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ORIGINAL PAPER

Breeding maize as biogas substrate in Central Europe: I. Quantitative-genetic parameters for testcross performance

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Abstract Biofuels have gained importance recently and the use of maize biomass as substrate in biogas plants for production of methane has increased tremendously in Germany. The objectives of our research were to (1) estimate variance components and heritability for different traits relevant to biogas production in testcrosses (TCs) of maize, (2) study correlations among traits, and (3) discuss strategies to breed maize as a substrate for biogas fermenters. We evaluated 570 TCs of 285 diverse dent maize lines crossed with two flint single-cross testers in six environments. Data were recorded on agronomic and quality traits, including dry matter yield (DMY), methane fermentation yield (MFY), and methane yield (MY), the product of DMY and MFY, as the main target trait. Estimates of variance components showed general combining ability (GCA) to be the major source of variation. Estimates of heritability exceeded 0.67 for all traits and were even much greater in most instances. Methane yield was perfectly correlated with DMY but not with MFY, indicating that variation in MY is primarily determined by DMY. Further, DMY had a larger heritability and coefficient of genetic variation than MFY. Hence, for improving MY, selection should primarily focus on DMY rather than MFY. Further, maize breeding for biogas production may diverge from that for forage production because in the former case, quality traits seem to be of much lower importance.

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Introduction

Renewable sources of energy, such as biomass, wind, and solar radiation, have tremendously grown in importance during the past decade. The reasons for this development include environmental concerns (e.g., reduction in CO_2 emissions), energy security, and economic issues (e.g., foreign exchange savings, rural development) (Demirbas 2009). For example, the European Union (EU) has set targets of 20% renewable energy in the overall energy consumption by 2020 with a substantial proportion coming from bioenergy (European Parliament and Council 2009). Bioenergy refers to energy derived from organic materials and the three major products are bioethanol, biodiesel, and biogas (Yuan et al. 2008).

In Germany, the production of biogas through fermentation of different types of plant biomass and organic waste together with animal slurry has become a major branch of production for many farmers. Compared with liquid biofuels of the first generation such as bioethanol from starch and sugar or biodiesel from rapeseed, production of biogas from plant biomass is about three times as efficient in terms of the net energy produced per unit area of crop (Faulstich and Greiff 2008). In Germany, biomass from maize (*Zea mays* L.) is the most frequently used substrate in biogas plants (Weiland 2006) and the acreage of maize grown for biogas production has exponentially increased during the past decade to more than 0.5 Mio ha in 2010 (BMELV 2011). This corresponds to more than 20% of the total maize acreage of 2.3 Mio ha (Deutsches Maiskomitee 2011) and supplied ~1% of the total primary energy consumption in Germany.

Methane is the energy carrier in biogas. Thus, maximizing the volume of methane produced per unit area is the key to the economic success of biogas production from maize. Hereafter, we refer to maize used for biogas production as biogas maize. Methane yield (MY) of cultivars in cubic decimeters per hectare is the product of two components: (1) dry matter yield (DMY) in kilograms per hectare and (2) volume of methane produced per unit of dry matter in cubic decimeters per kilograms, which we termed methane fermentation yield (MFY) in a recent publication (Grieder et al. 2011a). Obviously, each of these components may be considered in breeding biogas maize.

Under the agroclimatic conditions of Central Europe, DMY of maize can be increased by exploiting late-maturing material and introgressing short-day genes from exotic populations (Schmidt 2003). However, prolonged vegetative growth period of genotypes is usually associated with reduced ear development and, consequently, reduced contents of easily degradable components by bacteria. In forage maize, a certain proportion of grain is required to warrant a high digestibility of the whole plant silage by ruminants (Johnson et al. 1999), which limits the use of late-maturing or exotic germplasm. Later onset of grain development reduces digestibility of forage maize and the same effect may also apply to MFY. Alternatively, breeders may select for high MFY by paying special attention to the proportion of easily degradable, energy-rich components like starch, sugars, and fat (Lübken et al. 2010). Moreover, MFY might also be increased by decreasing the proportion of non-degradable components like lignin, which may additionally inhibit the degradation of other substances in the substrate (Lübken et al. 2010).

There is no consensus among breeders on the ideotype of biogas maize and relative weights that should be given to various components in order to increase MY. In forage maize breeding, digestibility traits play a paramount role beside DMY (Barrière et al. 1997). If greater emphasis is placed on degradability and MFY of the plant material, maize cultivars developed for forage could also be used for biogas production. However, digestibility traits might be of lower importance in biogas maize than in forage maize because of the different conditions in a biogas fermenter versus rumen (Grieder et al. 2011a). In such a scenario, more emphasis might be given to DMY than MFY. Consequently, breeding programs for biogas maize may on the long run diverge from those for forage maize.

Recent studies investigated MFY and MY in maize but only with a small number (5–25) of genotypes (Eder et al. 2009; Oslaj et al. 2010; Schittenhelm 2008). Therefore, the information was rather limited and not representative for a wider range of germplasm. To draw general conclusions, investigations with larger, more representative sets of germplasm, including also exotic materials, are required. Furthermore, for developing optimum breeding strategies for biogas maize, information of genotypic and masking variance components of the relevant traits as well as on the trait correlations is needed. Therefore, we studied a large population of 570 testcross progenies (TC) of 285 inbred lines crossed with two testers.

The objectives of our research were to (1) estimate variance components and heritability for agronomic and quality traits relevant to biogas production in TCs of maize, (2) study correlations among traits, and (3) discuss strategies for breeding of biogas maize.

Materials and methods

Germplasm

Our study is based on 570 TCs developed by pollinating 285 inbred parents with two testers in two isolation plots. The lines belong to the dent heterotic pool (European dent, US Corn Belt dent and tropical germplasm) and covered a broad range of maturity (Supplemental Table 1). The testers were two single crosses, i.e., F052 × F047 (T1) and F084 × F055 (T2), between flint lines developed by the University of Hohenheim; their testcross progenies are referred to as TC1 and TC2, respectively.

Field experiments and data collection

Based on the maturity of the inbred parents, TC1 and TC2 were divided into three maturity groups, each comprising 95 TCs. Together with five common check hybrids, the six groups of 95 TCs were evaluated in separate but adjacent experiments, each laid out as a 20 × 5 alpha designs (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 with diverse agroecological conditions (Supplemental Table 2). 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 90,000 plants per hectare. Fertilizer application and plant protection measures were undertaken following good agronomical practice.

Days to silking (DTS) was recorded in four environments only (Eckartsweier and Hohenheim during both the years), while the other traits were measured in all six environments. DTS was recorded as the number of days from sowing to silk emergence of 75% of the plants in a plot. Plant height (PHT) was measured as the distance from soil surface to the base of the first tassel branch. Experiments were harvested separately depending on the maturity of the material. Above-ground fresh biomass of each plot was machine harvested with a chopper and directly weighted. A sample of about 1.5 kg was taken from each plot using an automatic cyclone sampler on the chopper and dried at 60°C to a constant weight to determine dry matter concentration (DMC). Dry matter yield in Mg ha^{-1} was calculated as the product of fresh biomass and DMC.

Concentrations of fat, starch, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) in the whole plant dry matter (DM) (g kg^{-1}) as well as MFY per DM (dm^3 kg^{-1}) and metabolizable energy concentration (MEC) per DM (MJ kg^{-1}) were determined by near infrared reflectance (NIR) spectroscopy. Dried samples of about 0.4 kg taken for determination of DMC were ground using a ZM 200 rotor mill (Retsch GmbH, Haan, Germany) with a 1 mm mesh. Near infrared reflectance spectra of these ground samples were collected in the range of 400 to 2500 nm employing a laboratory NIRSystems 6500 spectrometer (FOSS NIRSystems, Inc. Silver Spring, MD, USA). For determination of MEC, the reference method of which is described by Boguhn et al. (2003), the official NIRS calibration of the German association of agricultural research institutes (VDLUFA) was used. Development of the NIRS calibrations for all other traits was described in detail elsewhere (Grieder et al. 2011b); coefficients of determination (R^2) obtained with validation were 0.90 for fat and starch, 0.84 for ADF, 0.81 for NDF, 0.76 for ADL, and 0.77 for MFY.

As a reference assay for determination of MFY in the NIRS calibration, the Hohenheim biogas yield test (Helffrich and Oechsner 2003) was used. With this method, 300 mg of ground whole plant material are inoculated with 30 g of standard slurry in glass syringes of 100 ml capacity. During fermentation at 37°C, the syringes are kept on a rotor inside a heating cabinet to assure continuous mixing. The volume of gas produced and its methane content are measured several times during fermentation and these values are aggregated over time, so MFY for each measurement interval and the complete fermentation period (35 days) can be calculated. Since NIRS calibrations referred to MFY in organic DM, these values were multiplied by 0.96 to obtain MFY in total DM, assuming a constant ash content of 4% for all samples. Owing to the high labor input required for collection and preparation of samples for NIRS measurements, prediction of the quality traits could only be performed for samples from one replication of each environment. Methane yield (dm^3 ha^{-1}) was calculated as the product of MFY with DMY.

Statistical analyses

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

$$\begin{aligned} y_{ijklmno} = {} & \mu + (1-\theta)c_i + \theta t_j + \theta g_k + \theta \mathrm{tg}_{jk} + e_l \\ & + (1-\theta)\mathrm{ce}_{il} + \theta \mathrm{te}_{jl} + \theta \mathrm{ge}_{kl} + \theta \mathrm{tge}_{jkl} \\ & + h_{lm} + r_{lmn} + b_{lmno} + \varepsilon_{ijklmno}. \end{aligned} \tag{1}$$

Here, $y_{ijklmno}$ represents the plot observation for trait y, μ denotes the overall mean, c_i is the effect of common check i, t_j the effect of tester j, g_k the general combining ability (GCA) of line k, tg_{jk} the specific combining ability (SCA) of the hybrid between tester j and line k, e_l the effect of environment l, ce_{il} is the interaction between common check i and environment l, te_{jl} the interaction between tester j and environment l, ge_{kl} the interaction between GCA of line k and environment l, tge_{jkl} the interaction between SCA of cross $j \times k$ and environment l, h_{lm} the effect of experiment m within environment l, r_{lmn} the effect of replication n within experiment m, b_{lmno} the effect of incomplete block o within replication n, and $\varepsilon_{ijklmno}$ is the residual. The dummy variable θ is used to distinguish the TCs from checks (Piepho et al. 2006b) and assumes a value of 1 for TC and 0 for check observations. All effects in the model except μ, c_i, and t_j were considered random. Estimates of variance components due to GCA of lines (σ^2_{gca}), SCA of the hybrids (σ^2_{sca}), interaction between GCA and environments ($\sigma^2_{\text{gca}\times e}$), interaction between SCA and environments ($\sigma^2_{\text{sca}\times e}$), and residual ($\sigma^2_\varepsilon$) 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 $[\sigma^2_\varepsilon]_{lm}$. Heritabilities ($h^2$) on a the basis of GCA effects of lines were calculated as

$$h^2=\sigma^2_{\text{gca}}/\left(\sigma^2_{\text{gca}}+\sigma^2_{\text{sca}}/T+\sigma^2_{\text{gca}\times e}/E+\sigma^2_{\text{sca}\times e}/\mathrm{ET}+\sigma^2_\varepsilon/\mathrm{ETR}\right)$$

where E is the number of environments, T the number of testers and R the number of replications. The denominator of the equation for h^2 equals 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 $100\sigma_p/\overline{X}$ and $100\sigma_g/\overline{X}$, respectively, where σ_g refers to the square root of the GCA variance and $\overline{X}$ to the mean performance of the TCs.

For the estimation of various covariances among traits (GCA, SCA, GCA-by-environment interaction, SCA-by-environment interaction, and error), 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 as described by Gilmour et al. (2006, p 173).

For the quality traits recorded in only one replication, the terms θtge_{jkl}, r_{lmn}, b_{lmno} were dropped from the model equation. As a result, estimate of $\sigma^2_{\mathrm{sca}\times e}$ was confounded with the estimate of σ^2_{ε}. However, estimates of σ^2_{gca} and σ^2_{sca}, $\sigma^2_{\mathrm{gca}\times e}$, h^2, r_p and r_g could still be calculated. All calculations were performed within the R-environment (R Development Core Team 2009). Mixed model analyses were performed using the package *ASReml* for the R-environment (Butler et al. 2007).

Results

Means and ranges

The average performance of TCs was 20.3 Mg ha^{-1} for DMY, 305 dm^3 (kg DM)$^{-1}$ for MFY, and 5,520 m^3 ha^{-1} for MY (Table 1). There was a wide range among the TCs for agronomic as well as quality traits. The range was from 14.5 to 25.9 Mg ha^{-1} for DMY, 289–322 dm^3 kg DM^{-1} for MFY, and 3,990–7,120 m^3 ha^{-1} for MY. The TC having the highest performance for DMY also showed highest MY. The effect of the tester was significant ($P < 0.05$) for DMY and MEC, highly significant ($P < 0.01$) for DMC, MY, MFY, and ADL, but non-significant for the other traits. Differences between the two testcross series were generally low: mean performance of TC2 was higher for DMY (0.6 Mg ha^{-1}), MY (240 m^3 ha^{-1}), MFY (3.9 dm^3 kg DM^{-1}), and MEC (0.13 MJ kg^{-1}), but lower for DMC (14 g kg^{-1}) and ADL (1 g kg^{-1}) compared with TC1 (data not shown).

Variance components and heritabilities

Estimates of σ^2_{gca} were highly significant ($P < 0.01$) for all traits (Table 1). Estimates of σ^2_{sca} were highly significant ($P < 0.01$) for all agronomic traits and significant ($P < 0.05$) for MEC, but not for other quality traits. Their magnitudes were considerably smaller than those of σ^2_{gca}. Estimates of $\sigma^2_{\mathrm{gca}\times e}$ were highly significant ($P < 0.01$) for all agronomic traits, MFY, and fat, significant ($P < 0.05$) for starch and MEC, and not significant for ADF and ADL concentrations. They were much smaller than those of σ^2_{gca}. Estimates of $\sigma^2_{\mathrm{sca}\times e}$ were not significant.

Estimates of h^2 were high (0.82–0.98) for all agronomic traits, being highest for DTS and lowest for MY (Table 1). The estimates for the quality traits were also high in the case of fat and starch (0.87–0.93), but slightly lower for the other traits (0.67–0.82). The lowest value was obtained for MFY.

Correlations among traits

Estimates of r_g were always higher than those of r_p but had always the same sign (Table 2). Most estimates of r_p were highly significant ($P < 0.01$) and r_g estimates larger than thrice their standard error. Exceptions were the significant but weaker correlation between MFY and DMY and the non-significant correlations of MFY with MY and starch.

High DMY was tightly associated with high MY (Table 2). Both high DMY and high MY were associated with large PHT, late flowering and low DMC. Among the quality traits, low MEC, low fat, and low starch concentrations but high ADF and ADL were associated with high DMY and high MY. The MFY, on the other hand, did not show any relationship to the final MY, and only a very weak negative relationship to DMY. Contrastingly to MY and DMY, high MFY was positively associated by early flowering, short PHT, high MEC, high fat contents, low ADF and low ADL, but most of these relationships were rather lose.

Discussion

The use of maize biomass as a biogas substrate has boomed in the recent years. Since hybrids specifically bred for biogas were not available, varieties developed for forage maize have been cultivated. However, the conditions for digestion in the rumen are quite different from those for fermentation in a biogas fermenter. Retention time of solid substrate in the complete gastrointestinal tract of ruminants is around 2 days (Hartnell and Satter 1979), whereas it is up to 60 days or even longer in a biogas fermenter (Weiland 2006). Owing to the longer retention time, slowly degradable chemical components are metabolized to a larger extent in a fermenter. Consequently, the importance of high concentrations of easily degradable components like starch (Lübken et al. 2010) can be expected to be reduced in biogas maize compared to forage maize, where digestibility plays a paramount role besides DMY (Barrière et al. 1997). We therefore hypothesize that the restrictions in forage maize breeding, e.g., high ear proportion and sufficient DMC to ensure adequate digestibility and intake by ruminants, do not apply to biogas maize. Thus, it may be possible to exploit exotic genetic resources such as tropical germplasm to develop hybrids with long vegetative growth, resulting in increased photosynthetic capacity, high biomass and, thereby, increased MY. This, in turn

Table 1 Means, ranges and variance components with standard errors (SE) for GCA of lines (σ^2_{gca}), SCA of testcrosses (σ^2_{sca}), line GCA-by-environment interactions ($\sigma^2_{\text{gca}\times e}$), SCA-by-environment interactions ($\sigma^2_{\text{sca}\times e}$) and error ($\sigma^2_{\varepsilon}$), and heritabilities ($h^2$) for various agronomic and quality traits determined from testcrosses of 285 dent lines in combinations with two flint testers, evaluated in four to six environments

Trait[a]	Mean	Range	Variance components					h^2 ± SE
			σ^2_{gca} ± SE	σ^2_{sca} ± SE	$\sigma^2_{\text{gca}\times e}$ ± SE	$\sigma^2_{\text{sca}\times e}$ ± SE	σ^2_{ε} ± SE	
Agronomic traits								
DTS (d)	78.1	70.2–93.6	16.373 ± 1.454**	0.347 ± 0.048**	0.211 ± 0.029**	0.000 ± 0.000	1.740 ± 0.070	0.98 ± 0.002
PHT (cm)	263	215–304	155.9 ± 14.4**	5.6 ± 1.0**	9.6 ± 1.2**	0.0 ± 0.0	81.6 ± 2.6	0.95 ± 0.005
DMC (g kg^{-1})	345	252–416	930.0 ± 83.8**	26.9 ± 4.0**	60.4 ± 4.8**	0.0 ± 0.0	245.9 ± 5.9	0.96 ± 0.004
DMY (Mg ha^{-1})	20.3	14.5–25.9	1.975 ± 0.912**	0.212 ± 0.032**	0.330 ± 0.034	0.026 ± 0.037	1.743 ± 0.046	0.89 ± 0.012
MY (10 m^3 ha^{-1})	552	399–712	1,371 ± 145**	148 ± 43**	352 ± 63**	1,923 ± 72	[b]–	0.82 ± 0.021
Quality traits								
MFY (dm^3 kg DM^{-1})	305	289–322	8.7 ± 1.2**	0.9 ± 0.7	2.9 ± 1.2**	40.4 ± 1.5	[b]–	0.67 ± 0.041
MEC (10^{-2} MJ kg^{-1})	1,232	1,132–1270	206 ± 24**	20 ± 12*	10 ± 18*	662 ± 24	–	0.75 ± 0.031
Fat (g kg^{-1})	26	17–32	3.99 ± 0.41**	0.15 ± 0.10	0.48 ± 0.16**	5.56 ± 0.20	–	0.87 ± 0.016
Starch (g kg^{-1})	350	127–429	1,431 ± 136**	26 ± 19	61 ± 31*	1,110 ± 40	–	0.93 ± 0.009
ADF (g kg^{-1})	215	180–261	83.8 ± 10.2	4.2 ± 5.2	0.0 ± 0.0	330 ± 8.6	–	0.74 ± 0.032
ADL (g kg^{-1})	21	15–27	1.77 ± 0.19**	0.00 ± 0.00	0.01 ± 0.12	4.78 ± 0.16	–	0.82 ± 0.017

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

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

[b] $\sigma^2_{\text{sca}\times\text{e}}$ confounded with σ^2_{ε} as methane yield and quality traits were determined by using samples from only one replication

Table 2 Phenotypic (above the diagonal) and genotypic (below the diagonal) correlations among traits based on GCA of inbred lines

Trait[a]	DTS	PHT	DMC	DMY	MY	MFY	MEC	Fat	Starch	ADF	ADL
DTS		0.83**	−0.88**	0.44**	0.56**	−0.31**	−0.76**	−0.81**	−0.85**	0.80**	0.68**
PHT	0.86 ++		−0.64**	0.81**	0.73**	−0.22**	−0.61**	−0.63**	−0.62**	0.67**	0.65**
DMC	−0.90 ++	−0.66 ++		−0.53**	−0.36**	0.22**	0.62**	0.74**	0.78**	−0.69**	−0.57**
DMY	0.45 ++	0.85 ++	−0.56 ++		0.98**	−0.13*	−0.42**	−0.47**	−0.47**	0.54**	0.59**
MY	0.64 ++	0.80 ++	−0.41 ++	1.00 ++		0.00	−0.35**	−0.39**	−0.41**	0.46**	0.50**
MFY	−0.40 ++	−0.31 ++	0.23 ++	−0.24 ++	−0.15		0.34**	0.36**	0.00	−0.40**	−0.53**
MEC	−0.86 ++	−0.73 ++	0.68 ++	−0.58 ++	−0.52 ++	0.30 ++		0.85**	0.85**	−0.84**	−0.74**
Fat	−0.87 ++	−0.70 ++	0.77 ++	−0.55 ++	−0.48 ++	0.40 ++	0.91 ++		0.84**	−0.81**	−0.72**
Starch	−0.89 ++	−0.67 ++	0.80 ++	−0.53 ++	−0.48 ++	−0.02	0.91 ++	0.86 ++		−0.73**	−0.56**
ADF	0.92 ++	0.81 ++	−0.75 ++	0.71 ++	0.65 ++	−0.47 ++	−0.86 ++	−0.86 ++	−0.75 ++		0.88**
ADL	0.76 ++	0.75 ++	−0.59 ++	0.72 ++	0.65 ++	−0.58 ++	−0.73 ++	−0.75 ++	−0.57 ++	0.90 ++	

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

+, ++ Genotypic correlation coefficient exceeds twice, thrice its standard error, respectively

[a] See Table 1 for abbreviation of traits

raises the question of whether separate breeding programs for forage and biogas maize are needed.

Means, variances and heritabilities

Mean DMY obtained in this study (20.3 Mg ha^{-1}) was similar to the mean DMY of current maize hybrids recommended for biogas production, which was 22.6 Mg ha^{-1} in 2009 and 20.0 Mg ha^{-1} in 2010, based on trials at different locations in Bavaria, Germany (LfL 2010). The DMY of the best TC (26 Mg ha^{-1}) was lower than 30 Mg ha^{-1}, the level of DMY considered to be a realistic goal for biogas maize by Schmidt (2003). However, we investigated the possibility of exploiting the genotypic variation only in the dent heterotic group and our two testers were ordinary flint single-cross hybrids selected for forage production. Expecting a similar magnitude of genotypic variance in the flint as in the dent heterotic group, the goal of 30 Mg ha^{-1} DMY seems realistic.

Estimates of σ^2_{gca} indicated the presence of significant differences for GCA among the lines, and in view of the high h^2 values, selection should be highly effective for all traits. Significant estimates of $\sigma^2_{\text{gca}\times e}$ for most traits indicated the need for evaluating the materials in several environments. Our estimate of σ^2_{gca} as well as h^2 for DMY was larger or at least of equal magnitude compared with earlier results on forage maize (Dhillon et al. 1990; Geiger et al. 1992; Gouesnard et al. 1996; Krützfeldt 2004; Lübberstedt et al. 1997).

The large estimates of h^2 may be due to the very broad range of germplasm evaluated in our study, which also included tropical lines. Further, the relatively large number of environments used for evaluating the materials has contributed to high h^2 estimates. The medium h^2 for MFY is largely attributable to the high experimental error in the lab assay used for development of the NIRS calibration, as discussed in detail by Grieder et al. (2011a).

Estimates of σ^2_{sca} for all traits were less than 10% of the corresponding estimates of σ^2_{gca}. Since we used single-cross testers, σ^2_{sca} corresponds to one half of the variance of SCA effects expected with inbred testers. Thus, even considering this effect, SCA effects were of low importance in our experiment. However, in view of the low number of testers used in our study and the fact the tester lines were all derived from the same breeding pool, further research with larger factorial mating designs is warranted to assess the importance of SCA and, therefore, dominance variance.

Methane fermentation yield and plant chemical composition

Our results corroborate earlier findings of Schittenhelm (2008) and Eder et al. (2009), who reported no correlation of starch concentration with MFY after complete fermentation of whole plant maize. Grieder et al. (2011a) investigated the kinetics of methane production in biogas maize by measuring MFY during the entire fermentation period. They concluded that due to the long fermentation time in the reference assay (35 days), variation in MFY due to easily degradable chemical components diminished over time because of complete or partial degradation of all chemical components. This especially holds true for starch, which is a carbohydrate, having the same stoichiometric value for MFY as all other carbohydrates like sugars, celluloses, and hemicelluloses (Lübken et al. 2010). Thus, these results support the hypothesis that in biogas maize, high ear and starch proportions might be of lower importance than in forage maize.

The lack of correlation between MFY and starch concentration seems contradictory to official guideline values for MFY based on multiple experiments (KTBL 2010), which are 340 dm^3 kg DM^{-1} for whole plant maize silage and 380 dm^3 kg DM^{-1} for maize kernels. However, Zilic et al. (2011) reported concentrations of ADF and ADL in maize hybrid kernels close to 40 and 5 g kg^{-1}, respectively, which is much lower than observed for the whole plant material in our study. Hence, most likely the lower content of potentially lignified fibers as discussed below, but not the higher starch concentration might be responsible for the higher observed MFY of kernels compared to whole plant material.

Significant correlations of MFY with fat, ADF and ADL observed in our study were contrary to the results reported earlier by Schittenhelm (2008) and Eder et al. (2009). Fat has a high energy concentration and its stoichiometric value for MFY (708 dm^3 kg^{-1}) is much higher than that of carbohydrates (397 dm^3 kg^{-1}) (Lübken et al. 2010). Therefore, the positive but weak correlation of fat concentration in whole plant material with MFY could be expected and is in agreement with the strong, positive regression coefficient of fat in the model for MFY prediction in whole plant maize from Amon et al. (2007). Contrarily, lignin (measured as ADL) is not degradable in biogas fermenters and may additionally reduce the degradability of other structural carbohydrates (Lübken et al. 2010). This recalcitrance of lignin and its inhibitory effects on other components may explain the negative correlation of ADL with MFY. To avoid a dilution effect in the lignin content by the variable starch content, we calculated also the ADL:NDF ratio and found it to be negatively correlated with MFY ($r_g = -0.58$). Thus, the effect of ADL on MFY is not only attributable to varying grain proportion in the harvested material, but also seems to depend on the lignin content in the maize stover. Besides cellulose, ADL is a fraction of ADF. Expectedly, ADF was also negatively correlated with MFY, yet the correlation was weaker than that for ADL. MEC only showed weak correlation with MFY, indicating that this important parameter for forage maize only has low predictive value for MFY in biogas maize.

Determinants of methane yield

The correlations of MY with DMY ($r_g = 1.00$) and MFY ($r_g = -0.15$) observed with our material indicate that the variation in MY was primarily attributable to genetic differences in DMY, whereas MFY was only of minor importance. Since DMY and MFY were only weakly correlated ($r_g = -0.24$) and CV_g was much higher for DMY (6.9%) than for MFY (1.0%), this interpretation is also supported by theoretical derivations given in the Appendix. In agreement with conclusions drawn by Schittenhelm (2008) and Eder et al. (2009), our study showed that MY can be most efficiently improved by selecting genotypes with high DMY rather than high MFY. Further, as DMY and MFY showed only a weak, negative correlation, selection for DMY would have hardly an adverse impact on MFY.

Since breeders select genotypes within a given range of maturity, we divided our material also into groups with small ranges (i.e., 10 g kg^{-1}) of DMC. Even within these narrow maturity groups, estimates of r_p among MY, MFY, and DMY confirmed the dominating influence of DMY on MY. However, in regions where shortage of water limits plant biomass growth and sets an upper limit to DMY, MFY, and quality aspects might gain importance. When we divided our material into groups with narrow DMY ranges of 1 Mg ha^{-1}, MFY showed a stronger correlation with MY within these groups. Thus, improvement of MY via MFY within a given range of DMY would be feasible.

For temperate climates with cool autumn temperatures and low solar irradiance during the grain filling phase as in Central Europe, maize is considered to be mainly source limited (Borrás et al. 2004; Coors et al. 1997). Hence, complete ear development may not be essential for maximizing DMY in such regions, as the stalk can act as an alternative sink for assimilates additionally to the ear (Coors et al. 1997; Tollenaar 1977). In addition, late flowering, tall growing genotypes with large foliage and thereby increased photosynthetic apparatus can be expected to produce more assimilates. Hence, these genotypes show a higher DMY and in turn also higher MY, as observed in our study. There was a weak negative correlation between DTS and MFY in our study, which was most probably caused by a lower ear proportion in the later flowering and taller growing material, leading to decreased concentration of starch and fat, and higher content of lignin in the whole plant material. However, this relationship was obviously overcompensated by the positive correlation with DMY. This is supported by the moderate, positive correlation of DTS with MY. From a physiological point of view, the positive correlation between DTS and DMY can be explained by the longer vegetative growth period of late flowering genotypes, leading to higher biomass accumulation. From a genetic point of view, pleiotropy and close linkage of genes controlling for DTS and DMY are possible reasons for the correlation between these traits (Falconer and Mackay 1996), and further research is warranted to disentangle these different possibilities.

Separate analyses of maturity groups

The high h^2 estimates for all traits, the tight association of MY with DMY as well as the correlation pattern between MFY and plant chemical composition obtained in the combined analyses across maturity groups, were mostly confirmed by the analyses of each maturity group separately (Supplemental Tables 3, 4). One exception was MFY, which showed a significantly negative correlation with starch concentration in the intermediate maturity group, which was mainly caused by five genotypes with low starch concentration and high MFY. However, these genotypes fitted well into the negative correlation between MFY and ADL, which was consistent over all maturity groups, again supporting the importance of the lignification on MFY. Further, MFY was not negatively correlated with DTS and PHT, as observed in the early and late material. Thus, depending on the group of material, later flowering, tall growing genotypes are not necessarily associated with lower quality for biogas production. In general, estimates of h^2, r_p and r_g across all maturity groups (Tables 1, 2) were slightly larger compared with the separate analysis of maturity groups (Supplemental Tables 3, 4), because in the former case, these statistics are influenced by both, the genotypic differences within as well those among maturity groups.

Implications for biogas maize breeding

Our results suggest that DMY is the primary determinant for MY, whereas MFY is of little importance. Furthermore, compared with DMY, MFY had smaller CV_g and h^2, so that response to selection for this trait is expected to be smaller than for DMY. Hence, determination of MFY seems not rewarding, as even its prediction by NIRS requires labor-intensive sample preparation and time. Further, our study showed that tropical germplasm such as CML lines from CIMMYT or NC lines from North Carolina State University, the testcrosses of which showed the highest DMY in our experiment, can be easily exploited to improve biomass production and MY in breeding biogas maize. Consequently, there is potential to exploit exotic germplasm in biogas maize, because the restrictions of adequate ear proportion and digestibility are less rigid compared to forage maize. This is also reflected by the only weak correlation between MFY and MEC ($r_g = 0.30$), implying that lower quality for forage maize (i.e., MEC) is not necessarily accompanied with lower quality for biogas maize (i.e., MFY). Hence, DMY may be improved by making use of late flowering germplasm and short-day genes as proposed by Schmidt (2003). Breeding programs for biogas maize might, therefore, diverge from forage maize breeding, as for the latter quality aspects are of higher relevance.

For storage, biogas maize is usually ensiled. The optimum range of DMC for silage preparation is within the range of 280–350 g kg^{-1} (Barrière et al. 1997, Eder et al. 2009). If DMC is too low, formation of leachate in the silage will occur, necessitating its elaborate disposal and leading to loss of energy. With lowest values around 250 g kg^{-1}, DMC of late maturing TCs was below the optimum range. The observed strong, negative correlation between DTS and DMC, therefore, places some restrictions to the use of late maturing material for increasing DMY, because the recommended lower limit of 280 g kg^{-1} for DMC could not be reached in late flowering genotypes. Furthermore, early frost in September leading to premature termination of plant growth entails a high risk for farmers in growing late maturing cultivars.

For high dry matter yielding genotypes with low ear proportion, cell wall characteristics of the stover might deserve greater attention if the aim is to achieve higher MFY. Thus, selection for reduced lignin concentration in the stover, which showed a negative effect on MFY in our study, could be performed to indirectly maximize MFY. One option might be to exploit brown midrib (*bm*) mutants that are characterized by reduced lignin content and have been shown to increase forage digestibility, yet at the expense of reduced grain and DM yields (Sattler et al. 2010). However, high cell wall digestibility due to low lignin content is also reported to be associated with higher pest (Ralph et al. 2004) and lodging susceptibility (Esechie et al. 2004). Thus, selection for pest and lodging resistance would need to be emphasized when exploiting *bm* mutants or other genetic resources with lower lignin concentration.

While MFY had a minor impact on MY in our study, its general importance for the operational efficiency of biogas plants has still to be assessed. In particular, relative economic weights for MFY and DMY need to be determined for the construction of appropriate selection indices, adapting a similar approach as for forage maize (Mistele et al. 1994; Utz et al. 1994). Moreover, the relationship between line per se and testcross performance deserves further research in order to optimize schemes for biogas maize breeding.

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Appendix

We show that for two stochastically independent random variables X and Y with mean μ unequal to zero, the coefficient of variation CV_{XY} of their product variable XY can be expressed in terms of the CV's of X and Y:

$$\mathrm{CV}_{XY} = \sqrt{\mathrm{CV}_X^2 + \mathrm{CV}_Y^2 + \mathrm{CV}_X^2\mathrm{CV}_Y^2}, \tag{1}$$

and the correlation of XY with Y $\left(r_{XY,Y}\right)$ can be expressed as

$$r_{XY,Y} = \frac{\mathrm{CV}_Y}{\sqrt{\mathrm{CV}_X^2 + \mathrm{CV}_Y^2 + \mathrm{CV}_X^2\mathrm{CV}_Y^2}} \tag{2}$$

Proof Let μ_X, μ_Y, μ_{XY} and $\sigma_X^2, \sigma_Y^2, \sigma_{XY}^2$ denote the means and variances of X, Y, XY, respectively. Then, σ_{XY}^2 is given by Mood et al. (1974, p 180) as

$$\sigma_{XY}^2 = \mu_Y^2\sigma_X^2 + \mu_X^2\sigma_Y^2 + \sigma_X^2\sigma_Y^2. \tag{3}$$

Dividing this equation by $\mu_{XY} = \mu_X\mu_Y$ and taking the square root proves Eq. (1), because $\sigma^2 = \mathrm{CV}^2\mu^2$.

Bohrnstedt and Goldberger (1969, Eq. 13), give the covariance of XY with another product variable UV. By assuming $U = Y$ and $V = 1$, the covariance between XY and Y can then be expressed with their formula as $\mathrm{COV}_{XY,Y} = \mu_X\sigma_Y^2$, and using Eq. (3) for σ_{XY}^2, we obtain

$$r_{XY,Y} = \frac{\mu_X\sigma_Y^2}{\sqrt{(\mu_Y^2\sigma_X^2 + \mu_X^2\sigma_Y^2 + \sigma_X^2\sigma_Y^2)\sigma_Y^2}}.$$

Dividing the nominator and denominator of this equation by $\mu_X\mu_Y^2$ proves Eq. (2).

From Eq. (1) it follows that $\mathrm{CV}_{XY} \geq \max(\mathrm{CV}_X, \mathrm{CV}_Y)$. Hence, if CV_X is much larger than CV_Y and both CV's are considerably smaller than 1, then $\mathrm{CV}_{XY} \approx \mathrm{CV}_X$. From Eq. (2) it follows that the ratio of the correlations $r_{XY,X} : r_{XY,Y} = \mathrm{CV}_X : \mathrm{CV}_Y$. Consequently, if CV_X is much larger than CV_Y, then X is much tightly correlated with the product variable XY than Y.

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