows that it is possible to develop high-yielding early- to medium-maturing maize hybrids based on favorable combining ability values for grain-filling rate and duration.

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**Abbreviations:** AD, anthesis date; ASI, anthesis–silking interval; DPM, days to physiological maturity; EGFD, effective grain-filling duration; GCA, general combining ability; GCAf, general combining ability of the females (lines); GCAm, general combining ability of the males (testers); GFR, grain-filling rate; GY, grain yield; KR, kernels per row; SCA, specific combining ability; TGFD, total grain-filling duration.

Globally, there is an increasing demand for coarse grains such as maize to meet the demand for food, feed, and energy for a rising human population. Therefore, maize production has to increase more than twofold to compensate for the supply gaps, especially in developing countries (Pingali and Pandey, 2001). Early-maturing maize genotypes are useful because they secure harvests against fluctuating weather conditions and ensure an early supply of needed grain. Early-maturing maize genotypes typically take about 130 d to reach physiological maturity in southern Africa, according to the International Maize and Wheat Improvement Center (CIMMYT) (Magorokosho et al., 2009). However, under optimum growing conditions in southern Africa, early-maturing maize genotypes yield between 15 and 30% less than late-maturing ones (Magorokosho et al., 2009), probably due to either limited source efficiency or sink capacity (Dwyer

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et al., 1994; Lee and Tollenaar, 2007). More than 80% of maize genotypes grown in southern Africa farming systems, many of which are in marginal and drought-prone areas, are either early-maturing hybrids or early-maturing open-pollinated varieties (Pswarayi and Vivek, 2008). The challenge, therefore, is to improve the grain yield of early-maturing maize while maintaining earliness, to provide reliable harvests.

Genetic variability for grain yield in early-maturing maize has been reported and could be used to improve yield (Magorokosho et al., 2009; Pswarayi and Vivek, 2008). Selection for high yield is associated with a high environmental influence. Therefore, selection for improved yield using secondary traits that are strongly correlated to yield and are highly heritable is desirable. Secondary traits such as anthesis–silking interval, ears per plant, and the rate of leaf senescence have been used to select high-yielding genotypes under drought and low N conditions (Derera et al., 2007; Banziger et al., 2004). In other studies, the single or combined use of delayed leaf senescence (stay-green characteristic), kernel number, and cob size have been used to indirectly select for grain yield in maize (Lee and Tollenaar, 2007; Wang et al., 1999; Zheng et al., 2009).

In maize, grain-filling rate and grain-filling duration affect yield and could be used as indirect selection traits for grain yield in both early- and late-maturing maize. Grain filling in maize occurs in three stages: the lag phase, during which there is rapid cell division and differentiation, the linear phase, during which rapid dry matter accumulation occurs, and the final phase, when the seed attains physiological maturity (Lee and Tollenaar, 2007). Over 90% of the total dry matter in the grain is accumulated during the linear phase and this period is therefore called the effective grain-filling duration (Lee and Tollenaar, 2007). The effective grain-filling duration can be extended by selecting for earlier flowering and/or shorter lag and final grain-filling phases. There is limited information on the inheritance of grain-filling rate and effective grain-filling duration, and their use as indirect selection traits for high yield in early-maturing maize. Maize genotypes with varying grain-filling rate and grain-filling duration have been reported (Wang et al., 1999; Borras et al., 2009; Gambin et al., 2007; Lee and Tollenaar, 2007; Magorokosho et al., 2009) thus suggesting the possibility for using grain-filling traits for indirect selection for high yield in early-maturing maize. The objective of this study is to investigate the use of grain-filling traits as secondary traits in improving grain yield in early-maturing tropical maize.

# MATERIALS AND METHODS

## Description of the Experimental Sites

The experiments were conducted in Zimbabwe at the CIMMYT-Harare station located at an altitude of 1500 m above sea level and longitude and latitude of 31° E and 17°43′ S, respectively. The mean annual rainfall exceeds 700 mm, mostly occurring during a single growing season that ranges from early November to mid April each year. During this period, mean monthly temperatures range from 24 to 26°C. The field experiments received 350 kg $ha^{-1}$ of basal fertilizer, compound D with N:P:K ratio of 7:14:7, and a top dressing of 300 kg $h^{-1}$ $NH_4NO_3$ (ammonium nitrate) with 37.5% N. Hand weeding was done to control the weeds. The experiments were planted at the same time, but one trial was evaluated under irrigation as the first environment while a second trial was evaluated under rain-fed conditions as the second environment. Day was used as a unit of time, instead of thermal unit, because the temperature was very moderate throughout the growing season, with little variation during the grain-filling period of the two experiments (27–28°C for day and 19–20°C for night).

**Table 1. The maize inbred lines used in the study.**

| Name | Heterotic group | Pedigree |
|---|---|---|
| Lines | | |
| VL08528 | B | ZEWBc2F2-101-2-B |
| VL058014 | A | ZEWAc1F2-254-2-1-B-1-BBB |
| C329-2 | B | 02SADVE2B-#-20-2-1-1-2-BBB |
| CML537 | A | MAS[206/312]-23-2-1-1-B*7 |
| C389-92 | B | ZM523B-29-2-1-1-B*6 |
| CML539 | A | MAS[MSR/312]-117-2-2-1-B*9 |
| VL0536 | B | [CML389/CML176]-B-29-2-2-B*5 |
| CML202 | B | ZSR923-B*4-5-1-B |
| CML491 | A | 6207QB/6207QA)-1-4-#-2-2-B |
| CML395 | B | 90323B-1-B-1-B |
| CML444 | B | P43-C9-1-1-1-1-1-B |
| Testers | | |
| VL055063 | A | Ent320:92SEW277/[DMRESR-W]EarlySel-#1-2-4-B/CML386]-B-11-3-B-2-#-B*4-B-B |
| CML197 | A | Ent52:92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-B*5-B-B |
| CML506 | B | [EarlyMid1/KatumaniSR]-#-169-2-4-B-1-#-BBB |
| VL05615 | B | ZEWBc1F2-216-2-2-B-2-B*4-2-4-BB-B-B |

## Plant Materials and Experimental Design

Fifteen early- to medium-maturing maize inbred lines were crossed in a North Carolina design II mating scheme with 11 females and four males (Table 1). The resulting 44 $F_1$ hybrids were evaluated in both trials or environments using an α-lattice design with two replications. The hybrids were evaluated under irrigation and rain-fed conditions as described earlier. A plot consisted of three rows, 4 m long, spaced 75 cm apart with 25 cm spacing between plants within the row in all trials.

## Data Collection

To assess various grain-filling parameters, destructive sampling was performed weekly by removal of developing maize cobs, starting from 2 wk after pollination. In each plot, plants from which developing cobs were removed were left standing to maintain the initial plant density. From each sampled cob, 10 g of grain (fresh weight) was obtained from the middle part of the cob to reduce variation that might result from sampling

different parts of the same cob. Dry weights were measured after drying the grains in a forced-air oven at 80°C for at least 96 h. The same procedure was repeated weekly until the crop reached physiological maturity indicated by the formation of a black layer at the point where the kernel is attached to the cob. At physiological maturity there is no further increment in grain weight due to grain filling.

When a narrow range of temperatures prevail during the reproductive stage as observed in our experiment, Stewart et al. (1998) showed that maize kernel development follows a log-linear pattern. Therefore, a log-linear equation, $y = \ln(x) + b$, was fitted on the data, in which $y$ is the percentage of dry matter at sampling time $x$, $b$ is the slope of the curve (rate of percentage dry matter increase on a log scale), and $x$ is the sampling point in time (weekly basis). The start of the linear phase is when the maize kernels reach 87% moisture content (13% dry matter content) and end when the kernels reach 36% moisture content (64% dry matter content) (Borras et al., 2009). This equation was fitted to the weekly dry matter content data per plot to predict the start of the linear phase. The period before the linear phase was designated the lag phase duration. The period from the start of the linear phase until physiological maturity was considered the effective grain-filling duration (EGFD). Days to physiological maturity (DPM) were recorded as the days from sowing until the kernels develop a black layer at the point of their attachment to the cob. The grain-filling rate (GFR) (g $d^{-1}$) was calculated as the final grain yield (GY) per plant divided by the EGFD.

The total grain-filling duration (TGFD) was calculated by subtracting the days to silking from the DPM. Data were also recorded on anthesis date (AD), anthesis–silking interval (ASI), number of kernels per row (KR), and GY. Grain yield per plant was obtained by dividing the total grain weight per plot by the number of harvested cobs per plot. Days to anthesis and silking were recorded as the number of days from planting to when 50% of plants in each plot had shed pollen or had emerged silks, respectively. The ASI was calculated as days to 50% silking minus days to 50% anthesis.

## Data Analyses

Line × tester analyses were performed for all traits on plot means of GY, GFR, EGFD, TGFD, AD, ASI, and KR using the SAS PROC GLIMMIX for the general linear mixed model (where inbred lines are fixed while environments are random) of ANOVA for progenies generated using the North Carolina design II mating design (SAS, 2009) with the following model:

$$X_{ijkq} = \mu + g_i + g_j + s_{ij} + \gamma_q + r_k(\gamma_q) + (g\gamma)_{iq} + (g\gamma)_{jq} + (s\gamma)_{ijq} + e_{ijkq},$$

in which $i = 1, 2, \ldots, 11$; $j = 1, 2, 3, 4$; $k = 1, 2$; $q = 1, 2$; and $X_{ijkq}$ denotes the value of the hybrid obtained by mating the $i$th female line and the $j$th male line in the $k$th replication and the $q$th environment. The term $\mu$ is the grand mean, $g_i$ is the general combining ability (GCA) effect common to all progenies of the $i$th female line, $g_j$ is the GCA effect common to all progenies of the $j$th male line, $s_{ij}$ is the specific combining ability (SCA) effect specific to the progeny obtained by mating the $i$th female line and the $j$th male line, $\gamma_q$ is the average effect of the $q$th environment, $r_k(\gamma_q)$ is the effect of the $k$th replication nested within the $q$th environment, $(g\gamma)_{iq}$ and $(g\gamma)_{jq}$ are the interactions between the GCA effects and environment, $(s\gamma)_{ijq}$ is the interaction between the SCA effect and the environment, and $e_{ijkq}$ is the random experimental error. The variance components attributable to each source of variation were computed and used to estimate Baker's ratio, $[\sigma^2_{gca\ (male)} + \sigma^2_{gca\ (female)}]/[\sigma^2_{gca\ (male)} + \sigma^2_{gca\ (female)} + \sigma^2_{sca\ (female \times male)}]$ (Baker, 1978), in which $\sigma^2_{gca\ (male)}$ is the genetic variance due to males lines, $\sigma^2_{gca\ (female)}$ is the genetic variance due to female lines, and $\sigma^2_{sca\ (female \times male)}$ is the genetic variance due to the interaction between males and females lines, and the coefficient of genetic determination (the fixed parent equivalent of heritability) (Lee et al., 2005). The genotype × environment interaction ANOVAs were performed on all recorded data based on the general linear mixed model (where hybrids are fixed while environments were random) as implemented by the SAS PROC GLIMMIX in SAS software version 9.2 (SAS, 2009) using the following model of Dabholker (1999):

$$P_{ijk} = \mu + h_i + t_j + (hg)_{ij} + e_{ijk},$$

in which $P_{ijk}$ is the phenotypic value of the hybrid $i$ when tested in replicate $k$ in environment $j$, $\mu$ is the population mean, $h_i$ is the effect of the hybrid $i$, $t_j$ is the effect of the environment $j$, $(ht)_{ij}$ is the hybrid × environment interaction effect associated with hybrid $i$ and environment $j$, and $e_{ijk}$ is the within environment error associated with hybrid $i$, environment $j$, and the replicate $k$.

A $t$ test was used to compare the mean performance for GY and other traits between the top 10 and the worst 10 performers. The 44 hybrids developed were grouped into three based on heterotic groups of their parental inbred lines. The three groups were hybrids formed by crossing inbred lines only from the heterotic group A (A × A), inbred lines only from heterotic group B (B × B), and then inbred lines from opposite heterotic groups (either A × B or B × A). A $t$ test was used to compare the mean of hybrids formed from inbreds of opposite heterotic groups with those formed from the same heterotic group.

For each trait the GCA estimates ($g_i$ and $g_j$) for all parental lines were calculated as

$$g_i = (\gamma_{i.} - \gamma_{..}) \text{ and } g_j = (\gamma_{.j} - \gamma_{..}),$$

in which $\gamma_{i.}$ is the mean of all hybrids involving the $i$th female parent, $\gamma_{.j}$ is the mean of all hybrids involving the $j$th male parent, and $\gamma_{..}$ is the mean of all hybrids. The standard errors for $g_i$ or $g_j$ estimates were calculated as $SE_{GCA} = \{MS_{fe}[(f-1)/mfer]\}^{1/2}$ or $\{MS_{me}[(m-1)/mfer]\}^{1/2}$ for females and males, respectively, in which mfer is males × females × environments × replications. The $MS_{fe}$ and $MS_{me}$ are the respective female × environment and male × environment mean squares and are multiplied by the appropriate proportion of the total number of observation [males × females × replications (environments)]. When the $MS_{fe}$ and $MS_{me}$ were nonsignificant, they were replaced in the calculations by the pooled error.

The phenotypic correlations among traits were computed as described by Singh and Chaudhary (2004) as $r_p = [Cov_p/(\delta_{P(X)}\delta_{P(Y)})]$, in which $r_p$ is the phenotypic correlation between $X$ and $Y$, $Cov_p$ is the phenotypic covariance between $X$ and $Y$, $\delta_{P(X)}$ is the phenotypic standard deviation of $X$, and $\delta_{P(Y)}$ is

Table 2. Mean square values for the combined ANOVA for maize grain yield and other related traits of hybrids across the two environments.

| Source of variation | Degrees of freedom | Grain yield | Grain-filling rate | Effective grain-filling duration | Total grain-filling duration | Days to anthesis | Anthesis–silking interval | Kernels per row |
|---|---|---|---|---|---|---|---|---|
| | | g per plant | g d$^{-1}$ | d | | | | |
| Environment (Env) | 1 | 53.6 | 0.039 | 2.03 | 10.15 | 3.00 | 2.12 | 45.10 |
| Replication/Env | 2 | 122.5 | 0.014 | 8.23 | 9.42** | 16.69 | 1.11 | 6.37 |
| Hybrids | 43 | 371.90* | 0.260*** | 59.73*** | 74.28*** | 35.15*** | 6.02*** | 18.29*** |
| Hybrids × Env | 43 | 211.7* | 0.086 | 3.948 | 2.35 | 2.651 | 1.95 | 6.929 |
| Line | 10 | 471.6* | 0.330** | 50.95*** | 46.62*** | 89.77*** | 10.6** | 26.55* |
| Tester | 3 | 94.3 | 0.097 | 95.62* | 190.75** | 67.59* | 19.44*** | 22.02* |
| Env × line | 10 | 123.4 | 0.058 | 5.8* | 1.87 | 1.58 | 1.39 | 8.24 |
| Env × tester | 3 | 3.3 | 0.005 | 3.89 | 3.48 | 3.37 | 0.42 | 0.76 |
| Line × tester | 30 | 366.4 | 0.253** | 59.07*** | 71.85*** | 13.70*** | 3.15 | 15.17* |
| Env × line × tester | 30 | 261.9** | 0.100 | 3.31 | 2.40 | 2.94 | 2.3 | 7.11 |
| Pooled error | 77 | 119.4 | 0.068 | 2.84 | 1.86 | 2.39 | 1.58 | 6.21 |

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

the phenotypic standard deviation of *Y*. The genotypic correlations among traits were computed as $r_A = [Cov_A)/(\delta_{A(X)}\delta_{A(Y)})]$, in which $r_A$ is the genetic correlation between *X* and *Y*, $Cov_A$ is the genetic covariance between *X* and *Y*, $\delta_{A(X)}$ is the genetic standard deviation of *X*, and $\delta_{A(Y)}$ is the genetic standard deviation of *Y*. The genetic variances and covariances were obtained by subtracting the error variances and covariances from their respective phenotypic variances and covariances based on the 44 genotype means. The covariance values were obtained from a covariance analysis using the same structure as the ANOVA. Gain from indirect selection of yield based on GFR and EGFD was computed as described by Dabholker (1999). The percentage gain through indirect selection of GY (*y*) was based on secondary trait (*x*) and was obtained using

$$(r_{xy}) \times (h^2_x/h^2_y)^{1/2},$$

in which $r_{xy}$ is the correlation between *y* and *x*, $h^2_y$ is the narrow-sense heritability for GY, and $h^2_x$ is the narrow-sense heritability for the secondary trait.

# RESULTS

## Grain Yield among Hybrids with Varying Maturity Periods

Highly significant variations ($P < 0.001$) in GY among the 44 $F_1$ hybrids were detected (Table 2). The average yield per hybrid ranged from 84 to 130 g per plant (equivalent to 4.5 to 7.0 t ha$^{-1}$). The mean yield of the top 10 hybrids was greater than that of the worst 10 hybrids by 27.3% (Table 3). The top 10–yielding hybrids had 22% higher GFR of 2.40 g d$^{-1}$ compared to 1.97 g d$^{-1}$ for the worst 10–yielding hybrids. They also had a 1.9 d longer EGFD. The highest-yielding hybrids were VL08528 × CML197, C389-92 × CML197, and CML444-B × VL055063, with 130.4, 117.8, and 116.3 g per plant respectively (equivalent to 7.0, 6.4, and 6.3 t ha$^{-1}$, respectively). These hybrids were all medium-maturing genotypes that flowered in 64 to 71 d and took between 134 and 139 d to reach physiological maturity. The best early-maturing hybrid VL058014 × CML506 ranked fourth in performance and yielded 114.9 g per plant (equivalent to 6.2 t ha$^{-1}$). This hybrid reached physiological maturity in 127 d and flowered in 60 d (50% anthesis and silking). The yield of VL058014 × CML506 was not significantly different from that of the top three medium-maturing hybrids (Table 3). In general, the high-yielding hybrids also reached anthesis a day earlier and had a significantly lower ASI than that of the lower-yielding group. The effects of heterotic groups on grain-filling attributes were not significant.

## Contribution of Grain-Filling Traits to the Best Yielding Hybrids

The highest-yielding medium-maturing hybrid, VL08528 × CML197, was derived from a cross between the inbred line VL08528, characterized by positive GCA values for GFR and EGFD, and the tester CML197, which also had a high and positive GCA value for GFR (Table 4). Furthermore, the highest-yielding early-maturing hybrid was a cross between the inbred line VL058014, with a significant positive GCA value for GFR, and the tester CML506, with an average GCA value for GFR (Table 4). This hybrid had a negative GCA for TGFD. Among the highest-yielding early- and medium-maturing hybrids, the average GCA contribution to yield, GFR, and EGFD were 1.7 g per plant, 0.04 g d$^{-1}$, and −0.02 d, respectively. For the 10 highest-yielding hybrids, the average SCA contributions to yield, GFR, and EGFD were 10.7 g per plant, 0.18 g d$^{-1}$, and 1.06 d, respectively.

## Correlations between Traits

Correlation analysis showed that GFR had a moderately strong and positive genotypic association with GY ($r = 0.57$) (Table 5). Grain yield was also positively correlated with KR

Table 3. Maize grain yield, grain-filling rate and duration, ear size-related traits, and maturity attributes of the top 10 and worst 10 yielding hybrids.

| Line name† | Grain yield | Grain-filling rate | Effective grain-filling duration | Total grain-filling duration | Days to anthesis | Anthesis–silking interval | Days to physiological maturity† | Number of kernels per row |
|---|---|---|---|---|---|---|---|---|
| | g per plant | g d$^{-1}$ | d | | | | | |
| VL08528 | 130.3 | 2.46 | 53.0 | 73.8 | 64.3 | 1.0 | 139 | 42.2 |
| C389-92 | 117.8 | 2.26 | 52.2 | 74.5 | 65.5 | –1.0 | 139 | 36.8 |
| CML444 | 116.3 | 2.68 | 43.4 | 63.8 | 71.0 | –0.8 | 134 | 36.9 |
| VL058014 | 114.9 | 2.60 | 45.1 | 65.3 | 60.3 | 1.5 | 127 | 36.8 |
| CML491 | 113.0 | 2.20 | 51.5 | 71.0 | 65.5 | 2.5 | 139 | 40.1 |
| VL08528 | 111.8 | 2.21 | 50.6 | 71.8 | 61.8 | 1.5 | 135 | 38.7 |
| CML395 | 111.3 | 2.42 | 46.0 | 66.5 | 68.0 | 0.5 | 135 | 39.7 |
| VL058014 | 110.0 | 2.64 | 41.6 | 63.3 | 63.0 | 0.8 | 127 | 37.2 |
| CML395 | 109.5 | 2.39 | 45.8 | 62.8 | 69.8 | 0.4 | 133 | 38.2 |
| CML444 | 109.5 | 2.17 | 50.6 | 70.3 | 69.5 | –0.8 | 139 | 36.7 |
| Mean | 114.4 | 2.40 | 48.0 | 68.3 | 65.9 | 0.6 | 135 | 38.3 |
| Standard deviation among hybrids | 6.3 | 0.19 | 4.0 | 4.5 | 3.6 | 1.1 | 5 | 1.9 |
| VL058014 | 93.8 | 2.18 | 43.0 | 65.5 | 61.3 | 0.3 | 127 | 35.6 |
| VL0536 | 93.5 | 2.09 | 44.8 | 61.8 | 70.8 | 0.5 | 133 | 37.7 |
| CML202 | 92.3 | 1.81 | 51.1 | 70.5 | 67.0 | 3.5 | 141 | 40.3 |
| CML537 | 91.0 | 1.86 | 48.5 | 70.3 | 66.8 | 1.9 | 139 | 39.2 |
| C329-2 | 91.0 | 1.89 | 48.0 | 67.0 | 67.5 | 0.5 | 135 | 33.8 |
| CML491 | 89.5 | 1.77 | 50.9 | 68.0 | 71.3 | 1.8 | 141 | 38.3 |
| CML202 | 89.5 | 1.81 | 49.5 | 68.5 | 66.0 | 4.5 | 139 | 35.4 |
| VL08528 | 89.3 | 2.34 | 38.2 | 55.0 | 68.8 | 3.3 | 127 | 32.0 |
| VL0536 | 85.8 | 2.16 | 39.6 | 59.0 | 66.3 | 1.8 | 127 | 37.2 |
| C329-2 | 83.5 | 1.77 | 47.3 | 67.0 | 66.0 | 2.0 | 135 | 33.3 |
| Mean | 89.9 | 1.97 | 46.1 | 65.3 | 67.2 | 2.0 | 134 | 36.3 |
| Standard deviation among hybrids | 3.2 | 0.21 | 4.6 | 5.1 | 2.8 | 1.4 | 5.7 | 2.7 |
| Difference | 24.5 | 0.43 | 1.9 | 3.0 | –1.3 | –1.4 | 0.3 | 2.0 |
| Percent difference | 27.3 | 22.08 | 4.1 | 4.6 | –1.9 | –71.9 | 0.2 | 5.6 |
| Standard error of the difference | 2.2 | 0.09 | 1.9 | 2.1 | 1.5 | 0.6 | 2.3 | 1.0 |
| *t*-value | 11.0 | 4.89 | 1.0 | 1.4 | –0.9 | –2.5 | 0.1 | 2.0 |
| *t* probability | 0.00 | 0.00 | 0.34 | 0.18 | 0.38 | 0.02 | 0.9 | 0.07 |
| Minimum value | 83.5 | 1.77 | 38.2 | 55.0 | 60.3 | –1.3 | 127 | 32.0 |
| Mean value | 101.6 | 2.18 | 46.7 | 66.4 | 66.8 | 1.1 | 134 | 37.3 |
| Maximum value | 130.3 | 2.68 | 55.0 | 74.8 | 74.3 | 4.5 | 141 | 42.8 |
| CV, % | 11.4 | 12.00 | 3.8 | 2.1 | 2.3 | 121.0 | | 6.8 |
| Probability value for the 44 hybrids | *** | *** | *** | *** | *** | *** | | *** |
| LSD (5%) | 16.3 | 0.37 | 2.5 | 1.9 | 2.1 | 1.8 | | 3.5 |

***Significant at the 0.001 probability level.

†Trait recorded in one replication of the two environments

($r$ = 0.57) but not with DPM ($r$ = 0.02), TGFD ($r$ = 0.31), or EGFD ($r$ = 0.21). The EGFD was, however, positively correlated with KR ($r$ = 0.43). As expected, the EGFD was negatively correlated to GFR ($r$ = −0.68). However, some hybrids performed distinctly above the regression line.

## Variance Components for Grain-Filling Traits and Grain Yield

The GCA of the females (lines) (GCAf) was significant ($P < 0.01$) for most traits, including GFR and EGFD. The GCA of the males (testers) (GCAm) was similarly significant for most traits including EGFD, TGFD, KR, AD, and ASI (Table 2). Significant SCA ($P < 0.05$) was found for most traits. The SCA terms for GY and ASI were not statistically significant because of a relatively large SCA × environment term. Hybrid × environment interactions were significant only for GY, which had a relatively large SCA × environment interaction. Interactions between the environment and GCAm were nonsignificant for all traits. However, a significant interaction ($P < 0.05$) between the environment and GCAf was observed for the EGFD.

The variance components due to error were large for GY and GFR but small for other traits (Table 6). The SCA × environment interaction variance component was large for GY but small for other traits (Table 6). The high [(GCAf + GCAm)/(GCAf + GCAm + SCAmf)] (Baker's

**Table 4. Line and tester general combining ability effects values for maize grain yield and other yield-determining traits across environments.**

| Line | Heterotic group† | Grain yield | Grain-filling rate | Effective grain-filling duration | Total grain-filling duration | Days to anthesis | Anthesis–silking interval | Kernels per row |
|---|---|---|---|---|---|---|---|---|
| | | g per plant | g d$^{-1}$ | d | | | | |
| VL08528 | B | 4.47 | 0.06 | 0.49 | 0.64 | –1.98 | 0.47 | –0.53 |
| VL058014 | A | 4.02 | 0.19 | –1.80 | 0.20 | –5.24 | –0.33 | –1.02 |
| C329-2 | B | –10.73 | –0.29 | 1.08 | 1.14 | –1.73 | 0.47 | –2.73 |
| CML537 | A | –1.31 | 0.01 | –0.99 | –0.17 | 0.09 | 0.21 | 0.45 |
| C389-92 | B | 3.07 | 0.05 | 0.36 | 0.64 | 2.07 | –1.83 | –0.33 |
| CML539 | A | 3.59 | 0.14 | –0.74 | –0.34 | –1.48 | –0.29 | 1.26 |
| VL0536 | B | –7.48 | –0.04 | –3.20 | –3.86 | 1.20 | –0.20 | 0.55 |
| CML202 | B | –2.51 | –0.10 | 1.23 | 0.33 | 1.07 | 1.24 | 1.46 |
| CML491 | A | –2.16 | –0.20 | 3.53 | 2.64 | 2.07 | 0.67 | 1.53 |
| CML395 | B | 1.49 | 0.06 | –0.82 | –2.04 | 1.40 | 0.28 | –0.05 |
| CML444 | B | 7.54 | 0.12 | 0.86 | 0.83 | 2.51 | –0.70 | –0.60 |
| Standard error | | 2.73 | 0.065 | 0.42 | 0.34 | 0.39 | 0.31 | 0.62 |
| Tester | | | | | | | | |
| VL055063 | A | –1.05 | 0.06 | –1.63 | –2.29 | 1.55 | –0.60 | –0.87 |
| CML197 | A | 1.86 | –0.06 | 1.93 | 2.74 | –0.28 | –0.08 | 0.88 |
| CML506 | B | –0.16 | 0.00 | –0.15 | –0.38 | –1.45 | 0.94 | –0.06 |
| VL05615 | B | –0.65 | –0.01 | –0.15 | –0.06 | 0.17 | –0.27 | 0.06 |
| Standard error | | 1.65 | 0.039 | 0.25 | 0.21 | 0.23 | 0.19 | 0.38 |
| Grand mean | | 102.01 | 2.19 | 46.94 | 66.61 | 66.98 | 1.076 | 37.40 |

†The pedigrees of these CIMMYT inbred lines are given in Table 1.

**Table 5. Genotypic (lower diagonal) and phenotypic (upper diagonal) correlations of maize grain yield, grain-filling traits, ear-related traits, and maturity traits among 44 maize hybrids.†**

| | Grain yield | Grain-filling rate | Effective grain-filling duration | Total grain-filling duration | Days to anthesis | Anthesis–silking interval | Days to physiological maturity | Kernels per row |
|---|---|---|---|---|---|---|---|---|
| | g per plant | g d$^{-1}$ | d | | | | | |
| Grain yield, g per plant | **1.00** | 0.69 | 0.16 | 0.25 | –0.19 | –0.39 | 0.01 | 0.46 |
| Grain-filling rate, g d$^{-1}$ | 0.57 | **1.00** | –0.60 | –0.49 | –0.14 | –0.32 | –0.64 | 0.16 |
| Effective grain-filling duration, d | 0.21 | –0.68 | **1.00** | 0.94 | –0.01 | 0.02 | 0.89 | 0.30 |
| Total grain-filling duration, d | 0.31 | –0.58 | 0.96 | **1.00** | –0.22 | –0.08 | 0.79 | 0.33 |
| Days to anthesis, d | –0.22 | –0.18 | 0.02 | –0.19 | **1.00** | –0.26 | 0.39 | 0.04 |
| Anthesis–silking interval, d | –0.57 | –0.41 | –0.02 | –0.10 | 0.14 | **1.00** | –0.01 | –0.31 |
| Days to physiological maturity, d | 0.02 | –0.76 | 0.92 | 0.80 | 0.37 | 0.02 | **1.00** | 0.33 |
| Kernels per row | 0.57 | 0.08 | 0.43 | 0.44 | 0.08 | –0.13 | 0.40 | **1.00** |

†The *r* critical values at 10, 5, 1, and 0.1% probability levels are 0.24, 0.29, 0.38, and 0.47, respectively. The genotypic and phenotypic correlations were calculated based on genotype means across environments.

ratio), in which SCAmf is the genetic variance due to the interaction between males and female lines, indicated the importance of additive gene action compared to nonadditive gene action. Ratios exceeding 0.70 were observed for reproductive traits such as AD and ASI. The ratios were however moderate (0.26–0.45) for TGFD, GY, GFR, and EGFD (Table 6). Higher ratios were obtained for GY (0.45) than GFR (0.32) and EGFD (0.26). The narrow-sense coefficients of genetic determination (the fixed parent equivalent of heritability based on single plots) were low for GY (10%) but slightly higher for GFR (13%) and EGFD (22%). Based on correlation analysis (Table 5) and the coefficient of genetic determination values (Table 6), the expected gains through indirect selection of GY using GFR and EGFD were predicted to be 65 and 31% of direct selection, respectively.

## DISCUSSION

The major objective of this study was to investigate the use of grain-filling-associated traits as secondary traits for breeding high-yielding early-maturing maize hybrids. There is need to expand the range of tools available for breeding for higher yields in early-maturing maize, since their shorter stature, shorter growth cycle, and reduced leaf area index result in considerably lower yield than late-maturing maize (Dwyer et al., 1994).

In this study, the highest-yielding hybrids were categorized as medium maturing (134–139 DPM). Only one

Table 6. Components of variance and other genetic parameters for maize grain yield and other related traits of 44 maize hybrids.

| | Grain yield | Grain-filling rate | Effective grain-filling duration | Total grain-filling duration | Days to anthesis | Anthesis–silking interval | Kernels per row |
|---|---|---|---|---|---|---|---|
| | g per plant | g $d^{-1}$ | d | | | | |
| Hybrids variance component | 40.05 | 0.043 | 13.95 | 17.98 | 8.12 | 1.02 | 2.84 |
| Line variance component | 21.76 | 0.017 | 2.82 | 2.80 | 5.46 | 0.56 | 1.14 |
| Tester variance component | –0.57 | 0.001 | 2.08 | 4.26 | 1.46 | 0.41 | 0.36 |
| Line × tester variance component | 26.13 | 0.038 | 13.94 | 17.36 | 2.69 | 0.21 | 2.02 |
| Hybrids × environment (Env) variance component | 46.15 | 0.009 | 0.56 | 0.25 | 0.13 | 0.19 | 0.36 |
| Env × line variance component | 0.50 | –0.001 | 0.37 | 0.00 | –0.10 | –0.02 | 0.25 |
| Env × tester variance component | –5.28 | –0.003 | 0.05 | 0.07 | 0.04 | –0.05 | –0.25 |
| Env × line × tester variance component | 71.25 | 0.016 | 0.24 | 0.27 | 0.28 | 0.36 | 0.45 |
| Pooled error mean square | 119.40 | 0.068 | 2.84 | 1.86 | 2.39 | 1.58 | 6.21 |
| CV, % | 11.40 | 12.0 | 3.8 | 2.1 | 2.3 | 121.0 | 6.8 |
| Baker's ratio | 0.45 | 0.316 | 0.26 | 0.29 | 0.72 | 0.82 | 0.43 |
| Narrow-sense coefficient of genetic determination | | | | | | | |
| Single plot basis | 0.10 | 0.133 | 0.22 | 0.27 | 0.57 | 0.33 | 0.15 |
| Genotype mean basis (two replications and two environments) | 0.21 | 0.228 | 0.25 | 0.28 | 0.67 | 0.58 | 0.29 |
| Broad-sense coefficient of genetic determination | | | | | | | |
| Single plot basis | 0.22 | 0.420 | 0.85 | 0.92 | 0.79 | 0.40 | 0.35 |
| Genotype mean basis (two replications and two environments) | 0.47 | 0.722 | 0.95 | 0.98 | 0.94 | 0.71 | 0.67 |
| Fraction of phenotypic variance | | | | | | | |
| Hybrids variance component | 0.19 | 0.326 | 0.63 | 0.68 | 0.67 | 0.35 | 0.28 |
| Line variance component | 0.10 | 0.128 | 0.13 | 0.11 | 0.45 | 0.19 | 0.11 |
| Tester variance component | 0.00 | 0.005 | 0.09 | 0.16 | 0.12 | 0.14 | 0.04 |
| Line × tester variance component | 0.12 | 0.287 | 0.63 | 0.65 | 0.22 | 0.07 | 0.20 |
| Hybrids × Env variance component | 0.22 | 0.068 | 0.03 | 0.01 | 0.01 | 0.06 | 0.04 |
| Env × line variance component | 0.00 | –0.010 | 0.02 | 0.00 | –0.01 | –0.01 | 0.03 |
| Env × tester variance component | –0.02 | –0.022 | 0.00 | 0.00 | 0.00 | –0.02 | –0.02 |
| Env × line × tester variance component | 0.33 | 0.120 | 0.01 | 0.01 | 0.02 | 0.12 | 0.04 |
| Pooled error mean square | 0.56 | 0.512 | 0.13 | 0.07 | 0.20 | 0.54 | 0.62 |

early-maturing hybrid (127 DPM) was among the top four performers (Table 3). The 10 best hybrids produced up to 7 t $ha^{-1}$, with 27.3% more GY than the 10 lowest-yielding hybrids. These high-yielding hybrids were characterized by early flowering, a significantly lower ASI, and a high GFR (at 2.40 g per plant $d^{-1}$), which was 18% higher than that obtained for the lowest-yielding hybrids. These hybrids also had an EGFD (an estimator for linear phase of grain filling) of between 38 and 53 d after flowering. In maize, the GFR is influenced by sink capacity, a trait that is dependent on the total number of kernels, which in turn is related to efficiency in kernel set, and is influenced by the ASI (Carvoca and Otegui, 2007; Bolanos and Edmeades, 1993). In this study, the high-yielding hybrids had comparatively lower ASI values than the low-yielding ones. A long ASI reduces the number of grains per plant and GY in maize (Edmeades et al., 1993; Anderson et al., 2004) and partly explains why the high-yielding hybrids outperformed the low-yielding ones in this study. The dependence of GY on ASI increases under stress conditions. Surprisingly, the effects of heterotic groups on grain-filling attributes were not significant. We attribute this result to the fact that the parentage of many of these lines involves selections from populations that are themselves very diverse. Such lines often do not adhere to strict two-classification heterotic group assignments (Pswarayi and Vivek, 2008).

The results of this study also demonstrated the strong linkage between grain-filling patterns and their allied traits and the overall GY. Indeed, GFR had a moderately strong positive genotypic association with GY ($r$ = 0.57). The EGFD was also positively correlated with KR ($r$ = 0.43). These results are consistent with the findings of Wang et al. (1999) and Lee and Tollenaar (2007) who reported GFR to be positively correlated with grain-filling duration, flowering traits, and GY. The strong association between the grain-filling and flowering traits and GY suggests that they could be used as indirect selection tools for high-yielding early-maturing maize as they are predictive of the genotype's sink capacity and hence yield. Despite the negative correlation observed between GFR and EGFD, there were some exceptional cases in which

some hybrids combined both long EGFD and high GFR and translate this to high yield. For example, the highest-yielding hybrid was developed from a cross between the inbred line, VL08528, with positive GCA values for GFR and EGFD, and the tester, CML197, also with a positive GCA value for GFR. In addition, this hybrid had a very high SCA value for yield (not shown).

These results show that it is possible to develop high-yielding early- to medium-maturing maize hybrids by combining long EGFD and high GFR. The expected gain through indirect selection for GY using GFR and EGFD are 65 and 31%, respectively. Although these gains are well below 1.0 (indirect selection is equally effective as direct selection), they are high enough to indicate potential for simultaneous selection involving these traits and yield to provide gains better than yield alone.

The variance components and their fractional contribution to the phenotypic variance shows that selection based on GY alone is associated with several challenges that include the high experimental error and large hybrid × environment interaction due to the very large SCA × environment interaction. Grain yield also has lower broad-sense coefficient of genetic determination and high coefficient of variation that reduces the repeatability of the obtained results. However, secondary traits that include GFR, EGFD, ASI, and KR have shown to be associated with proportionately less experimental error and less genotype × environment interaction and SCA × environment interaction. These traits have high broad-sense coefficient of genetic determination and hence the repeatability of their data is high. These secondary traits together with GFR and EGFD can be used to formulate a selection index that can be used to select for high GY in early-maturing maize. The higher contribution of experimental error and SCA × environment to GY than to GFR and EGFD suggest that evaluation of hybrids for GY will need many environments and replications whereas the evaluation of GFR, EGFD, and allied traits will need relatively less environments and replications. This suggests that including these correlated traits could make selection cheaper and effective than direct selection based on GY alone. Furthermore, the variance components due to the testers, tester × environment interaction, and line × environment interaction were very negligible for these traits. This suggests that selection of desired parental lines based on the GCA values of these traits might be effective even without extensive multienvironmental trials. The little contribution of the testers' variance component to the phenotypic variance component could be attributed to the fact that CIMMYT has chosen testers based on the wide adaptability thus making them ideal in the subsequent test-cross evaluations.

For effective use of GFR and EGFD for GY improvement in maize, it must be easy to obtain the accurate measurement of these traits. Estimation of the start and end of the linear phase requires considerable improvement. Visual assessment of the blister stage of grain development could be used to show the start of the linear phase. Since the EGFD is highly correlated to TGFD ($r = 0.96$), DPM may be adequate to infer the end of the linear phase instead of the much more difficult method of using days to 36% kernel moisture content as described by Borras et al. (2009). Grain-filling rate can then be estimated as the total grain weight over the EGFD.

The GCA values for GFR and EGFD can thus be used to reduce the number of potential inbred lines and hence the number of hybrid combinations to be made while developing high-yielding early-maturing hybrids. The identification of lines and testers with positive GCA values for these two traits and other complementary yield traits is therefore critical in the selection of suitable parents for hybrid development. Based on significant GCA values for grain-filling traits and yield, a three-step selection scheme based on grain filling and allied traits is proposed. The first step involves evaluation of candidate inbred lines in single rows for traits that may show reasonable correlations between inbred and hybrid, such as AD, silking date, ASI, DPM, EGFD, number of kernel rows, and KR. In the second step, crosses between promising inbred lines and two to four testers can be made and the testcross hybrids evaluated together with their parents. General combining ability values, heritability values, and inbred–hybrid correlations must be estimated. In the final step, the data from the selected traits can then be used to develop a selection index for improving GY of hybrids. It should, however, be noted that large SCA effects will still require the evaluation of numerous inbred combinations. Furthermore, large genotype × environment variance components for GY will necessitate wide multilocation evaluation of hybrids for stability. However, the results presented here indicate potential for using EGFD and GFR to reduce the number of hybrids that must be produced and tested and to reduce the numbers that are advanced to testing in a large number of environments. Therefore, it would be worthwhile to evaluate the correlation and stability of yield-related traits in the hybrids and the consistency of inbred–hybrid relationships of EGFD, ASI, and other traits using a wide range of genetic materials in different environments. This information would allow assessment of the feasibility of the proposed selection scheme and would facilitate the identification of stable quantitative trait loci for GFR, EGFD, and GY for marker-assisted selection.

## CONCLUSIONS

Hybrids that are early maturing and high yielding have been identified in this study. The high-yielding hybrids possessed both high GFR and long EGFD thus making these two traits candidate supplemental traits for selection for improved GY in early maize. Furthermore, unlike

GY, GFR, EGFD, and allied traits are associated with less influence of experimental error, less genotype × environment interaction, and less SCA × environment interaction, making them suitable traits to aid in yield selection. A methodology to improve the measurement of these grain-filling traits have been proposed together with a selection scheme based on GFR and EGFD and allied traits for enhancing breeding selection for high-yielding and early-maturing maize. In subsequent work, we recommend the testing of the effectiveness of this strategy and using the data generated to also identify stable molecular markers for GFR and EGFD for use in marker-assisted selection.

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