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Design of breeding strategies for energy maize in Central Europe

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Contents

Abbreviations

ADF	acid detergent fiber
ADL	acid detergent lignin
AIC	Akaike information criterion
BFY	biogas fermentation yield
BLUE	best linear unbiased estimate
CHO	carbohydrate
CV	coefficient of variation
DM	dry matter
DMC	dry matter concentration
DMY	dry matter yield
GCA	general combining ability
IVDOM	*in vitro* digestible organic matter
LP	line *per se* performance
LSR	least squares regression
maxR	maximum methane production rate
MC	methane concentration in biogas
ME	metabolizable energy
MFY	methane fermentation yield
MY	methane yield
NDF	neutral detergent fiber
NfE	nitrogen-free extract
NIRS	near infrared spectroscopy
ODM	organic dry matter
PHT	plant height
PLSR	partial least squares regression
R^2	coefficient of determination
RMSEP	root mean square error of prediction
RPD	ratio performance deviation
SCA	specific combining ability
SEP	standard error of prediction
T_{95}	time needed to reach 95% of final methane fermentation yield
TC	testcross

1. General Introduction

Owing to the past and ongoing extensive use of fossil fuels, their stocks will be depleted within decades (Shafiee and Topal 2009) and supply shortfall will occur when trying to meet the rising energy demand of the growing world population. Rising oil prices as experienced during the past decade are a first symptom for the increasing scarcity of fossil fuels. Further, global warming that is effected by the emission of greenhouse gases from fossil fuels and other sources (Lashof and Ahuja 1990) is a well known threat with ecological (Hughes 2000) and economical (Tol 2005) consequences. For example, rising sea level (Meehl et al. 2005) threatens the living space of millions of people and higher variability in regional climatic condition with drought spells and floods (Lehner et al. 2006) can cause reduced agricultural production. To reduce the dependency on fossil fuel reserves and reduce the negative impacts of their use, the implementation of alternative, renewable, and climate neutral energy sources is a major issue. With the Kyoto protocol, the European Union committed itself to reduce the emission of CO_2 and other greenhouse gases by 8% in 2010 compared to 1990 (Gerin et al. 2008). As further measures to reduce emission of greenhouse gasses and dependency on fossil fuels beyond 2010, the European Union has set target to increase the share of renewable sources on total energy consumption to 20% and a mandatory share of biofuels in transportation of 10% by 2020 (European Parliament and Council 2009). Since different goals have been set for the EU member states, Germany has to increase the share of renewable energy sources on total energy consumption from 11% in 2010 to 18% in 2020.

Besides other renewable energy sources like solar radiation, wind and hydropower, energy production from biomass is well suited to reach the goals described above. Already in 2010, biomass contributed to 71% of total final energy consumption from renewables in Germany, whereby a large proportion arose from the use of biogenic solid fuels used for heat and power production (AGEE-Stat 2011). However, further potential is seen also for other forms of energy from biomass like biogas or liquid biofuels, and these are promoted by the German renewable energy sources act. Thereby, biogas had drawn the most attention. The number of biogas plants as well as the installed electric power has increased tremendously during the past decade (Fachverband Biogas e.V. 2011). For example in 2010, 5905 biogas plants produced 14.8 TWh electric power, corresponding to an avoidance of 11 million tons CO_2 equivalents based on calculations from Fachverband Biogas e.V. (2010).

For biogas production, biomass (e.g., from organic waste, energy crops) is fermented under anaerobic conditions. Biogas is a mixture of different gases, containing 50 to 75% of methane (CH_4), which is the energy containing component, CO_2 (25-50%), H_2O (2-7%), H_2S (~2%), N_2 (< 2%) and H_2 (< 1%) (KWS Mais GmbH 2009). Currently, most of the biogas is used in combined heat and power (CHP) units adjacent to the plant to produce electric power for the grid, while the accumulating heat is not always completely used. However, new techniques are now available to clean the biogas at low costs, making it possible to inject the produced methane directly into the gas grid (Drescher 2011). These new technologies bear several advantages over the direct electric power production on the plant like reduced CH_4 losses, possibility of storing energy in the gas grid, higher efficiency in use of excess heat when electricity is produced in large scale CHP units and possibility for farmers to bring their methane directly on the market instead of just being producer of crude biogas (Drescher 2011; Klinkert et al. 2010). Hence, continuous innovation is on the way and biogas technology can be expected to gain further efficiency and importance in the future.

Biomass for biogas production

As consequence of the rapidly increasing biogas production, the demand for organic substrates has also increased tremendously. In terms of mass, 46% of the substrate currently used in German biogas plants is derived from plants grown especially for this purpose (energy crops), whereas 45% are from animal excrements, and 7% from biological waste (DBFZ 2011). However, the corresponding values are 80% from energy crops, 11% from animal excrements, and 7% from biological waste in terms of the energy produced (DBFZ 2011). Hence, the use of energy crops is essential to reach high methane production rates in biogas plants. Since energy crops are increasingly applied in most biogas plants, also the area under energy crops increased. For example the area under crops grown for biogas production increased from 650’000 ha in 2010 to approximate 800’000 ha in 2011 within one year only (FNR 2011).

To be suitable for biogas production, a crop has to fulfill certain requirements (Eder 2010; Schittenhelm 2008): good suitability for storage, high methane yield per area land, low costs of production, and easy integration into existing farming systems. Maize fulfills these criteria to a high degree. The storage of maize as whole plant silage can be easily performed and comes along with only small losses in quality. Under Central European conditions, the dry matter yield (DMY) potential of maize is among the highest of the commonly grown crops. Further characteristics making maize advantageous for biogas production are its high nitrogen- and water efficiency (Eder 2010). Silage maize, which contributes to 76% of the biomass from energy crops used for biogas production (DBFZ 2011), is nowadays the most widely used energy crop in Germany and the area under maize grown for biogas production (biogas maize) was about 500’000 ha in 2010 (BMELV 2011).

Maize for biogas production

For the choice of a maize cultivar, different criteria such as type of use (grain, silage, biogas), adequate maturity, yield potential, nutritional value, resistance to pests and diseases, lodging tolerance, and cold tolerance during early development are important (Eder 2010). With biogas maize being a relatively new type of use, the importance of these different criteria is still being discussed. Hitherto, mostly normal silage maize hybrids have been used for biogas production. The large area cultivated with biogas maize goes along with a high demand of hybrid seeds, making it worthwhile for seed companies to establish separate market segments with cultivars especially adapted for biogas production. However, this premises detailed knowledge about the requirements for biogas maize.

Since the cultivation of biogas maize should be as economic as possible without having negative impact on the environment, the energy output in relation to given inputs (e.g., acreage, labor, fuel, fertilizer, et.) needs to be maximized. This aspect also needs emphasis in view of the possible competition between agricultural production of energy and food. Since methane is the energy carrier in biogas, maximizing its volume produced per unit area is key to the success of biogas production from maize. Methane yield (MY) of a cultivar in m^3 ha^{-1} is the product of two components: (i) DMY in kg ha^{-1} and (ii) volume of methane produced per unit of dry matter in m^3 kg^{-1}, which subsequently is referred to as methane fermentation yield (MFY). Obviously, each of these two components may be considered for improving MY of biogas maize.

Breeding targets for improving methane yield

Improvement of MY might be accomplished by selecting for higher DMY. Under the agroclimatic conditions of Central Europe, later maturing genotypes are observed to have a longer vegetative development, resulting in larger leaf area, increased light interception and higher DMY. For maturity classification of the worldwide maize germplasm, the FAO system is often used. With this system, genotypes are assigned by numbers of three digits (100-900), whereby the first digit indicates the belonging to one of the nine major maturity groups, the second digit is used for further differentiation within the maturity group and the third gives the color of the kernels. Generally, an increase in 10 FAO units is accompanied by a delay in ripening of 1 to 2 days and a decrease in dry matter concentration (DMC) at harvest of 1 to 2%. Since for optimal preparation of silage DMC of 28 to 35 % are required (Barrière et al. 1997; Eder et al. 2009), the use of late maturing material is restricted in order to reach the minimum DMC standards. Up to a certain maturity level, later maturing genotypes were observed to have a higher DMY and MY potential and recommendations were so far to grow slightly (40 to 50 FAO units) later maturing cultivars for biogas maize compared to locally adapted silage maize cultivars (Eder and Eder 2009; Degenhardt 2005) in order to take advantage of the higher yield potential without risking to low DMC at harvest. Breeding of adapted but late maturing genotypes with faster dry down of the stover at the end of the season can help to overcome the problem of too low DMC at harvest experienced with late maturing material. Schmidt (2003) hypothesized that DMY of maize can be significantly increased by exploiting late-maturing material and introgressing short-day genes from exotic populations into adapted material.

For MFY, results from earlier studies on silage maize have been reported to range from 195 to 700 l kg^{-1}, while the majority of the studies was within the range between 300 and 400 l kg^{-1} (Eder 2010). Since the different chemical components (e.g., carbohydrates, protein, fat, and lignin) differ for their degradability and stoichiometric expected MFY (Buswell 1936; Baserga 1998), MFY of different substrates can be expected to depend on their chemical composition. Fat for example has a high theoretically expected MFY (Buswell 1936) and has been shown to be positively correlated with MFY (Amon et al. 2007). Protein too is accompanied with high theoretically expected MFY (Buswell 1936), but high concentrations are considered to inhibit bacterial growth in the fermenter, and different studies found no correlation with MFY (Eder 2010; Schittenhelm 2008). Carbohydrates show the lowest expected MFY (Buswell 1936) and degradability of the different carbohydrate fractions is expected to vary strongly: sugars and starch can be easily accessed by bacteria, whereby lignin is non-degradable (Lübken et al. 2010) and can additionally reduce the degradability of the cellulose and hemicelluloses from the cell wall matrix.

Prolonged vegetative growth period, as would result from the breeding strategy of Schmidt (2003) for increasing DMY, is usually also associated with reduced ear development. Consequently, late maturing genotypes would also show reduced contents of components that are easily degradable by bacteria like starch. In forage maize breeding, digestibility traits play a paramount role beside DMY (Barrière et al. 1997) and a certain proportion of grain is required to warrant a high digestibility of the whole-plant silage by ruminants (Johnson et al. 1999). This limits the use of late-maturing or exotic germplasm in forage maize. Since digestibility is negatively affected by late maturity, the same might also apply to MFY, the important quality criterion for biogas maize. If greater emphasis is placed on degradability and MFY of biogas maize plant material, maize cultivars developed for forage could also be used for biogas production. However, conditions in a biogas fermenter differ from those in an animal rumen: beside different microflora, the retention time of 60 to 90 days in a biogas fermenter (Weiland 2006) is much longer than in a ruminant, where material only stays for ~2 days (Hartnell and Satter 1979). Hence, digestibility traits might be of lower importance in biogas maize than in forage maize and more emphasis might be given to DMY than MFY. As a consequence, breeding programs for biogas maize may diverge from those for forage maize on the long run.

Yet, there is no consensus among breeders on both the ideotype of biogas maize and the relative weights that should be given to DMY and MFY in order to increase MY. This becomes obvious by looking at advertisements for biogas maize varieties of different seed companies: some put emphasis on digestibility and other quality aspects of their varieties, whereas others highlight the yield potential of their varieties. To answer the question regarding the importance of DMY vs. MFY for biogas maize, it is of outmost interest to assess the dependence of MFY from the chemical composition in the relevant materials and the correlation of MFY with the different agronomic and quality traits relevant for animal nutrition. Different studies examining these relationships have been performed (Eder 2010; Schittenhelm 2008; Oslaj et al. 2010), but these were based on a rather restricted set of genotypes (5 - 25), thus not allowing estimation of quantitative genetic parameters, which are needed to draw general conclusions and for the formulation of optimum breeding strategies.

High throughput assessment of quality traits

The measurement of MFY, the second component of MY, is difficult and resource intensive. So far, there is also no consensus on a standard procedure for its determination. Different discontinuous assays have been developed in which samples are kept for a defined time period (usually about 30-40 days) in small biogas fermenters and methane production is recorded during that period (Owen et al. 1979; Helffrich and Oechsner 2003; Kaiser and Gronauer 2007). Some of the fermentation assays can be partially automated, e.g., by automatic gas counters (Kaiser and Gronauer 2007). However, their limited capacity and the long time needed to perform the complete fermentation restrict their application on a large scale. For example in plant breeding, the annual number of plots evaluated by a large multi-national company can surpass millions. Thus, fast and cost effective methods are required for determination of MFY and related quality traits with adequate accuracy (proximity between the measured and the true value of the material) and precision (proximity of replicated measurements of the same material).

Near infrared spectroscopy (NIRS) has proven to be a powerful tool to determine chemical composition, digestibility and metabolizable energy in animal feeds (Roberts et al. 2004; Zimmer et al. 1990; Andrés et al. 2005b). Further, gas production and its kinetic parameters obtained from *in vitro* digestibility trials have been directly predicted by NIRS in several studies performed in animal nutrition (Andrés et al. 2005a; Kruse et al. 2008; Herrero et al. 1996). Because NIRS is rapid and can handle a large number of samples with fewer human and financial resources compared to standard reference assays, it is of interest to assess the potential of this technique for prediction of relevant biogas maize quality traits.

Objectives of this study

The goal of this research was to determine and examine relevant traits for breeding maize for biogas production in a broad, representative panel of maize inbred lines and their testcrosses, as needed for the formulation of optimum breeding strategies. Further, high throughput phenotyping methods like NIRS for determination of quality traits were to be developed and employed for a fast measurement of these traits. In particular, the objectives were

(1) determine MFY and its production kinetics as well as the chemical composition in a diverse core set of maize genotypes;
(2) examine the relationship of MFY and traits related to its kinetics with plant chemical composition and silage quality traits in a diverse core set;
(3) examine and compare the potential of NIRS and chemical composition for determination of traits related to methane production;
(4) examine a large population of 285 inbred lines and their 570 testcrosses with two testers of maize for agronomic and quality traits by NIRS;
(5) estimate variance components and heritabilities for agronomic and quality traits relevant to biogas production in inbred lines and testcrosses of maize;
(6) study correlations among traits as well as between testcross and inbred line performance; and
(7) draw conclusions for breeding maize as a substrate for biogas production.

References

AGEE-Stat, Arbeitsgruppe Erneuerbare Energien - Statistik (2011) Entwicklung der erneurbaren Energien in Deutschland im Jahr 2010. http://www.erneuerbare-energien.de/files/pdfs/allgemein/application/pdf/ee_in_deutschland_graf_tab.pdf. Accessed 25 July 2011

Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, Hrbek R, Friedel J, Pötsch E, Wagentristl H, Schreiner M, Zollitsch W (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 98:3204-3212

Andrés S, Calleja A, López S, González JS, Rodríguez PL, Giráldez FJ (2005a) Prediction of gas production kinetic parameters of forages by chemical composition and near infrared reflectance spectroscopy. Anim Feed Sci Technol 123-124 Part 1:487-499

Andrés S, Murray I, Calleja A, Giráldez FJ (2005b) Nutritive evaluation of forages by near infrared reflectance spectroscopy. J Near Infrared Spectrosc 13:301-311

Barrière Y, Argillier O, Michalet-Doreau B, Hébert Y, Guingo E, Giauffret C, Émile JC (1997) Relevant traits, genetic variation and breeding strategies in early silage maize. Agronomie 17:395-411

Baserga U (1998) Landwirtschaftliche Co-Vergärungs-Biogasanlagen. Tänikon, Switzerland, Eidgenössische Forschungsanstalt für Agrarwirtschaft und Landtechnik (FAT), Report No. 512, pp 11.

BMELV, Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz (2011) Pressemitteilung Nr. 045 des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz. http://www.bmelv.de/SharedDocs/Pressemitteilungen/2011/045-AI-EEGKongress.html. Accessed 25 July 2011

Buswell AM (1936) Anaerobic fermentations. Bulletin No. 32. Division of the State Water Survey. Univ. of Illionois.

DBFZ, Deutsches Biomasse Forschungszentrum (2011) Monitoring zur Wirkung des Erneuerbare-Energien-Gesetz (EEG) auf die Entwicklung der Stromerzeugung aus Biomasse. Zwischenbericht März 2011. http://www.dbfz.de/web/fileadmin/user_upload/Userupload_Neu/Stromerzeugung_aus_Biomasse_Zwischenbericht_Maerz_2011.pdf. Accessed 1 September 2011

Degenhardt H (2005) Optimierung des Biogasertrages durch angepasste Maissorten und richtiges Anbaumanagement. In: Proceedings of the First International Energy Farming Congress, Papenburg, Germany, March 2-4 2005.

Drescher B (2011) Einspeisung von Biomethan - Wettbewerbsfähigkeit gegenüber Erdgas. 3. Einbecker Energiepflanzenkolloquium, Einbeck, Germany, August 29-30 2011. http://www.kws.de/aw/KWS/germany/innovation/Energiepflanzen/~dvig/KWS_Energiepflanzen_Kolloquium/. Accessed 15.09.2011

Eder B (2010) Pflanzenbauliche Untersuchungen zum Einfluss von Genotyp und Anbauverfahren auf die Ertragsbildung und das Methanbildungspotenzial von Mais (*Zea mays* L.). Dissertation, Technische Universität München, Germany

Eder B, Eder J, Papst C, Darnhofer B, Mukengele M, Heuwinkel H, Oechnser H (2009) Welcher Maissortentyp zur Biogaserzeugung? In: Internationale Wissenschaftstagung Biogas Science, Erding, Germany, December 2-4 2009

Eder J, Eder B (2009) Silomais für die Biogasproduktion. Freising, Germany, Bayerische Landesanstalt für Landwirtschaft, Institut für Pflanzenbau und Pflanzenzüchtung, pp 8.

European Parliament and Council (2009) Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official Journal of the European Union 52:16-62

Fachverband Biogas e.V. (2011) Biogas Segment Statistics 2011. http://biogas.org/edcom/webfvb.nsf/id/DE_Branchenzahlen/$file/11-11-15_Biogas%20Branchenzahlen%202011_eng.pdf. Accessed 02.01.2012

Fachverband Biogas e.V. (2010) Stellungnahme des Fachverbandes Biogas e.V. zur öffentlichen Anhörung des Bundestagsausschusses für Ernährung, Landwirtschaft und Verbraucherschutz zum Thema „Landwirtschaft und Klimaschutz" am 22. Februar 2010, insbesondere auch in Bezug auf die Fragen 9, 15, 17, 24, 25, 27, 28 und 30 des Fragenkatalogs. http://biogas.org/edcom/webfvb.nsf/id/DE_Positionspapiere_FvB. Accessed 15.11.2011

FNR, Fachagentur Nachwachsende Rohstoffe e.V. (2011) Anbau nachwachsender Rohstoffe in Deutschland (ha) 2011 http://www.nachwachsenderohstoffe.de/service/daten-und-fakten/anbau/. Accessed 16.11.2011

Gerin PA, Vliegen F, Jossart JM (2008) Energy and CO_2 balance of maize and grass as energy crops for anaerobic digestion. Bioresour Technol 99:2620-2627

Hartnell GF, Satter LD (1979) Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. J Anim Sci 48:381-392

Helffrich D, Oechsner H (2003) Hohenheimer Biogasertragstest - Vergleich verschiedener Laborverfahren zur Vergärung von Biomasse. Landtechnik 58:148-149

Herrero M, Murray I, Fawcett RH, Dent JB (1996) Prediction of the *in vitro* gas production and chemical composition of kikuyu grass by near-infrared reflectance spectroscopy. Anim Feed Sci Technol 60:51-67

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? Trends in Ecology & Evolution 15:56-61

Johnson L, Harrison JH, Hunt C, Shinners K, Doggett CG, Sapienza D (1999) Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review. J Dairy Sci 82:2813-2825

Kaiser F, Gronauer A (2007) Methanproduktivität nachwachsender Rohstoffe in Biogasanlagen. Freising, Germany, Bayerische Landesanstalt für Landwirtschaft (LfL), pp 21.

Klinkert V, Bothendorf E, Urban W, Meyer-Prescher B, Berger R (2010) Studie zu den Auswirkungen der Biogaseinspeisung in das Erdgasnetz auf den Netzbetrieb und Endverbraucher. In Urban W (ed.) Gasnetze der Zukunft, Oberhausen, Germany.

Kruse S, Herrmann A, Loges R, Taube F (2008) Prediction of gas production kinetics of maize stover and ear by near infrared reflectance spectroscopy. J Anim Feed Sci 17:422-433

KWS Mais GmbH (2009) Biogas: Grundlagen der Gärbiologie. http://www.kws.de/go/id/cjhf/. Accessed 12.11.2011

Lashof DA, Ahuja DR (1990) Relative contributions of greenhouse gas emissions to global warming. Nature 344:529-531

Lehner B, Döll P, Alcamo J, Henrichs T, Kaspar F (2006) Estimating the impact of global change on flood and drought risks in Europe: A continental, integrated analysis. Clim Change 75:273-299

Lübken M, Gehring T, Wichern M (2010) Microbiological fermentation of lignocellulosic biomass: Current state and prospects of mathematical modeling. Appl Microbiol Biotechnol 85:1643-1652

Meehl GA, Washington WM, Collins WD, Arblaster JM, Hu A, Buja LE, Strand WG, Teng H (2005) How much more global warming and sea level rise? Science 307:1769-1772

Oslaj M, Mursec B, Vindis P (2010) Biogas production from maize hybrids. Biomass Bioenergy 34:1538-1545

Owen WF, Stuckey DC, Healy Jr JB (1979) Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res 13:485-492.

Roberts CA, Stuth J, Flinn P (2004) Analysis of Forages and Feedstuffs. In: Barbarick KA, Dick WA, Roberts CA, Workman J, Reeves JB, Al-Amoodi L (eds) Near-infrared spectroscopy in agriculture. Madison, Wisconsin, USA, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America Inc., pp 231-267

Schittenhelm S (2008) Chemical composition and methane yield of maize hybrids with contrasting maturity. Eur J Agron 29:72-79

Schmidt W (2003) Hybridmaiszüchtung bei der KWS SAAT AG. In: Bericht über die 54. Tagung 2003 der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, BAL Gumpenstein, pp 1-6

Shafiee S, Topal E (2009) When will fossil fuel reserves be diminished? Energy Policy 37:181-189

Tol RSJ (2005) The marginal damage costs of carbon dioxide emissions: An assessment of the uncertainties. Energy Policy 33:2064-2074

Weiland P (2006) Biomass digestion in agriculture: A successful pathway for the energy production and waste treatment in Germany. Eng Life Sci 6:302-309

Zimmer E, Gurrath PA, Paul C, Dhillon BS, Pollmer WG, Klein D (1990) Near infrared reflectance spectroscopy analysis of digestibility traits of maize stover. Euphytica 48:73-81

2. Publication 1: Kinetics of methane fermentation yield in biogas reactors: Genetic variation and association with chemical composition in maize

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Abstract

In Germany, maize (*Zea mays* L.) is the most frequently use substrate for methane production in biogas reactors. Methane yield per area is determined by methane fermentation yield per unit of dry matter (MFY), but still little information is available on the latter trait. Our objectives were to investigate the kinetics of MFY during fermentation of maize, estimate quantitative-genetic parameters for different traits related to MFY and examine the relationship of MFY with chemical composition and silage quality. For this purpose, we analyzed methane production from whole-plant material of 16 inbreds and their 32 testcrosses during 35 days of fermentation using a discontinuous laboratory assay. The plant material was also analyzed for chemical composition and *in vitro* digestible organic matter (IVDOM). At early fermentation stages (up to 5 days), significant genotypic variances and high heritabilities were observed, most probably caused by different concentrations of easily degradable chemical components. However, complete or partial degradation of all chemical components with progressing fermentation reduced genotypic variances and heritability of MFY at later fermentation stages. Correlations of MFY with chemical components were strong at early, but not at later fermentation stages. Hence, MFY at later stages does not seem to be amenable to selection, although it is closer to potential MFY. IVDOM could be used for preliminary or indirect selection due to its high heritability and strong correlation with MFY in testcrosses. Besides MFY, dry matter yield (DMY) is the second component of methane yield. Since DMY showed much larger genetic variation than MFY, more emphasis on breeding for DMY seems appropriate.

3. Publication 2: Determination of methane fermentation yield and its kinetics by near infrared spectroscopy and chemical composition in maize

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Abstract

Among substrates used in biogas reactors, maize (*Zea mays* L.) got the most emphasis in Germany and its use is still increasing. Methane fermentation yield (MFY), i.e. the amount of methane produced per unit of dry matter can be determined by laboratory assays, but these are costly and complex. Near infrared spectroscopy (NIRS) is already successfully used for fast and cost-effective examination of animal feeds. Thus, its employment for determination of MFY would be a valuable tool. The objectives of this study were to examine the potential of employing NIRS to predict MFY as measured in a discontinuous fermenter, investigate the reliability of prediction of parameters related to the kinetics of MFY and compare models based on NIRS with that on chemical composition for reliable prediction of MFY. Using a discontinuous fermentation assay, dried whole plant material samples, derived from 55 maize genotypes grown in six environments, were analyzed for their MFY after different fermentation times. Additionally, the samples were analyzed for their chemical composition and their NIR spectra were measured. For prediction of MFY and related traits, calibration models based on NIRS and chemical composition were developed. Prediction of MFY after short fermentation time (R^2 = 0.88 after 5 days) was better than after complete fermentation (R^2 = 0.77 after 35 days). Chemical composition models were always inferior to NIRS models what can be explained by the higher information content in the NIR spectra. Contrary to NIRS models, chemical models showed a strong decrease in performance to predict MFY with ongoing fermentation time. Our study showed the NIRS can be used for fast determination of MFY, making it a valuable tool in maize breeding, where large numbers of samples have to be analyzed within short time.

4. Publication 3: Breeding maize as biogas substrate in Central Europe: I. Quantitative-genetic parameters for testcross performance

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Abstract

The importance of biofuels is increasing worldwide. In Germany, maize (*Zea mays* L.) biomass is used for production of methane in biogas plants and its use has increased tremendously. 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. A diverse set of 570 TCs, derived by crossing 285 dent maize lines with two flint single-cross testers, was evaluated 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. General combining ability (GCA) showed to be the major source of variation and heritabilities were high to very high. Variation in MY was mainly determined by DMY, because MY showed almost perfect correlation with DMY, but not with MFY. Additionally, DMY showed larger heritability and coefficient of genetic variation than MFY, making DMY the primary selection target for improving MY. It can be expected that in the future genotypes bred for biogas production will diverge from those for forage production, because our study showed that quality traits seem to be of much lower importance in the former case.

5. Publication 4: 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

The amount of maize (*Zea mays* L.) being used for production of biogas (biogas maize) is steadily increasing, wherefore breeding maize for this type of use has recently gained importance. In addition to performance of testcrosses, optimization of hybrid breeding programs requires information about line per se performance (LP) of inbreds and its relation to their general combining ability (GCA). 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. A diverse set of 285 inbred lines was evaluated 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. Variation in MY was mainly determined by DMY, what confirmed observations based on GCA in a companion study. MFY revealed only low genotypic variation, showing moderate correlation with lignin but only weak correlation with starch. In consequence, selection of genotypes with high DMY and less focus on ear proportion should be favored for biogas maize. For maturity traits (days to silking, dry matter concentration) highest (≥0.94) genotypic correlations between LP and GCA [r_g (LP, GCA)] were observed, whereas these correlations were moderate (≥0.65) for DMY and MY. Multistage selection is recommended, whereby selecting for GCA of maturity traits, plant height, and to some extent also quality traits and DMY on the level of LP would be effective.

6. General Discussion

The design of optimum breeding strategies requires the knowledge on different quantitative genetic parameters, calculated based on genetically broad based populations (Hallauer et al. 2010). Since hybrid varieties are normally grown on farmers' fields in the case of maize, both, performance of inbred lines *per se* (LP) and performance of their testcross progenies (TP) have to be taken into account. Thus, information is required on the different sources of variation (genotype, genotype-by-environment interaction, error) and heritability (h^2) of relevant traits for LP and TP, the correlations among these traits within LP and TP, and the correlation between LP and TP. For traits with presence of non-additive gene action or epistasis, performance of a specific hybrid is not only determined by the average contribution of each parental line, referred to as general combining ability (GCA), but also by an interaction effect between these two specific parental lines, generally referred to as specific combining ability (SCA) (Falconer and Mackay 1996). Hence, in order to assess the contribution of GCA and SCA to the genotypic variation in the testcrosses, these have further to be produced by mating the inbred lines under study with at least two different testers. For reliable estimation of quantitative genetic parameters and separation of sources of variance (genotype, environment), the number of genotypes has to be sufficiently large and the evaluation should be conducted in different environments. Hence, in this study, we examined a large set of 285 inbred lines of the dent heterotic pool as well as their testcross progenies derived from mating the inbred lines with two different flint single-cross testers during two years at three locations. In total, this resulted in a large experiment with 10'800 double-rowed field plots with an area of more than 8 ha.

Regarding the acquisition of genotypic data, the number of genetic markers and genotypes that can be assessed by given resources has markedly increased during the past years (Gupta et al. 2008) and calls for similar improvements for assessing phenotypic data. Development and employment of fast phenotyping methods is inevitable in order to provide the necessary phenotypic data for large association studies or breeding programs. Due to the large size, the experiments conducted within this thesis also called for the application of fast phenotyping methods for chemical composition and methane production of harvested plant material.

Phenotyping for methane production

Determination of chemical composition by measuring NIR spectra of the dried, ground sample material (laboratory NIRS) is nowadays routinely performed in plant breeding trials (Montes 2006). Successful development of laboratory NIRS calibrations has been reported for concentrations of protein, starch, fat, sugars, and different fiber fractions (Welle et al. 2003; Roberts et al. 2004; Míka et al. 2003; Amari and Abe 1997; Zimmer et al. 1990). Also for different biological parameters from the area of animal nutrition like metabolizable energy concentration (de Boever et al. 1995; Zijlstra et al. 2011), *in vitro* digestibility of organic matter (Barber et al. 1990; Van Waes et al. 1997; Andrés et al. 2005), or *in vitro* gas production after short fermentation time (Andrés et al. 2005; Herrero et al. 1996; Kruse et al. 2008), prediction by laboratory NIRS is possible. Hence, determination of the biological parameter MFY by laboratory NIRS is also desirable and we have assessed its potential within this thesis.

Analysis of methane production in a NIRS calibration set

A core set of 320 field plot samples from genotypes with diverse agronomic properties (maturity, yield) was analyzed for its chemical composition and MFY. To better understand the biology of MFY and to make results comparable to gas production assays from animal nutrition, which generally use shorter fermentation times (Menke et al. 1979; Theodorou et al. 1994), we also examined the temporal development of MFY during the 35 days of fermentation by different non-linear regression models (Grieder et al. 2012). This procedure allowed for interpolating the gas production curves and comparing MFY of different samples after precisely defined fermentation periods (e.g., 3 days, 5 days after start of fermentation), whereby the MFY commonly reported in literature would correspond to MFY after complete fermentation (35 days).

Mean MFY after 35 days fermentation of 327 l (kg OTS)$^{-1}$ in inbred lines and 337 l (kg OTS)$^{-1}$ in testcrosses of the core set was in agreement with earlier studies on maize (Schumacher 2008; Kaiser et al. 2004; Schittenhelm 2008; Eder 2010). For MFY after short fermentation time of 3 to 5 days, as generally employed for gas production assays in animal nutrition, high h^2 indicated a large genotypic variation in relation to the error of the reference method (Grieder et al. 2012). However, owing to the decreasing genotypic variance and, in the case of inbred lines, the increasing error variance, h^2 for MFY decreased towards day 35. This indicated that after complete fermentation, differences for MFY among genotypes in the core set were only small, although significant variation was observed for concentration of different chemical components (Grieder et al. 2012).

After short fermentation time (3-5 days), the situation may be comparable to a ruminant and only the easily accessible chemical components can be converted into biogas by the microbial community. Thus, genotypes that differ for their chemical composition also show variation in MFY. However, degradation of all chemical components with ongoing fermentation time reduces the genotypic variation in MFY, as it was indicated by decreasing correlation coefficients between MFY and single chemical components from 5 till 35 days after start of fermentation (Grieder et al. 2012). Low variation in MFY, which is caused by leveling of differences among genotypes due to the long fermentation time, complicates the development of successful NIRS calibrations and was seen as the main reason by Darnhofer et al. (2009) for unsatisfactory performance of NIRS to predict MFY. In order to be successful in calibration development, given a true relationship between variation in spectra and target traits is present, precision of the reference values entering the calibration must be maximized. This can be achieved on the one hand by increasing the number of laboratory replications per reference data point and on the other hand by optimizing the precision of the reference assay.

Development of NIRS calibrations

Owing to non-significant variation between field-replications of the same genotype, which was lower than the measurement error, values of field plot samples were averaged over field-replications in order to get more precise estimates (Grieder et al. 2011a). Regarding the reference assay for determination of MFY, the Hohenheim Biogas Yield Test (HBT), no modifications could be performed to increase precision of the measurements due to external accomplishment. Three aspects are making the HBT different from other assays for determination of MFY (Eder 2010): the low amount of sample material (300 mg), the fact that fresh material is not ensiled but just dried at 55 °C, and the fine grinding of the material to 1 mm. Low sample amounts bear the risk of errors while sampling (amount, representativeness). Increasing the sample amount in the HBT can reduce the sampling error (Mittweg et al. 2012), but an upper limit is given because the inoculum:test-substrate ratio (in terms of ODM) should not get below 2:1 according to VDI-guideline 4630, in order to avoid inhibitory effects during fermentation. Further, electrostatic effects may influence the sampling and require standardized procedures (Mittweg et al. 2012). Since preparation of silage bears the risk of different additional error sources, simple drying of the fresh material, as performed with the HBT, is accompanied with higher repeatability of MFY measurements, especially when comparing materials with very diverse DMC (Eder 2010; Mittweg et al. 2012; Mukengele and Oechsner 2007), as present in this thesis. Fine grinding as done with the HBT, bears the risk of strong disintegration of the material and, therefore, too high MFY. However, grinding to 1mm was generally observed not to affect the integrity of the cell walls and particle size is assumed to have no influence on MFY (Darnhofer et al. 2009; Helffrich and Oechnser 2003).

In harmony with estimates of h^2 (Grieder et al. 2012), performance of NIRS to predict MFY after short fermentation time of 3 days (R^2 = 0.85) was higher compared to MFY after complete fermentation during 35 days (R^2 = 0.77) (Grieder et al. 2011a). While results for short fermentation are comparable to results obtained from fermentation studies in the area of animal nutrition (Andrés et al. 2005; Herrero et al. 1996), results for complete fermentation were better than in a recent study (Darnhofer et al. 2009). Although the NIRS-performance criteria of a minimum ratio performance deviation of 2.5 (RPD, calculated as the standard deviation in the reference samples divided by the standard error of NIRS-prediction) and minimum R^2 of 0.80 (Wiliams and Sobering 1993; Wiedower et al. 2009) were not completely met by MFY after complete fermentation (RPD = 2.1, R^2 = 0.77), this calibration can be regarded as satisfactory. Since MFY after 35 days is the most important parameter, this trait could successfully be predicted in the main experiment within this thesis.

Transferability from laboratory assays to practical conditions

As described by Helffrich and Oechsner (2003), discontinuous biogas assays are used to determine the potential methane production from a substrate, wherefore MFY values determined by the HBT have to be seen as the maximum attainable under optimum conditions (Schumacher 2008). When trying to transfer results from these discontinuous batch assays to small scale continuous fermenter systems or to commercial biogas plant conditions, usually a deduction in MFY has to be performed due to scouring of organic material or permanent production of acids with inhibitory effects on methanogenesis in the continuous systems (Schlattmann et al. 2004). Different factors (procedural parameters like hydraulic retention time, technical setup of the plant, etc.) can further influence the MFY attainable under practical conditions, which is assumed to be 10 to 15% lower than indicated by guideline values derived from discontinuous fermentation assays (Schlattmann et al. 2004; Schwab and Reinhold 2006).

Alternatively, the examination of the methane production kinetics of the discontinuous assay (Grieder et al. 2012) might be used to find better predictors of the MFY attainable under practical conditions with a continuous flow reactor. For example the average time needed to reach 90% (100% - deduction of 10% as explained above) of the final MFY of a sample was around 9 days after start of fermentation. Thus, MFY of a substrate obtained after 9 days of fermentation might be a more realistic estimator for the MFY attainable with a larger scale continuous fermenter. However, this would require further investigations and analysis of the same material with the HBT and a continuous assay in order to find the fermentation time with the HBT that is most informative for MFY obtained by the continuous fermenter.

Ideotype of biogas maize

Application of the developed phenotyping platforms to the main field experiments (10’800 field plots) allowed for the detailed examination of variation of the different agronomic and quality traits as well as the correlations among them. Our main emphasis hereby was on the comparison of biogas maize with normal forage maize and possible implications for biogas maize breeding. In particular, the question whether biomass quality aspects are of the same importance in biogas maize as in forage maize was of main interest.

At first, the correlation of MY with its two components was examined. In agreement with earlier studies (Schumacher 2008; Eder 2010; Oechsner et al. 2003; Böhmel and Claupein 2007), this revealed a very strong dependence of MY on DMY, whereas MFY was of lower importance. Hence, biomass production of the cultivar seems to be the most important component of MY (Grieder et al. 2011b; Grieder et al. 2011c). However, also in forage maize, the target trait metabolizable energy yield (MEY) is strongly determined by DMY (Geiger et al. 1992), but nevertheless, metabolizable energy concentration (MEC) is of high relevance in forage maize breeding. The reason here for is that for ruminants, feed intake is restricted (e.g., to approx. 20-25 kg DM d^{-1} for a lactating dairy cow (Allen

2000)) and the animals are therefore dependent on a high MEC in the feed to meet their energy needs. Since Mistele et al. (1994) determined increasing economic weights of MEC for cows with higher average milk yield, this especially holds true for ruminants with a high performance. On the contrary, "feed intake" of a biogas plant is not that strongly restricted as for a ruminant and due to the long retention times of 60 to 90 days in a biogas fermenter (Weiland 2006), degradation of the material is much stronger. Even in the diverse set of testcrosses examined (Grieder et al. 2011b), the genotypic coefficient of variation (CV_g) for MFY was only small (1%). In commercial biogas plants, a mixture of different substrates with varying MFY is fermented. The MFY of cattle slurry, which is applied in most commercial plants (DBFZ 2011) is around 180 l (kg ODM)$^{-1}$ (KTBL 2010) and therefore significantly lower than that of maize. Hence, methane production of the biogas plant can be expected to depend much more on the mixture of the different substrates than on the MFY of the maize component, again reducing the importance of MFY in maize.

Additionally, the two main quality parameters for biogas and forage maize, i.e., MFY and MEC, respectively, may be compared. MEC was found to depend much stronger on the chemical composition than MFY, i.e., positive correlations of MEC with the easily degradable components starch and fat and negative correlations with different fiber fractions were more pronounced than for MFY (Grieder et al. 2011b). Starch is the main component of the ear and the lack of correlation between starch and MFY indicated that, in contrary to forage maize, the ear proportion is only of minor importance. Owing to a later change to generative growth, late maturing genotypes were characterized by lower starch and higher sugar and fiber concentrations (Grieder et al. 2011b), most likely caused by lower ear proportion. This might have a potential negative effect on MFY. However, shorter generative growth is also accompanied with decreased lignification and, therefore, increased digestibility of the stover (Eder 2010), having a potentially positive influence on MFY. Obviously, these two opposite effects balance each other quite well, because later flowering was observed to be only weakly negatively correlated with MFY in the experiments of this thesis (Grieder et al. 2011b). Contrary, MEC showed strong negative correlation with DTS, again highlighting the importance of the availability of easily degradable starch from the ear.

Breeding goals for biogas maize

From the correlations among traits as discussed above, we conclude (Grieder et al. 2011b) that biogas maize and forage maize are two types of use that clearly differentiate and require on the long run separate breeding programs. The lower importance of a high cob proportion in biogas maize relaxes the restrictions that apply to forage maize. Hence, a faster selection gain for DMY, the component that most strongly determines the target trait MY, can be attained.

Late maturity to increase yield potential

For Central European conditions, different studies showed an increasing DMY potential of hybrids by delaying maturity to, at least, a certain level (Eder 2010; Grieder et al. 2011b), whereas others could not confirm a higher yield potential of later maturing varieties (Schittenhelm 2008; Böhmel and Claupein 2007; Gröblinghoff et al. 2005; Amon et al. 2007). One factor influencing these opposing results can be the mix-up of late maturity and non-adaptiveness. Late maturing hybrid varieties with FAO values of 300 and higher are generally developed for Southern Europe (Frei 2000), which has more favorable climatic conditions compared to Central-Europe like Germany, where genotypes with FAO numbers <300 are normally grown for forage maize production. Furthermore, late maturing hybrids adapted to Southern Europe are mostly of the dent×dent type, whereas north of the alps, dent×flint hybrids are generally grown due to the better cold tolerance of the flint component (Frei 2000). If late maturing but non-adapted varieties are grown for biogas production, their reduced early vigor and cold tolerance (Gröblinghoff et al. 2005) can lead to reduced growth and their advantage of longer vegetative growth will only be realized late in the season. For example Eder (2010) observed late maturing varieties (FAO 260 - 400) and very late maturing varieties (> FAO 400) to outperform the adapted reference variety in terms of biomass just after mid of September and beginning of October, respectively.

For the dent inbred lines examined within this thesis, late maturity was also associated with reduced early growth for LP and TP, but the late maturing genotypes were still associated with higher DMY (Strigens et al. 2011). Since variation for early growth and cold tolerance is present within the dent and flint heterotic pools (Strigens et al. 2011; Peter et al. 2009; Presterl et al. 2007; Rodríguez et al. 2010), the combination of a long vegetative growth with high growth rates might result in genotypes with even better DMY performance (Strigens et al. 2011).

Limitations to maturity

For optimal silage preparation, the DMC of the harvested material should range between 280 and 350 g kg^{-1} (Barrière et al. 1997; Eder et al. 2009). Whereas higher DMC values will lead to problems in compacting the material, lower values will lead to formation of leachate that is accompanied by a loss of energy. Thus, a sufficient dry down of the material at harvest time is required. Length of the vegetation phase depends on the regional conditions and genotypes with appropriate maturity have to be grown. If maturity is too late, sufficient DMC will be reached only very late in the season (late October, early November), when climatic conditions might already be unfavorable (e.g., wet soils, frost events), hampering machine harvest in the field. In order to avoid the risk of unfavorable harvesting conditions, maturity of the genotype has to be chosen accordingly.

However, even if restrictions are imposed by the maturity of the material, DMY remains the dominant factor determining MY. Also when dividing the complete set of testcrosses into narrow DMC groups (each covering a range of 10 g kg^{-1}), MY always showed a very strong association with DMY, but not with MFY. Thus, breeding of biogas maize should focus on increasing DMY by combining good early growth and cold tolerance with an adequately later maturity and fast dry-down of the stover at the end of the season. Consequently, our results largely confirm the strategy of Schmidt (2003), who proposed to change to later maturity groups, improve cold tolerance, introgress short-days genes, and optimize maize for a C3/C4 crop rotation.

Yield limiting conditions

Besides the length of vegetation period as discussed above, other factors like water or nutrient supply might also limit the biomass production potential. For regular yield levels (~20 Mg ha^{-1}), maize requires approximately 500-800 mm of water (Critchley et al. 1991) and even larger amounts of water can be expected to be required for high biomass yielding genotypes. Annual precipitation in certain parts of Germany (e.g. Eastern Germany) is as low as 400 mm (KIT 2011) and in these regions, water availability might restrict the cultivation of high biomass yielding varieties. Since additional irrigation would reduce the economic as well as ecological efficiency of biogas maize cultivation, limits are given to DMY for increasing MY in this case and MFY might gain in importance. By dividing the genotypic means of testcross performance into narrow DMY groups (each covering a range of 1 Mg ha^{-1}), Grieder et al. (2011b) observed a stronger relationship between MFY and MY within these groups. For example within the DMY groups 19-20, 20-21 and 21-22 Mg ha^{-1}, these two traits were positively correlated and a MFY increase of 1 l (kg ODM)$^{-1}$ was associated with an increase in MY of 24, 20, and 21 m^{3} ha^{-1}, respectively. Hence, if limits are given to the yield potential, it will be worthwhile to grow genotypes that exhibit a maximum MFY at this DMY level. Owing to the fact that lignin concentration in the whole plant, but also the lignin : NDF ratio were among the quality traits showing the strongest (negative) correlation with MFY (Grieder et al. 2011b), normal silage maize hybrids that exhibit a good digestibility of the stover (e.g. low lignin : NDF ratio) might be chosen in this case.

Alternatively, other crops for biogas production can be grown in such regions. Sorghum (*Sorghum bicolor, Sorghum sudanese*) is generally known to have a higher water use efficiency than maize (Zacharias 2011). Thus, adoption of sorghum in water limited regions would be a further alternative to increase DMY and, therefore, methane yields per hectare, given regional climatic conditions support growth of the not yet widely chilling tolerant (Yu et al. 2004) sorghum plant.

Adaption to crop rotation

Continuous monocropping with maize and removal of all organic material is associated with negative effects like reduced soil organic matter (Nardi et al. 2004), higher pest and disease incidence (Seran and Brintha 2010) and loss of nutrients (nitrogen) in the case of open soils during winter (Böhmel 2007). Further, continuous monocropping reduces biodiversity and policies therefore favor the utilization of a variety of co-substrates in addition to maize for biogas production (Böhmel and Claupein 2007). For example with the latest amendment to the renewable energy sources act in Germany, the application of maize in biogas plants is capped to 60% of the substrate (Fachverband Biogas e.V. 2011). Although from a purely economical point of view, monocropping of maize would be most efficient (Böhmel and Claupein 2007; Hubert et al. 2011), the different concerns and legal restrictions named above favor an efficient crop rotation. The goal of a certain crop rotation is to combine a high productivity per area with a high sustainability of the cropping system (Böhmel 2007). Between two subsequent years of maize as main crop, catch crops like winter rye can be sown to reduce nitrogen losses and soil erosion. Further, maize can be followed by other main crops like sorghum, sunflower, Jerusalem artichoke, winter wheat, pasture or other crops in the subsequent year.

Since the goal is to increase DMY or MY of the complete cropping system, requirements on biogas maize might again be different. For example in the study of Böhmel and Claupein (2007), DMY of the cropping system was higher for early maturing maize with short growth period compared to late maturing maize with longer growth period (earlier sowing, later harvest) due to higher yields of the catch crop in the former case. However, in this study late maturing genotypes had very high FAO numbers up to 700. Possible non-adaption of these late maturing genotypes to the test location (Ihinger Hof, also employed as testing region in the study of Grieder et al. (2011b, c)) might be responsible for the lack of responsiveness in DMY with longer maize growth. Whereas Böhmel and Claupein (2007) harvested their long duration maize from end of October till early November, even the late maturing flint×dent testcrosses with low ear proportion but high stover yield in the study of Grieder et al. (2011b) could be harvested between early September in the warm location Eckartsweier and early October in the cool location Ihinger Hof. Thus, different climatic conditions among environments require provision of specifically adapted material. Whereas in less favorable locations like Ihinger Hof, the reduced growth might require earlier maturing genotypes, later maturing, DMY accentuated biogas maize genotypes might profit from more favorable locations like Eckartsweier or Hohenheim without impairing on the yield level of the catch crop. For the varying conditions at the local level, choice of the appropriate maize variety within the crop rotation will depend on the experience of the farmer. However, this requires availability of suitable maize varieties on the market bred for biogas production.

Breeding and production of biogas maize

Since hybrid varieties are grown commercially, determination of the ideotype of biogas maize requires variance components of the different traits and correlations among them for testcross performance. However, hybrids are produced from inbred lines and in order to optimize breeding programs, knowledge on variation of the traits, correlations among them and between LP and TP are also of interest. Detailed results and discussion of TP, LP and their correlation is given by Grieder et al. (2011b) and Grieder et al. (2011c).

In breeding programs, large numbers of inbred lines are produced every year. Because evaluation of all inbred lines in testcrosses would not be feasible, their number has to be reduced prior to production of testcross progenies. Prediction of testcross performance from the inbred line's genotype has recently been shown to bear certain potential (Riedelsheimer et al. 2012) and may be used to reduce the number of possible candidate lines in a first step. However, this requires yet still expensive genotyping, wherefore selection of inbred lines based on LP of some easily assessable traits might still be more efficient. Pre-selection of inbred lines based on LP is efficient if it results in an adequate response for their GCA in testcrosses. The relative efficiency (*RE*) of indirect selection can be used as a criterion to judge whether for a certain trait selection on LP is worthwhile. *RE* gives the ratio of the indirect response to selection in GCA of inbred lines, if selection is performed for on LP, 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* is calculated as

$$RE = \frac{i_{LP} h_{LP} r_{g(}(LP, GCA)}{i_{GCA} h_{GCA}},$$

where i denotes the selection intensity, h the square root of heritability, and $r_g(LP,GCA)$ the genotypic correlation between LP and GCA. Besides the ratios i_{LP}:i_{GCA} and h_{LP}:h_{GCA} that are discussed in Grieder et al. (2011c), *RE* is mainly determined by $r_g(LP,GCA)$. Thus, with increasing correlation between LP and GCA, indirect selection for LP is getting more attractive.

Owing to high *RE* for maturity traits (DMC, DTS), plant height and quality traits, selection for GCA on the basis of LP might be promising for these traits in biogas maize (Grieder et al. 2011c). Lower *RE* for DMY and MY showed a lower potential to select for these traits on the basis of LP and laborious harvest of the inbred lines might not be rewarding. Thus, testing and selection for DMY would have to be performed within the testcrosses. However, in the experiments of this thesis a simple visual scoring of the DMY potential (scale from 1 = very poor to 9 = very good performance) showed good genotypic correlation with DMY, which were as high as 0.64 for LP and 0.82 for GCA in testcrosses. Since in a breeding program selection intensities (i) are commonly higher for selection in LP than selection in testcrosses ($i_{LP} > i_{GCA}$), the use of this visually determined yield potential in LP might, nevertheless, be rewarding to reduce the number of inbred lines entering the testcross stage.

With the time span from sowing to silking ranging up to 120 days, the late maturing inbreds examined by Grieder et al. (2011c) would not be able to produce mature seeds within the target environment. Thus, if such late maturing inbred lines would be used in a biogas maize breeding program, propagation of the line and hybrid seed development would not be possible within the target environment. Thus, evaluation of the inbred lines for seed yield and seed quality needs additionally to be selected in the environment designated for seed production.

Conclusion and outlook

The application of NIRS showed potential for prediction of MFY. However, the calibrations were based on spectra taken from dried, ground material, which still requires elaborate sampling preparation. Further, the results are specific to the Hohenheim Biogas Yield Test and comparison among results obtained with different fermenter types is not easy. Since the variation in MFY in the examined material was low, application of NIRS to predict MFY in large scale breeding trials may not be rewarding. However, by applying the NIRS model within this study, we could show that quality requirements on biogas maize are different from those on silage maize for use as forage. Because the market segment for biogas maize is large, breeding companies are already supplying this segment with special varieties. Since fewer restrictions apply to biogas maize, faster improvement in DMY will be possible for biogas maize germplasm, which can be expected to show stronger segregation from forage maize in the future. A broad range of maize varieties with either more emphasis on earliness and quality or on DMY will thus be available. It is up to the farmer to choose the variety fitting best the regional climatic conditions, his cropping system, and the type of use (pure biogas maize, mixed forage and biogas maize) on his farm.

References

Allen MS (2000) Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci 83:1598-1624

Amari M, Abe A (1997) Application of near infrared reflectance spectroscopy to forage analysis and prediction of TDN contents. Jpn Agric Res Q 31:55-63

Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, Hrbek R, Friedel J, Pötsch E, Wagentristl H, Schreiner M, Zollitsch W (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technol 98:3204-3212

Andrés S, Calleja A, López S, González JS, Rodríguez PL, Giráldez FJ (2005) Prediction of gas production kinetic parameters of forages by chemical composition and near infrared reflectance spectroscopy. Anim Feed Sci Technol 123-124 Part 1:487-499

Barber GD, Givens DI, Kridis MS, Offer NW, Murray I (1990) Prediction of the organic matter digestibility of grass silage. Anim Feed Sci Technol 28:115-128

Barrière Y, Argillier O, Michalet-Doreau B, Hébert Y, Guingo E, Giauffret C, Émile JC (1997) Relevant traits, genetic variation and breeding strategies in early silage maize. Agronomie 17:395-411

Böhmel C (2007) Energiepflanzen für die Biogaserzeugung. In: Böhmel C (ed) Comparative performance of annual and perennial energy cropping systems under different management regimes, Dissertation, University of Hohenheim, Stuttgart, Germany, pp 45-64

Böhmel C, Claupein W (2007) High quality production of energy maize (*Zea mays* L.) in crop rotation systems – biomass and methane yields and environmental impacts. In: Böhmel C (ed) Comparative performance of annual and perennial energy cropping systems under different management regimes, Dissertation, University of Hohenheim, Stuttgart, Germany, pp 65-89

Critchley W, Siegert K, Chapman C (1991) Water harvesting - A manual for the design and construction of water harvesting schemes for plant production. Food and Agriculture Organization of the United Nations, Rome

Darnhofer B, Eder J, Oechsner H, Mukengele M (2009) Entwicklung einer NIRS Kalibration zur Bestimmung der Biogasausbeute von Mais. In: Internationale Wissenschaftstagung Biogas Science, Erding, Germany, December 2-4 2009, pp 345-354.

DBFZ, Deutsches Biomasse Forschungszentrum (2011) Monitoring zur Wirkung des Erneuerbare-Energien-Gesetz (EEG) auf die Entwicklung der Stromerzeugung aus Biomasse. Zwischenbericht März 2011. http://www.dbfz.de/web/fileadmin/user_upload/Userupload_Neu/Stromerzeugung_aus_Biomasse_Zwischenbericht_Maerz_2011.pdf. Accessed 1 September 2011

de Boever JL, Cottyn BG, Vanacker JM, Boucqué CV (1995) The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle. Anim Feed Sci Technol 51:243-253

Eder B (2010) Pflanzenbauliche Untersuchungen zum Einfluss von Genotyp und Anbauverfahren auf die Ertragsbildung und das Methanbildungspotenzial von Mais (*Zea mays* L.). Dissertation, Technische Universität München, Germany

Eder B, Eder J, Papst C, Darnhofer B, Mukengele M, Heuwinkel H, Oechnser H (2009) Welcher Maissortentyp zur Biogaserzeugung? In: Internationale Wissenschaftstagung Biogas Science, Erding, Germany, December 2-4 2009, pp 57-72.

Fachverband Biogas e.V. (2011) Pressemitteilung 27/11: Biogas 2012 - Neue Herausforderungen für die Branche. http://www.biogas.org/edcom/webfvb.nsf/id/DE_PM_27_11. Accessed 11 December 2011

Falconer DS, Mackay TF (1996) Introduction to quantitative genetics. Longman Group Limited, Harlow

Frei OM (2000) Changes in yield physiology of corn as a result of breeding in northern Europe. Maydica 45:173-183

Geiger HH, Seitz G, Melchinger AE, Schmidt GA (1992) Genotypic correlations in forage maize I. Relationships among yield and quality traits in hybrids. Maydica 37:95-99

Grieder C, Mittweg G, Dhillon BS, Montes JM, Orsini E, Melchinger AE (2011a) Determination of methane fermentation yield and its kinetics by near infrared spectrsocopy and chemical composition in maize. J Near Infrared Spectrosc 19:463-477

Grieder C, Schipprack W, Dhillon BS, Melchinger AE (2011b) Breeding maize for use as biogas substrate in Central Europe I: Estimation of quantitative genetic parameters in testcrosses. Theor Appl Genet (in press)

Grieder C, Dhillon BS, Schipprack W, Melchinger AE (2011c) Breeding maize as a biogas substrate in Central Europe: II. Quantitative-genetic parameters for inbred lines and correlations with testcross performance. Theor Appl Genet (in press)

Grieder C, Mittweg G, Dhillon BD, Montes JM, Orsini E, Melchinger AE (2012) Kinetics of methane fermentation yield in biogas reactors: Genetic variation and association with chemical composition in maize. Biomass Bioenerg 37:132-141

Gröblinghoff FF, Lüdtke-Entrup N, Block K (2005) Mais liefert viel Methan. Landwirtschaftliches Wochenblatt Westfalen Lippe 13:34-36

Gupta PK, Rustgi S, Mir RR (2008) Array-based high-throughput DNA markers for crop improvement. Heredity 101:5-18

Hallauer AR, Carena MJ, Miranda Filho JB (2010) Quantitative genetics in maize breeding. 3rd edn. Springer Science+Business Media, New York

Helffrich D, Oechnser H (2003) Hohenheimer Biogasertragstest - Vergleich verschiedener Laborverfahren zur Vergärung von Biomasse. Landtechnik 58:148-149

Herrero M, Murray I, Fawcett RH, Dent JB (1996) Prediction of the *in vitro* gas production and chemical composition of kikuyu grass by near-infrared reflectance spectroscopy. Anim Feed Sci Technol 60:51-67

Hubert S, Schwarz C, Habeck B, Hinners-Tobrägel L, Pekrun C (2011) Alternativen zum Monomaisanbau? Praxisnah 1/2011: 8-9

Kaiser F, Diepolder M, Eder J, Hartmann S, Prestele H, Gerlach R, Ziehfreund G, Gronauer A (2004) Ertragspotenziale verschiedener nachwachsender Rohstoffe in landwirtschaftlichen Biogasanlagen. Schriftenreihe Bayerische Landesanstalt für Landwirtschaft 13:43-55

KIT, Karlsruher Institut für Technologie (2011) Langjährige Mittelwerte des Niederschlags in Deutschland. http://imk-tornado.physik.uni-karlsruhe.de/~muehr/precip_mean_d.html. Accessed 15 December 2011

Kruse S, Herrmann A, Loges R, Taube F (2008) Prediction of gas production kinetics of maize stover and ear by near infrared reflectance spectroscopy. J Anim Feed Sci 17:422-433

KTBL (2010) Gasausbeute in landwirtschaftlichen Biogasanlagen. Kuratiorium für Technik und Bauwesen in der Landwirtschaft e.V., Darmstadt

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979) The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci 93:217-222

Míka V, Pozdíšek J, Tillmann P, Nerušil P, Buchgraber K, Gruber L (2003) Development of NIR calibration valid for two different grass sample collections. Czech J Anim Sci 48:419-424

Mistele M, Zeddies J, Utz HF, Melchinger AE (1994) Economic aspects of breeding for yield and quality traits in forage maize. 1. Determination of economic weights. Plant Breed 112:102-109

Mittweg G, Oechsner H, Hahn V, Lemmer A, Reinhardt-Harnisch A (2012) Evaluation of a laboratory batch method to determinate biogas and methane production potentials. Eng Life Sci (in review)

Montes JM (2006) Application of near-infrared spectroscopy in plant breeding programs. Dissertation, University of Hohenheim, Stuttgart, Germany.

Mukengele M, Oechsner H (2007) Einflusss der Silierung auf den spezifischen Methanertrag bei Mais. Landtechnik 62:20-21

Nardi S, Morari F, Berti A, Tosoni M, Giardini L (2004) Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. Eur J Agron 21:357-367

Oechsner H, Lemmer A, Neuberg C (2003) Feldfrüchte als Gärsubstrat in Biogasanlagen. Landtechnik 58:146-147

Peter R, Eschholz TW, Stamp P, Liedgens M (2009) Swiss Flint maize landraces - A rich pool of variability for early vigour in cool environments. Field Crops Res 110:157-166

Presterl T, Ouzunova M, Schmidt W, Möller EM, Röber FK, Knaak C, Ernst K, Westhoff P, Geiger HH (2007) Quantitative trait loci for early plant vigour of maize grown in chilly environments. Theor Appl Genet 114:1059-1070

Riedelsheimer C, Czedik-Eysenberg A, Grieder C, Lisec J, Technow F, Sulpice R, Altmann T, Stitt M, Willmitzer L, Melchinger AE (2012) Genomic and metabolic prediction of complex heterotic traits in hybrid maize. Nat Genet (in press)

Roberts CA, Stuth J, Flinn P (2004) Analysis of Forages and Feedstuffs. In: Barbarick KA, Dick WA, Roberts CA, Workman J, Reeves JB, Al-Amoodi L (eds) Near-infrared spectroscopy in agriculture. Madison, Wisconsin, USA, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America Inc., pp 231-267

Rodríguez VM, Romay MC, Ordás A, Revilla P (2010) Evaluation of European maize (*Zea mays* L.) germplasm under cold conditions. Genet Resour Crop Evol 57:329-335

Schittenhelm S (2008) Chemical composition and methane yield of maize hybrids with contrasting maturity. Eur J Agron 29:72-79

Schlattmann M, Speckmaier M, Gronauer A (2004) Biogas-Gärtests in verschiedenen Fermentertypen. Landtechnik 59:338-339

Schmidt W (2003) Hybridmaiszüchtung bei der KWS SAAT AG. In: Bericht über die 54. Tagung 2003 der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, BAL Gumpenstein, pp 1-6

Schumacher B (2008) Untersuchungen zur Aufbereitung und Umwandlung von Energiepflanzen in Biogas und Bioethanol. Dissertation, University of Hohenheim, Stuttgart, Germany

Schwab M, Reinhold G (2006) Biogaserträge aus Energiepflanzen - Eine kritische Bewertung des Datenpotentials In: 3. Norddeutsche Biogastagung, Hildesheim, Germany, May 19-21 2006

Seran TH, Brintha I (2010) Review on maize based intercropping. J Agron 9:135-145

Strigens A, Grieder C, Haussmann BIG, Melchinger AE (2011) Genetic variation for early growth of maize inbred lines, their testcrosses and it's relationship to final dry matter yield. Crop Sci (in press)

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48:185-197

Van Waes J, Carlier L, Van Waes C, Van Bockstaele E (1997) Evaluation of quality characteristics in official trials with silage maize varieties in Belgium. Neth J Agric Sci 45:277-289

Weiland P (2006) Biomass digestion in agriculture: A successful pathway for the energy production and waste treatment in Germany. Eng Life Sci 6:302-309

Welle R, Greten W, Rietmann B, Alley S, Sinnaeve G, Dardenne P (2003) Near-infrared spectroscopy on chopper to measure maize forage quality parameters online. Crop Sci 43:1407-1413

Wiedower E, Hansen R, Bissell H, Ouellette R, Kouba A, Stuth J, Rude B, Tolleson D (2009) Use of near infrared spectroscopy to discriminate between and predict the nutrient composition of different species and parts of bamboo: Application for studying giant panda foraging ecology. J Near Infrared Spectrosc 17:265-273

Wiliams PC, Sobering DC (1993) Comparison of commerical near infrred transmittance and reflectance instruments for analysis of whole grains and seeds. J Near Infrared Spectrosc 1:25-32

Yu J, Tuinstra MR, Claassen MM, Gordon WB, Witt MD (2004) Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Res 85:21-30

Zacharias A (2011) Innovation in der Sorghumzüchtung. 3. Einbecker Energiepflanzenkolloquium, Einbeck, Germany, August 29-30 2011. http://www.kws.de/aw/KWS/germany/innovation/Energiepflanzen/~dvig/KWS_Energiepflanzen_Kolloquium/. Accessed 15.09.2011

Zijlstra RT, Swift ML, Wang LF, Scott TA, Edney MJ (2011) Short communication: Near infrared reflectance spectroscopy accurately predicts the digestible energy content of barley for pigs. Can J Anim Sci 91:301-304

Zimmer E, Gurrath PA, Paul C, Dhillon BS, Pollmer WG, Klein D (1990) Near infrared reflectance spectroscopy analysis of digestibility traits of maize stover. Euphytica 48:73-81

7. Summary

The area of maize (*Zea mays* L.) grown for production of biogas has tremendously increased in Germany during the past decade. Thus, breeding companies have a keen interest to develop special varieties for this new market segment. A high methane yield per area (MY), which depends multiplicatively on dry matter yield (DMY) and methane fermentation yield (MFY), is required to ensure the efficiency of biogas maize cultivation. However, information on the targeted biogas maize ideotype is still missing and estimates of relevant quantitative genetic parameters for representative material are required to design optimum breeding strategies.

We conducted a large field experiment to assess the relevant traits in biogas maize, their variation, and associations among them. In detail, our objectives were to (1) determine MFY and its production kinetics as well as the chemical composition, (2) examine the relationship of MFY and traits related to its kinetics with plant chemical composition and silage quality traits like *in vitro* digestible organic matter (IVDOM) and metabolizable energy concentration (MEC); (3) examine the potential of near infrared spectroscopy (NIRS) for prediction of traits related to methane production; (4) evaluate a large population of inbred lines and their testcrosses under field conditions for agronomic and quality traits; (5) estimate variance components and heritabilities (h^2) of traits relevant to biogas production; (6) study correlations among traits as well as between inbred line *per se* (LP) and testcross performance (TP); and (7) draw conclusions for breeding maize as a substrate for biogas production. For this purpose, a representative set of 285 dent inbred lines from diverse origins and their 570 testcross progenies with two adapted flint testers was produced. Both material groups were evaluated in field experiments conducted in six environments (three locations, two years) in Germany.

For analysis of MFY, samples of a diverse core set of 16 inbred lines and their 32 testcrosses were analyzed using the Hohenheim Biogas Yield Test, a discontinuous, laboratory fermentation assay. The kinetics of methane production was assessed by non-linear regression. Estimates of h^2 for MFY measured after short fermentation time (3 days) were high, but genotypic variance (σ^2_g) and, therefore, also h^2 decreased towards the end of the fermentation period (35 days). This was presumably the consequence of a nearly complete degradation of all chemical components during the long fermentation period. This interpretation was supported by strong correlations of MFY with chemical components, IVDOM and MEC for the early, but not the late fermentation stages.

Based on the samples in the core set, NIRS calibrations were developed for MFY, parameters related to the kinetics of methane production, and chemical composition. With a coefficient of determination from validation (R^2_V) of 0.82, accuracy of prediction was sufficiently high for the maximum methane production rate, which is related to the early fermentation phase, but not satisfactory for the time needed to reach 95% of a sample's final MFY ($R^2_V = 0.51$). In agreement with the trend of h^2, performance of NIRS to predict MFY on day 35 ($R^2_V = 0.77$) was lower than for MFY on day 3 ($R^2_V = 0.85$), but still at a satisfactory level, as was the case for concentrations of different chemical components. Hence, NIRS proved to be a powerful tool for prediction of MFY and chemical composition in the main experiment.

For TP, estimates of variance components from the main experiments revealed that general combining ability (GCA) was the major source of variation. The very tight correlation of MY with DMY but not with MFY indicated that variation in MY was primarily attributable to differences in DMY. Compared to MEC, MFY showed a weaker association with chemical composition. Genotypic correlation (r_g) of MFY was strongest with non-degradable lignin (-0.58). Correlation of MFY with starch was not significant and indicated a lower importance of high cob proportions for biogas maize than for forage maize. Hence, to improve MY, selection should primarily focus on increasing DMY. Results for LP in the main experiment largely confirmed results from testcrosses and favor selection for high dry matter yielding genotypes with less emphasis on ear proportion. Estimates of r_g between LP and GCA were highest (≥ 0.94) for maturity traits (days to silking, dry matter concentration) and moderate (≥ 0.65) for DMY and MY. Indirect selection for GCA on basis of LP looks promising for maturity traits, plant height, and to some extent also for DMY.

Our study revealed that biogas maize and forage maize are two types of use that clearly differ from each other. Forage maize requires varieties with high cob and starch proportions to meet the ruminant's need of easily accessible energy, whereas restrictions in terms of quality are much lower for biogas maize. This enables faster breeding progress in terms of DMY and MY for biogas maize. Longer vegetative growth by introgression of later maturing or exotic material can be used to increase DMY, as indicated by the positive correlation of time to flowering with DMY and the highest yield potential of testcrosses from exotic dent inbred lines. From our results, we conclude that varieties bred for biogas and forage maize can be expected to diverge more clearly in the future.

8. Zusammenfassung

In den letzten Jahren hat die Anbaufläche von Mais (*Zea mays* L.) zur Biogasproduktion in Deutschland stark zugenommen. Für Saatzuchtfirmen lohnt es sich deshalb, dieses Marktsegment mit speziell dafür entwickelten Sorten zu bedienen. Für einen effizienten Biogasmaisanbau muss der Methanertrag pro Fläche, welcher sich aus dem Trockenmasseertrag (TME) und der Methanausbeute zusammensetzt, möglichst hoch sein. Bislang ist der anzustrebende Biogasmais-Idealtyp jedoch noch offen und Schätzwerte für diverse quantitativ-genetische Parameter aus repräsentativem Zuchtmaterial werden benötigt, um effiziente Züchtungsstrategien zu formulieren.

Untersuchungsgegenstände der vorliegenden Arbeit waren: (1) die Bestimmung der Methanausbeute, deren Produktionskinetik sowie verschiedener relevanter Inhaltsstoffe; (2) die Assoziation dieser Parameter mit Inhaltsstoffen und Silomais-Qualitätsparametern wie *in vitro* verdauliche organische Substanz (IVDOM) und umsetzbare Energie (ME); (3) die Erforschung der Einsatzmöglichkeiten von Nah-Infrarot Spektroskopie (NIRS) zur Vorhersage der Methanausbeute und verwandter Merkmale; (4) die Evaluation von Inzuchtlinien und deren Testkreuzungsnachkommen bezüglich agronomischer Eigenschaften und Qualitätsmerkmalen; (5) die Schätzung von Varianzkomponenten und Heritabilität (h^2) der für die Biogasproduktion relevanten Merkmale; (6) die Schätzung der Korrelationen zwischen Merkmalen sowie zwischen der Eigenleistung der Inzuchtlinien (LP) und deren Testkreuzungs-Leistung (TP); und (7) Schlussfolgerungen für die Züchtung von Biogasmais. Zu diesem Zweck wurde ein repräsentativer Satz von 285 Dent-Inzuchtlinien verschiedener Herkunft (Europa, US Corn Belt, tropisch) sowie deren 570 Testkreuzungsnachkommen mit zwei adaptierten Flint-Testern erstellt. Beide Materialgruppen wurden in Feldexperimenten in sechs Umwelten (drei Orte, zwei Jahre) in Deutschland evaluiert.

Für die Untersuchung der Methanausbeute wurde ein Kernsatz von 16 Inzuchtlinien und deren 32 Testkreuzungsnachkommen mit dem Hohenheimer Biogasertragstest, einem diskontinuierlichen Fermentationsversuch, analysiert. Nicht-lineare Regressionsmodelle wurden verwendet, um die Methan-Produktionskinetik zu beschreiben. Für die Methanausbeute nach kurzer Fermentationszeit (bis 5 Tage) wurden hohe h^2-Werte erzielt. Die genotypische Varianz (σ^2_g), und somit auch h^2, nahm jedoch mit fortschreitender Fermentationszeit ab. Dies ist vermutlich darauf zurückzuführen, dass die meisten Inhaltsstoffe größtenteils abgebaut und somit Unterschiede zwischen Genotypen nivelliert

wurden. Diese Interpretation wird bestärkt durch enge Korrelationen zwischen Methanausbeute und diversen Inhaltsstoffen sowie IVDOM und ME nach kurzer, jedoch nicht nach längerer Fermentationszeit.

Basierend auf den Proben des Kernsatzes wurden NIRS Kalibrationen für verschiedene Methanmerkmale und Inhaltsstoffe erstellt. Wie für h^2 beschrieben, nahm die Güte der Kalibration für Methanausbeute mit zunehmender Fermentationszeit ab ($R^2_V = 0.85$ nach 3 und 0.77 nach 35 Tagen), war aber, wie auch für alle Inhaltsstoffe, auf einem akzeptablen Niveau. NIRS kann deshalb zur Bestimmung dieser Merkmale empfohlen werden.

Die Analyse der Testkreuzungsnachkommen im Hauptexperiment zeigte, dass die Allgemeine Kombinationsfähigkeit (GCA) die wichtigste Variationsursache war. Da der Methanertrag eine sehr enge Korrelation mit TME, nicht jedoch mit der Methanausbeute zeigte, wird er hauptsächlich durch den TME bestimmt. Die Methanausbeute zeigte eine geringere Abhängigkeit von den Inhaltsstoffen als der ME-Gehalt. Die Methanausbeute korrelierte dabei am stärksten mit dem Gehalt an nicht abbaubarem Lignin ($r_g = -0.58$), jedoch nicht mit dem Stärkegehalt. Dies widerspiegelt die geringere Bedeutung eines hohen Kolbenanteils für Biogasmais im Vergleich zu Silomais. Zur Steigerung des Methanertrags sollte folglich verstärkt auf einen hohen TME selektiert werden. Die Analyse der LP im Hauptexperiment bestätigte im Wesentlichen die TP-Ergebnisse und favorisiert somit auch eine Selektion auf einen hohen TME. Die genotypischen Korrelationen zwischen LP und GCA waren am stärksten (≥0.94) bei den Maturitätsmerkmalen (Blühzeitpunkt, Trockensubstanzgehalt) und lagen in einem mittleren Bereich (≥0.65) bei Trockenmasse- und Methanertrag. Indirekte Selektion für GCA auf Basis der LP während der Mehrstufenselektion scheint erfolgsversprechend für Maturitätsmerkmale, Pflanzenhöhe und, zu einem gewissen Grade, auch für TME.

Unsere Studie zeigte, dass Biogasmais und herkömmlicher Silomais zwei klar verschiedene Nutzungsrichtungen sind. Hohe Stärkegehalte, wie sie in Silomais zur Deckung des Bedarfs an leicht verfügbarer Energie wichtig sind, bilden in Biogasmais keine Restriktion. Dies erlaubt einen schnelleren Zuchtfortschritt im Trockenmasse- und Methanertrag. Das hohe Ertragspotential von Testkreuzungsnachkommen zwischen exotischen Dent-Linien und den adaptierten Testern sowie die positive Korrelation zwischen Blühzeitpunkt und TME belegen das große Potential von Ertragssteigerungen durch verlängertes vegetatives Wachstum. Als Schlussfolgerung ist zu erwarten, dass sich die Sorten für beide Nutzungsrichtungen in Zukunft noch stärker unterscheiden werden.

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10. Curriculum vitae

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11. Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst wurde und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

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Christoph Grieder

Hohenheim, 25.01.2012