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ORIGINAL PAPER

Breeding maize as biogas substrate in Central Europe: II. Quantitative-genetic parameters for inbred lines and correlations with testcross performance

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Abstract Breeding maize for use as a biogas substrate (biogas maize) has recently gained considerable importance. To optimize hybrid breeding programs, information about line per se performance (LP) of inbreds and its relation to their general combining ability (GCA) is required. The objectives of our research were to (1) estimate variance components and heritability of LP for agronomic and quality traits relevant to biogas production, (2) study correlations among traits as well as between LP and GCA, and (3) discuss implications for breeding of biogas maize. We evaluated 285 diverse dent maize inbred lines in six environments. Data were recorded on agronomic and quality traits, including dry matter yield (DMY), methane fermentation yield (MFY), and their product, methane yield (MY), as the main target trait. In agreement with observations made for GCA in a companion study, variation in MY was mainly determined by DMY. MFY, which showed moderate correlation with lignin but only weak correlation with starch, revealed only low genotypic variation. Thus, our results favor selection of genotypes with high DMY and less focus on ear proportion for biogas maize. Genotypic correlations between LP and GCA [r_g (LP, GCA)] were highest ($\geq$0.94) for maturity traits (days to silking, dry matter concentration) and moderate ($\geq$0.65) for DMY and MY. Multistage selection is recommended. Selection for GCA of maturity traits, plant height, and to some extent also quality traits and DMY on the level of LP looks promising.

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Introduction

The importance of plant biomass from maize (*Zea mays* L.) as a substrate for biogas production (hereafter referred to as biogas maize) has increased dramatically in Germany and other countries of Central Europe in recent years. To improve the economic as well as ecological efficiency of this renewable energy source, net energy yields per unit area under crop cultivation have to be maximized (Gerin et al. 2008). Since methane is the energy carrier in biogas, methane yield per unit area (MY) has to be enhanced, which depends multiplicatively on dry matter yield per unit area (DMY) and the amount of methane produced per dry matter unit, hereafter referred to as methane fermentation yield (MFY). However, information on the genotypic variation for MFY and MY as well as their genotypic correlations with other relevant agronomic and quality traits is not available for larger sets of maize germplasm.

In maize breeding, the final goal is to produce high performing hybrids, i.e., identify superior combinations of inbred lines. Therefore, inbred lines are evaluated in crosses with different testers for their testcross performance (TP). In a companion study based on TP, Grieder et al. (2011b) examined different traits in a broad germplasm collection and discussed their results with respect to breeding of biogas maize. They observed a preponderance of general combining ability (GCA) over specific combining ability (SCA) effects for agronomic as well as quality traits. However, line per se performance (LP) of

inbreds is also important for indirect selection to improve their performance in hybrid combinations. During line development by inbreeding, breeders select for LP in the different selfing generations, thus, limiting the number of potential candidate lines in later generations to be evaluated for TP. With the adoption of the doubled haploid technique (Prigge and Melchinger 2011) by which large number of inbred lines can be produced in one step, selection for LP has become even more important (Schmidt 2003).

Owing to the higher expected genotypic variance for LP compared with TP (Smith 1986), response to selection is expected to be high for LP. However, effective indirect selection for LP to improve TP requires a sufficiently high genotypic correlation (r_g) between these two criteria. Estimates of r_g between LP and TP depend on the predominant type of gene action (Hallauer and Miranda 1988; Mihaljevic et al. 2005; Smith 1986) and, therefore, vary for different traits. For forage maize, correlations between LP and TP for DMY, days to silking (DTS) and various quality traits ranged from moderate to strong (Argillier et al. 1995; Gurrath et al. 1991; Kreps et al. 1998; Seitz et al. 1992). Thus, estimates of r_g between LP and TP for novel traits like MFY and MY, which are important for biogas maize, are needed to develop optimum breeding strategies.

To the best of our knowledge, there is no information available on quantitative-genetic parameters for biogas relevant traits in maize with regard to LP and its correlation with TP or GCA of the lines. Thus, in a first attempt, we examined a large and diverse set of inbred lines from the dent heterotic pool, the GCA estimates of which have been reported in a companion study (Grieder et al. 2011b). The objectives of our study were to (1) estimate variance components and heritability of LP for agronomic and quality traits relevant to biogas production, (2) study correlations among traits as well as between LP and GCA, and (3) discuss implications for breeding of biogas maize.

Materials and methods

Germplasm, field experiments and data collection

Our study is based on 285 inbred lines from the dent heterotic pool (European dent, US Corn Belt dent, tropical germplasm) which cover a broad range of variation for maturity and other agronomic traits. A list of the inbred lines is given in the supplement of our companion study (Grieder et al. 2011b). Based on prior information about their flowering date, the inbred lines were divided into three maturity groups, each comprising 95 genotypes. Together with five common check inbred lines, the three groups of 95 inbred lines were evaluated in separate but adjacent experiments, each laid out as a 20-by-5 alpha design (Patterson and Williams 1976) with two replications. The experiments were conducted in 2008 and 2009 at three locations (Eckartsweier, Hohenheim, and Ihinger Hof) in Germany. We used two-row plots with 75 cm distance between rows and a length of 4 m at Eckartsweier and Ihinger Hof and 5 m at Hohenheim. Each plot was overplanted and later thinned to a final plant density of 100,000 plants ha^{-1}. Fertilizer application and plant protection measures were undertaken following good agronomical practice.

Data on various agronomic and quality traits were recorded as described in detail by Grieder et al. (2011b). The agronomic traits were DTS, plant height (PHT), dry matter concentration (DMC), DMY, and MY. Quality traits determined by near infrared spectroscopy (NIRS) on the basis of whole above-ground plant material were MFY, concentrations of fat, starch, acid detergent fiber (ADF), acid detergent lignin (ADL), and metabolizable energy concentration (MEC), an important quality parameter in forage maize production. Agronomic traits except MY were recorded in both replications, whereas quality traits were determined in only one replication.

In addition to the inbred lines, their testcrosses with two flint single-cross testers were evaluated in the same environments and the same traits were recorded. Testcrosses were grown adjacent to the inbred lines, using the same experimental design as described in detail in our companion study (Grieder et al. 2011b).

Statistical analyses

Data of LP were analyzed by a one-step approach using mixed models. Inbred lines from all three separate experiments were analyzed jointly following Piepho et al. (2006a). Data on the five common checks were used to estimate differences among the three experiments and to adjust for the same. Each location-year combination was regarded as an environment. The following model was used

$$y_{ijklmn} = \mu + (1-\theta)c_i + \theta g_j + e_k + (1-\theta)ce_{ik} + \theta ge_{jk} + h_{kl} + r_{klm} + b_{klmn} + \varepsilon_{ijklmn}.$$

Here, y_{ijklmn} represents the plot observation for trait y, μ denotes the overall mean, c_i is the effect of common check i, g_j the effect of inbred line j, e_k the effect of environment k, ce_{ik} the interaction between common check i and environment k, ge_{jk} the interaction between inbred line j and environment k, h_{kl} the effect of experiment l within environment k, r_{klm} the effect of replication m within experiment l, b_{klmn} the effect of incomplete block n within replication m, and ε_{ijklmn} the residual. The dummy variable θ is used to distinguish the inbred line from check observations (Piepho et al. 2006b) and assumes a value of 1 for inbred lines

and 0 for checks. All effects in the model except μ and c_i were considered random. Estimates of variance components due to genotypic effects of inbred lines (σ^2_g), interaction between inbred lines and environments (σ^2_{ge}), and residual (σ^2_ε) were computed by restricted maximum likelihood (REML). Heterogeneity of errors and incomplete block variances among environments and experiments were taken into account when estimating the variance components. The pooled σ^2_ε was calculated as the average of the individual estimates of $[\sigma^2_\varepsilon]_{kl}$. Heritabilities ($h^2$) on entry mean basis were calculated as:

$$h^2 = \sigma^2_g / \left(\sigma^2_g + \sigma^2_{ge}/E + \sigma^2_\varepsilon / ER \right),$$

where E is the number of environments and R the number of replications. The denominator of the equation for h^2 is equal to the phenotypic variance (σ^2_p). Standard errors of h^2 were calculated following Holland et al. (2003, p. 61). Phenotypic (CV_p) and genotypic (CV_g) coefficients of variation (%) were calculated as $100\sigma_p/\bar{X}$ and $100\sigma_g/\bar{X}$, respectively, where $\bar{X}$ is the mean LP.

For the estimation of various types of covariance (genotypic, genotype-by-environment interaction, and error) among traits, a bivariate mixed-model analogous to the univariate model described above was used. Phenotypic (r_p) and genotypic (r_g) correlation coefficients between traits and their associated standard errors were calculated following standard procedures described by Gilmour et al. (2006, p. 173). Estimates of GCA of the lines from the data of both testcross series were described in detail in our companion study (Grieder et al. 2011b). Estimates of r_p and r_g between LP and GCA for the same trait (LP, GCA) and between LP for a trait and GCA for MY (LP, GCA_{MY}) were calculated treating LP and GCA as two different traits and using the same approach as for calculating trait correlations, but ignoring replication and incomplete-block effects in the bivariate model.

For the quality traits, which had been determined for only one replication in each environment, the terms $(1 - \theta)ce_{il}$, θge_{jk}, r_{klm}, b_{klmn} were dropped from the model equation. As a result, estimates of σ^2_ε were confounded with the estimate of σ^2_{ge}. However, estimates of σ^2_g, h^2, r_p and r_g could still be calculated. All calculations were performed within the R-environment v. 2.9 (R Development Core Team 2009). Mixed model analyses were performed using the package *ASReml* v. 2.0 for the R-environment (Butler et al. 2007).

Results

Means and ranges

The average performance of inbred lines was 3,450 m^3 ha^{-1} for MY, 13.1 Mg ha^{-1} for DMY and 298 dm^3 (kg DM)$^{-1}$ for MFY (Table 1). Among the examined chemical components, average concentration was highest for starch (266 g kg^{-1}) and lowest for fat (19 g kg^{-1}). DTS ranged from 73 to 129 days and DMY from 5.6 to 19.7 Mg ha^{-1}. Apart from MFY and MEC, which only revealed a range of about 16% of the mean, all other agronomic and quality traits also showed a wide range that exceeded 60% of the mean performance.

Variance components and heritabilities

Estimates of σ^2_g were highly significant ($P < 0.01$) for all traits (Table 1). For DTS, PHT, DMC, and DMY, estimates of σ^2_{ge} were highly significant ($P < 0.01$), but of much smaller magnitude than those of σ^2_g (about three times for DMY to about 40 times for DTS). Estimates of h^2 were very high for all agronomic traits ($\geq$0.90). Estimates of h^2 were also very high for quality traits ($\geq$0.83) except for MFY, which showed a h^2 of 0.75.

Correlations among traits

Estimates of r_g were always higher than those of r_p with only few exceptions, where both were mostly of equal magnitude (Table 2). Further, the sign of r_g and r_p was the same in all instances. High MY was closely associated with high DMY and moderately with larger plant height. High ADL and high MFY values were also weakly associated with high MY, while the correlations between MY and the other quality traits as well as with DTS and DMC were non-significant. High DMY was moderately associated with large PHT and weakly with high ADL values and late flowering. High MFY tended to be associated with high MEC, high DMC, high fat and starch contents, but with low ADF and ADL contents and with early flowering and reduced PHT.

Correlations between line per se performance and GCA

Estimates of r_p (LP, GCA) for different traits were highly significant ($P < 0.01$), positive for all traits and consistently smaller than corresponding estimates of r_g (LP, GCA) (Table 3). Correlations were very strong for DTS and DMC [r_g (LP, GCA) $\geq$0.94] and moderately strong for PHT. For all quality traits except MFY, correlations between LP and GCA were strong [r_g (LP, GCA) $\geq$0.81]. Estimates of r_g (LP, GCA) were moderate (0.65–0.74) for MY and its two component traits DMY and MFY.

LP of the different agronomic traits showed moderate correlation with GCA of MY, with $|r_g$ (LP, $GCA_{MY})|$ ranging from 0.44 for DMC to 0.70 for DMY (Table 3). LP of MFY was not significantly correlated with GCA of MY. Estimates of r_g (LP, GCA_{MY}) of the other quality traits

Table 1 Means, ranges and variance components with standard errors (SE) for genotypes (σ^2_g), genotype-by-environment interactions (σ^2_{ge}), and residual (σ^2_ε), and heritabilities (h^2) for various agronomic and quality traits determined from 285 dent inbred lines, evaluated in four to six environments

Trait[a]	Mean	Range	Variance components			$h^2 \pm$ SE
			$\sigma^2_g \pm$ SE	$\sigma^2_{ge} \pm$ SE	$\sigma^2_\varepsilon \pm$ SE	
Agronomic traits						
DTS (d)	88.7	73.3–128.9	78.82 ± 6.78**	1.97 ± 0.15**	1.40 ± 0.12	0.99 ± 0.001
PHT (cm)	166	107–252	427.6 ± 37.4**	50.3 ± 3.7**	44.4 ± 3.5	0.97 ± 0.003
DMC (g kg^{-1})	337	190–523	4,582 ± 402**	519 ± 27**	228 ± 12	0.98 ± 0.002
DMY (Mg ha^{-1})	13.1	5.6–19.7	5.379 ± 0.496**	1.872 ± 0.100**	0.886 ± 0.042	0.93 ± 0.006
MY (10 m^3 ha^{-1})	345	139–497	3,815 ± 369**	2,449 ± 99	–[b]	0.90 ± 0.009
Quality traits						
MFY [dm^3 (kg DM)$^{-1}$]	298	271–316	37.1 ± 4.3**	72.5 ± 2.8	–[b]	0.75 ± 0.023
MEC (10^{-2} MJ kg^{-1})	1,192	1065–1269	794 ± 79**	722 ± 27	–	0.87 ± 0.012
Fat (g kg^{-1})	19	7–29	11.02 ± 1.05**	6.85 ± 0.26	–	0.91 ± 0.009
Starch (g kg^{-1})	266	15–474	5,058 ± 474**	1716 ± 66	–	0.95 ± 0.005
ADF (g kg^{-1})	227	160–320	273 ± 28**	338 ± 13	–	0.83 ± 0.016
ADL (g kg^{-1})	21	9–32	6.13 ± 0.60**	5.25 ± 0.20	–	0.88 ± 0.012

** Significant at the 0.01 probability level

[a] Traits are: *DTS* Days to silking, *PHT* plant height, *DMC* dry matter concentration, *DMY* dry matter yield, *MY* methane yield, *MFY* methane fermentation yield, *MEC* metabolizable energy concentration, *ADF* fat, starch, acid detergent fiber and *ADL* acid detergent lignin

[b] σ^2_{ge} confounded with σ^2_ε as methane yield and quality traits were determined using samples from only one replication

Table 2 Phenotypic (above the diagonal) and genotypic (below the diagonal) correlations among traits based on inbred line per se performance

Trait[a]	DTS	PHT	DMC	DMY	MY	MFY	MEC	Fat	Starch	ADF	ADL
DTS		0.48**	−0.88**	0.17**	0.05	−0.31**	−0.81**	−0.79**	−0.89**	0.75**	0.50**
PHT	0.49^{++}		−0.42**	0.64**	0.60**	−0.13*	−0.47**	−0.36**	−0.44**	0.41**	0.47**
DMC	-0.89^{++}	-0.43^{++}		−0.17**	−0.03	0.27**	0.62**	0.70**	0.83**	−0.51**	−0.32**
DMY	0.19^{++}	0.65^{++}	-0.18^{++}		0.98**	0.18**	−0.02	0.04	−0.09	0.12*	0.33**
MY	0.07	0.62^{++}	−0.04	1.00^{++}		0.29**	0.06	0.09	−0.03	0.06	0.26**
MFY	-0.36^{++}	-0.17^{+}	0.31^{++}	0.17^{+}	0.27^{++}		0.51**	0.33**	0.16**	−0.34**	−0.47**
MEC	-0.86^{++}	-0.52^{++}	0.66^{++}	−0.06	0.02	0.54^{++}		0.82**	0.83**	−0.84**	−0.66**
Fat	-0.83^{++}	-0.38^{++}	0.74^{++}	0.02	0.08	0.38^{++}	0.85^{++}		0.88**	−0.73**	−0.48**
Starch	-0.92^{++}	-0.47^{++}	0.85^{++}	−0.10	−0.05	0.20^{+}	0.86^{++}	0.90^{++}		−0.73**	−0.42**
ADF	0.83^{++}	0.45^{++}	-0.56^{++}	0.16^{+}	0.10	-0.41^{++}	-0.87^{++}	-0.77^{++}	-0.76^{++}		0.77**
ADL	0.54^{++}	0.51^{++}	-0.34^{++}	0.38^{++}	0.31^{++}	-0.54^{++}	-0.68^{++}	-0.49^{++}	-0.43^{++}	0.77^{++}	

*, ** Significant at the 0.05, 0.01 probability level, respectively

$^{+,\ ++}$ Genotypic correlation coefficient exceeds twice, trice its standard error, respectively

[a] See Table 1 for abbreviation of traits

were weak to moderate and generally of lower magnitude than those observed for agronomic traits.

Discussion

Amount of heritable variation

Ample heritable variation in relevant traits is a basic requirement for breeding. Significant and high estimates of σ^2_g and h^2 showed that large genotypic differences existed for all traits and effective selection can be carried out among the 285 inbred lines. Similar results for σ^2_g and high estimates of h^2 for these traits in the separate analyses of the three maturity groups strongly support this conclusion (Supplemental Table 1). Further, the separate analysis of maturity groups revealed that late material comprised the most diverse material according to DTS, resulting in highest genotypic variance within this group for all traits. Estimates of σ^2_g obtained for LP in the present study were

Table 3 Estimates of genotypic (r_g) and phenotypic (r_p) correlation coefficients of inbred line per se performance (LP) with general combining ability (GCA) of the same trait (LP, GCA) or with GCA of methane yield (LP, GCA_{MY}), as well as relative efficiency (RE) of indirect selection on LP to improve GCA

Trait[a]	GCA of same trait			GCA of MY		
	r_g (LP, GCA)	r_p (LP, GCA)	RE[b]	r_g (LP, GCA_{MY})	r_p (LP, GCA_{MY})	RE^b
Agronomic traits						
DTS	0.97^{++}	0.95**	0.98	0.62^{++}	0.55**	0.68
PHT	0.80^{++}	0.77**	0.81	0.67^{++}	0.61**	0.73
DMC	0.94^{++}	0.91**	0.95	-0.44^{++}	−0.40**	−0.48
DMY	0.70^{++}	0.65**	0.72	0.70^{++}	0.63**	0.75
MY	0.65^{++}	0.59**	0.68	–	–	–
Quality traits						
MFY	0.74^{++}	0.59**	0.79	−0.08	−0.07	−0.08
MEC	0.84^{++}	0.73**	0.90	-0.45^{++}	−0.40**	−0.46
Fat	0.85^{++}	0.78**	0.87	-0.38^{++}	−0.34**	−0.40
Starch	0.89^{++}	0.85**	0.90	-0.50^{++}	−0.45**	−0.54
ADF	0.81^{++}	0.69**	0.86	0.54^{++}	0.47**	0.54
ADL	0.81^{++}	0.72**	0.84	0.58^{++}	0.51**	0.60

** Significant at the 0.01 probability level

$^{++}$ Genotypic correlation coefficient exceeds trice its standard error

[a] See Table 1 for abbreviation of traits

[b] Calculated for equal selection intensities in LP and TP

2.7 times (DMY) to 4.8 times (DTS) larger than the estimates of variance due to GCA of lines (σ^2_{gca}) reported in our companion study (Grieder et al. 2011b). This is in agreement with quantitative-genetic expectations, as under the assumption of additive gene action only, σ^2_g among fully inbred lines is expected to be four times larger than σ^2_{gca} (Smith 1986).

Significant estimates of σ^2_{ge} for DTS, PHT, DMC, and DMY revealed the presence of interactions between inbred lines and test environments. Thus, evaluation of inbred lines for the traits under study warrants their testing in more than one environment to get reliable estimates of their genotypic values. The ratio σ^2_g:σ^2_{ge} (40 for DTS, 9 for DMC and PHT, and 3 for DMY) was approximately half the magnitude of corresponding ratio σ^2_{gca}:$\sigma^2_{gca\times e}$ observed in testcrosses (Grieder et al. 2011b). This reveals a higher relative importance of genotype-by-environment interactions in LP compared to TP. Although the ratio σ^2_g:σ^2_{ge} for LP was lower than the ratio σ^2_{gca}:$\sigma^2_{gca\times e}$ for TP, higher heritabilities were observed in LP for all traits. This can be attributed to the higher σ^2_ε observed in TP compared with LP and the additional contribution of σ^2_{sca} (SCA variance) and $\sigma^2_{sca\times e}$ (SCA-by-environment interaction variance) to the phenotypic variance in the case of TP. Thus, due to higher magnitudes of genotypic variance and heritability, response to selection in LP is expected to be larger compared with TP.

Correlations among traits

The correlations of MY with its two components traits were extremely high for DMY ($r_g = 1.00$) but low for MFY ($r_g = 0.27$) as already indicated by a higher CV_g of DMY (17.7%) than of MFY (2.0%). Further, we observed a moderate negative association of MFY with ADL and ADF, whereas fat showed a positive, weak and starch showed only very weak influence on this trait. All these correlations were in harmony with our companion study on TP (Grieder et al. 2011b), which may be seen for a detailed discussion on these associations.

Contrary to the non-significant correlation of MFY with MY and the negative correlation of MFY with DMY for TP based on GCA (Grieder et al. 2011b), these correlations were significantly positive but weak for LP. Similarly, the correlations of DMY with DTS ($r_g = 0.19$) and DMC ($r_g = -0.18$) were smaller for LP compared with the respective correlations for TP based on GCA. This may be attributable to a lack of adaptation of some late-maturing inbred lines of exotic origin. A lack of adaptation results in lower relative growth rates for LP than for TP (Strigens et al. 2011). Thus, due to non-optimal growth conditions at the beginning and end of the season, late-maturing, non-adapted inbred lines cannot exploit an extended vegetative growth period for increased biomass production. This is supported by the fact that DTS and DMY were positively

correlated ($r_g = 0.26$) in the early maturity group, which showed a range for DTS (72.6–92.2) equivalent to the complete range observed in TP, but not in the intermediate (DTS = 77.3–100.3) and late (DTS = 79.6–129.2) maturity groups (Supplemental Tables 1, 2). Later flowering was associated with lower DMC in all three maturity groups. Since only genotypes from the early maturity group showed higher DMY with later flowering, a negative correlation between DMC and DMY could only be observed for the early, but not for the intermediate and late material. All other correlations showed good agreement among the three maturity groups and with the correlations obtained from the combined analysis across maturity groups.

Correlations of line per se performance with GCA

The correlation r_g (LP, GCA) is of interest with regard to the comparison of the relative efficiency of indirect selection on LP for improvement of GCA of inbred lines. It also gives some indication about the predominant type of gene action of the genes underlying the investigated traits. Based on quantitative-genetic theory (Hallauer and Miranda 1988; Smith 1986), r_g (LP, GCA) is expected to be higher for traits with a preponderance of additive gene action compared to traits with substantial non-additive gene effects. In accordance with this expectation, r_g (LP, GCA) was among the lowest for DMY (0.70), for which earlier studies on forage maize (Barrière et al. 1993; Lübberstedt et al. 1997) also reported greater importance of non-additive gene action. As expected, r_g (LP, GCA) was also low for MY (0.65), because this trait is very closely associated with DMY. The very strong estimates of r_g (LP, GCA) for DTS (0.97) and DMC (0.94) are in accordance with the preponderance of additive gene action reported to be present for DTS (Buckler et al. 2009) and DMC in forage maize (Lübberstedt et al. 1997). High estimate of r_g (LP, GCA) for PHT (0.80) agrees with earlier results on forage (Kreps et al. 1998) and grain maize (Mihaljevic et al. 2005). Concentrations of fat, starch, ADF, and ADL, which had estimates of r_g (LP, GCA) intermediate to those of DTS and DMY (0.81–0.89), were largely determined by the ear proportion and, therefore, also by DTS. This was also observed by Argillier et al. (1995), who found estimates of r_p (LP, GCA) for concentrations of starch (0.83), ADF (0.73), and ADL (0.63) in forage maize which were similar to ours. Although MFY showed the lowest estimate of r_p (LP, GCA) among all traits due to its low heritability, the corresponding estimate of r_g (LP, GCA) (0.74) was only slightly lower than those for plant compositional traits.

Influence of tester

Weak testers with a low frequency of favorable, dominant alleles are expected to uncover best the effect of different alleles in the inbred lines (Rawlings and Thompson 1962) and consequently result in high values for σ^2_{gca} and r_g (LP, GCA) (Smith 1986). The four flint inbred lines that served as parents for the single-cross testers used for the production of TCs showed intermediate performance for biogas traits, when compared with the dent lines [9.8–16.2 Mg ha^{-1} for DMY, 300–310 dm^3 (kg DM)$^{-1}$ for MFY, and 2,603–4,445 m^3 ha^{-1} for MY]. Thus, even stronger r_g (LP, GCA) could be expected with poorer performing testers. In combination with the low importance of σ^2_{sca} compared with σ^2_{gca} for most traits (Grieder et al. 2011b), this suggests that employment of one single-cross or even one inbred line as tester should be effective for identifying promising inbred lines. In practical maize breeding, elite inbred testers are often preferred because they allow for the evaluation of experimental hybrids of potential use for direct commercialization (Hallauer 1990).

Efficiency of pre-selection among inbred lines

With the doubled haploid technology, huge number of lines are nowadays generated every season and evaluating all of them in testcrosses is impossible. Usually, breeders evaluate lines for LP before evaluating their TP in expensive multi-location trials. Since the ultimate aim is to improve the GCA of the inbred lines under evaluation in testcrosses, pre-selection for their LP is only efficient if it results in adequate response for their GCA. The effectiveness of this pre-selection can be determined by the relative efficiency (RE), i.e., the ratio of the indirect response to selection in GCA of inbred lines, if selection is performed for their inbred parents, over the response to direct selection in GCA of inbred lines, if selection is performed on the testcross progenies directly. Following Falconer and Mackay (1996), RE can be expressed as

$$\mathrm{RE} = \frac{i_{\mathrm{LP}} h_{\mathrm{LP}} r_g(\mathrm{LP}, \mathrm{GCA})}{i_{\mathrm{GCA}} h_{\mathrm{GCA}}},$$

where i denotes the selection intensity and h the square root of heritability. Thus, in addition to a high r_g (LP, GCA), RE of indirect selection depends on the ratios i_{LP}:i_{GCA} and h_{LP}:h_{GCA}. In our study, inbred lines were evaluated in six environments, which is more than usually used in practical plant breeding. Therefore, h_{LP} can be expected to be lower under real conditions and REs given in Table 3, which we calculated for equal selection intensities in LP and TP, are therefore expected to be overestimated.

High RE of indirect selection even with equal i in LP and TP evaluation for DTS (0.98) and DMC (0.95) would allow selecting for optimal maturity of hybrids at LP level. Owing to the low r_g (LP, GCA), RE of indirect selection for improving GCA of MY was lowest (0.68 with equal i in LP and TP) among all traits. The same applies for DMY,

which was strongly correlated with MY, and discloses the need for evaluating these two traits in testcrosses. However, taking into account that higher selection intensities are possible with LP evaluation due to larger numbers of genotypes being tested as compared to TP evaluation, even selection for LP based on direct measurement or visual scoring of DMY might reveal RE >1 and might therefore be effective for improving GCA of DMY and MY. Alternatively, PHT, which can be easily determined, would also be promising as selection criterion in LP for improving GCA of MY due to fairly high r_g and RE with GCA of the target trait MY [r_g (LP, GCA_{MY}) = 0.67, RE = 0.73].

If the aim is to improve plant quality (e.g., higher MFY and MEC, lower ADL), selection for quality traits based on LP for improving their GCA seems promising because REs under the assumption of equal i in LP and TP evaluation were intermediate (0.79–0.90) for all quality traits (Table 3). However, this would require fast methods for their determination as possible through the use of online-NIR spectrometers that can directly analyze harvested material on the chopper (Montes et al. 2007). The use of online-NIR spectrometers on choppers for determination of relevant quality traits for biogas maize seems promising based on the successfully developed laboratory NIRS calibrations for MFY and other quality traits (Grieder et al. 2011a). If selection is performed for quality parameters that relate to whole plant material like MEC, this might produce hybrids with increased grain or starch content, but poor cell wall digestibility of the stover (Argillier et al. 2000). This effect may also apply to MFY, although it showed a lower correlation with starch concentration than MEC. Thus, evaluation of MFY at the LP level for improvement of GCA may not be rewarding, because hybrids with lower ear proportions but high DMY are preferable for biogas maize. Alternatively, quality parameters relating to the stover material might be evaluated at the LP level.

Implications for biogas maize breeding

Since we observed a similar correlation pattern among traits for LP as reported for GCA in our companion study (Grieder et al. 2011b), our results support the conclusion that on the long run separate breeding programs for biogas and forage maize will be necessary. Whereas plant chemical composition is relevant for MEC in forage maize, it plays only a minor role in MFY for biogas maize. Moreover, the strong correlation of MY with DMY favors late-maturing hybrids with high DMY. Therefore, inbred lines may also be developed from base populations involving non-adapted or exotic germplasm (Schmidt 2003), but keeping in mind that DMC of the hybrids should not get below the optimum range of 280–350 g kg^{-1} as required for silage preparation (Eder et al. 2009).

Multi-stage selection is recommended in breeding for biogas maize. In the first stage, performance of doubled haploid lines would be assessed in observation plots in one or a few environments. Selection can be based on PHT, maturity (DTS and DMC), and to some extent on scoring of total biomass. Depending on the material, lodging and disease resistance are further important traits evaluated at the level of LP. The number of entries discarded would range between 50 and 90%. In the second stage, inbred lines would be tested for their GCA in topcrosses with a single-cross or inbred line tester from the opposite heterotic pool, further confining their number (Schmidt 2003). In addition to the traits reported in our study, special attention must be paid to lodging resistance of the tall growing biogas maize genotypes. The third stage involves evaluation of factorial crosses for determination of GCA and SCA effects.

In our study, estimates of r_g (LP, GCA) and h^2_{LP} for the examined biogas traits were higher than generally observed for grain yield. Consequently, more emphasis could be placed on selection for LP in breeding of biogas maize as compared to breeding of grain maize. However, owing to the later maturity of inbred lines used for biogas maize, seed production of the lines and hybrids might not be possible in the target environment (Central Europe). In this case, the inbred lines need additionally to be selected for seed yield and seed quality in the designated environment for seed production.

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