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# **Phenotypic and molecular analyses of grain and biomass productivity under irrigated and rainfed conditions in hybrid rye**

Dissertation  
zur Erlangung des Grades eines Doktors  
der Agrarwissenschaften  
vorgelegt  
der Fakultät Agrarwissenschaften

von  
Diplom-Agrarbiologin  
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Hofheim am Taunus

Stuttgart-Hohenheim  
2014

Die vorliegende Arbeit wurde am 05. Januar 2014 von der Fakultät Agrarwissenschaften der Universität Hohenheim als Dissertation zur „Erlangung des Grades eines Doktors der Agrarwissenschaften“ angenommen.

Tag der mündlichen Prüfung                      18. Februar 2014

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## Table of contents

|     |  |    |
|-----|--|----|
| 1.  | General introduction   | 1  |
| 2.  | Publication 1: Biomass yield of self-incompatible germplasm resources and testcrosses in winter rye <sup>1</sup>                               | 8  |
| 3.  | Publication 2: Impact of genotype, harvest time and chemical composition on the methane yield of winter rye for biogas production <sup>2</sup> | 10 |
| 4.  | Publication 3: Hybrid rye performance under natural drought stress in Europe <sup>3</sup>  | 12 |
| 5.  | Publication 4: Genetic architecture of complex agronomic traits in rye ( <i>Secale cereale</i> L.) <sup>4</sup>                                | 14 |
| 6.  | General discussion   | 16 |
| 7.  | References   | 37 |
| 8.  | Summary  | 51 |
| 9.  | Zusammenfassung  | 54 |
| 10. | Acknowledgements   | 57 |
| 11. | Curriculum Vitae   | 58 |
| 12. | Erklärung  | 59 |

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<sup>1</sup>Miedaner T, Hübner M, Koch S, Seggl A, Wilde P (2010) Biomass yield of self-incompatible germplasm resources and their testcrosses in winter rye. *Plant Breed* 129:369-375.

<sup>2</sup>Hübner M, Oechsner H, Koch S, Seggl A, Hrenn H, Schmiedchen B, Wilde P, Miedaner T (2011) Impact of genotype, harvest time and chemical composition on the methane yield of winter rye for biogas production. *Biomass Bioenergy* 35:4316-4323.

<sup>3</sup>Hübner M, Wilde P, Schmiedchen B, Dopierala P, Gowda M, Reif J, Miedaner T (2013) Hybrid rye performance under natural drought stress in Europe. *Theor Appl Genet* 126(2):475-482

<sup>4</sup>Miedaner T, Hübner M, Korzun V, Schmiedchen B, Bauer E, Haseneyer G, Wilde P, Reif JC (2012) Genetic architecture of complex agronomic traits examined in two testcross populations of rye (*Secale cereale* L.). *BMC Genomics* 13:706

## Abbreviations

|                |   |
|----------------|---|
| AFLP           | Amplified fragment-length polymorphism                    |
| cM             | Centimorgan   |
| DArT           | Diversity array technology                                |
| dt             | Deci tonne/ quintal                                       |
| EEG            | Erneuerbare-Energien Gesetz [Renewable Energy Source Act] |
| F <sub>2</sub> | Second filial generation                                  |
| ha             | Hectare   |
| HBT            | Hohenheim biogas yield test                               |
| MAS            | Marker-assisted breeding                                  |
| Pop-A          | Population A  |
| Pop-B          | Population B  |
| QTL            | Quantitative trait loci                                   |
| RAPD           | Random amplified polymorphic DNA                          |
| RFLP           | Restriction fragment length polymorphisms                 |
| R              | Rye chromosome  |
| SNP            | Single nucleotide polymorphism                            |
| SSR            | Simple sequence repeat                                    |

## 1. General introduction

Rye (*Secale cereale* L.) is a crop primarily cultivated in temperate regions. In Europe, compared to wheat and maize acreage, rye growing area is about one fifth and one third of the size, respectively (FAOSTAT 2011). In Germany, Russia, Belarus, Ukraine and Poland mainly grown as winter cereal, cultivated areas are mostly located in regions with sandy and infertile soils. About 60% of the rye growing acreage in Germany is accounted to hybrid cultivars (Anonymous 2010). In Germany, 2.9 Million tons were harvested in 2011. Approximately 24% of harvest is used for bread making, 57% for livestock feeding, and 14% as renewable energy resource, i.e. biogas and bioethanol production (Miedaner 2013a). These different purposes of usage are leading to different breeding goals. Besides 1000-kernel weight and pre-harvest sprouting resistance, measured as falling number, a balanced proportion between starch, pentosan and protein is required in order to achieve good baking properties (Weipert 1983). Contrary to rye used for baking, for livestock feeding high protein content is desirable and content of pentosan should be low (Boros 2007). In the last decades, new market segments were opened by using rye as renewable energy source. Rye for feeding and bioethanol usage share almost the same breeding goals, except for  $\alpha$ -amylase and crude protein content. When using rye as biogas substrate total dry matter at the individual harvest dates play the main role (Miedaner 2007, Grieder *et al.* 2012a). Two options of using rye for biogas production are possible. When growing forage rye in a crop rotation with maize with harvest at the end of April/begin of May, a fast growth in early spring is demanded. At this time dry matter yield is particularly high because of its outstanding long stems and large leaf volume. High biomass production and lodging resistance are necessary when rye is used as main crop and whole plant silage is used in biogas plants. The largest proportion of total dry matter yield is reached between milk ripening and dough ripening.

### ***Rye as renewable energy source***

Biogas production from agricultural products is of growing importance in many European countries. Germany is the largest biogas producer in the world (Weiland 2010) and the number of operating biogas plants increased in the last twenty years. In 1992, 139 biogas plants were installed, and for 2013 about 7900 installed plants are predicted (FVB 2012).

This tendency is associated with the guarantees by the Renewable Energy Source Act (EEG) in 2000. Compensatory payments up to 20 years have been awarded to holders of biogas plants, connected with an additional bonus when using plant biomass (Schittenhelm 2008). In 2012, approximately 2.5 Million hectares were used for cultivating energy crops, 960 000 hectares were used as biogas substrate (FNR 2012). A huge diversity of cultivars are suitable for bioenergy production and therefore were examined in many scientific studies (Amon *et al.* 2007, Heiermann *et al.* 2009, Lehtomäki *et al.* 2008, Rincón *et al.* 2010), but not much of them are used in practice. However, the largest part is taken by maize with 76%, followed by grass silage with 11%, cereals whole plant silage with 7%, the rest is divided by grain, sugar beet and others (Weiland 2006).

Winter rye is an economically interesting alternative and highly competitive to other small grain cereals, like triticale and wheat (Miedaner 2011). Caused by its high water- and nutrient use efficiency, high tolerance to acid soils, and lower input in fertilizer, rye coped well on soils with low supply of groundwater, poor of nutrients and in regions with low precipitation. Main rye growing areas are located in regions with sandy and infertile soils, like in Lower Saxony and Brandenburg. In these regions maize and forage rye/ maize rotation for biogas production is not efficient anymore. Therefore we propose using rye for biogas production as main substrate in these areas.

Biogas producers are only economically efficient when maximizing the methane yield production per hectare (Oslaj *et al.* 2010, Chynoweth *et al.* 1993, Walla *et al.* 2008, Lehtomäki *et al.* 2008). Methane yield consists multiplicatively of: (1) dry matter yield and (2) volume of methane produced per unit of dry matter. It is proposed, optimal biogas production is influenced by harvest times of individual crops, because methane yield highly depends on biomass lignin content (Amon *et al.* 2007, Oslaj *et al.* 2010, Alaru *et al.* 2011). There are also many other factors which are influencing the maximum biogas yield. Most important are the crop, genotype, harvest time and nutrient composition (Amon *et al.* 2003, Amon *et al.* 2007, Heiermann 2009, Bruni *et al.* 2010, Schittenhelm 2008, Petersson *et al.* 2007). It is known that a high correlation between dry matter yield and biogas yield exists for maize (Grieder *et al.* 2012a). Therefore we suggest breeding for biomass is more useful, because analyzing biogas yield is expensive and time consuming. However, little is known about the biomass potential, as well as the biogas potential of winter rye.

At present in Germany, the acreage of maize is still growing, especially for biogas production a significantly increase of the maize acreage during the past decades could be observed (BMELV 2011a). However, this is connected with a strong decline in biodiversity (Walhardt *et al.* 2011, Mühlenhoff 2011). Maize monocultures are adverse for diversity of species in the field. Therefore, revisions in the EEG are planned in order to counteract these problems (BMELV 2011a). On one hand using rye for the cultivation as alternative energy crop can offer the opportunity to disperse less diverse crop rotations of conventional farming. On the other hand, using winter rye as main substrate is an interesting opportunity and highly competitive caused by the modesty of rye in regions with sandy soils (Böse 2007).

### ***Climate change and the impact on rye***

Within recent years, farmers in Germany were touched by yield losses through periodical water shortness mainly in spring and early summer. Especially, April of 2007, 2009, 2010, and 2011 were among the driest in the last twenty years in Germany (DWD 2011). Particularly, Lower Saxony and East Germany, where 60% of rye growing area is located, were struck by drought. Precipitation rate in Poland and East Europe was after sowing and during plant development in 2010 and 2011 much lower than long-term average precipitation rate (DWD 2012). Drought periods run through whole temperate latitudes in Europe. Sandy soils are quite frequent in regions where rye is mainly grown, which possess a low water-retaining capability, low pH-value and nutrient content. Although rye is robust and tolerant to abiotic stresses, the average grain yield of Lower Saxony and Brandenburg declined sharply from 56 dt ha<sup>-1</sup> in 2009 to 45 dt ha<sup>-1</sup> in 2010 (BMELV 2011b). Rye is affected by the consequences of climate change drastically.

Conventional breeding for drought tolerance is done for several crops and genetic variation can be identified and implemented in cultivars through different mating designs and breeding tools (Ashraf 2010, e.g. maize: Bänziger *et al.* 2004, Bunce 2010; wheat: Villareal *et al.* 1994, Rajaram 2001, Valkoun 2001, Changhai *et al.* 2010; rice: MacLean *et al.* 2002; barley: Thomas and Fukai 1995). However, breeding drought resistant crops has been fully exploited only for arid and semi-arid regions, areas where rainless periods are expected. In Germany, water stress is not predictable and occurs only in some years. The final aim of breeders in Germany must be to develop genotypes which are performing well under

drought, but also possess high grain yield under non-stressed conditions. Hereafter, we suggest selection of drought-resistant germplasm in winter rye is needed.

Generally, drought escape, drought avoidance and drought tolerance are the mechanism behind drought resistance of plants (Levitt 1972), and like other abiotic stresses, drought stress is polygenically inherited (Zhao 2002, Mohammadi *et al.* 2005). Simultaneously, plants use more than one mechanism to withstand drought (Mitra *et al.* 2001). To our knowledge nothing is known on the physiological mechanisms of drought resistance in rye. However, when a genotype yields higher than another under severe drought stress it is relatively seen more drought resistant (Blum 2005). Unfortunately, most of specific adaptations to drought limit yield performance under normal conditions (Turner 1979). Consequently, crop adaptation must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity (Blum 2011).

Breeding for drought resistance is complicated by the fact that several types of abiotic stress can affect plants simultaneously and are often interrelated (Fleury *et al.* 2010), therefore the genetic control of drought resistance is complex. Selection under drought stress conditions is complicated concerning polygenic inheritance, low heritability and large genotype by environment interactions (Golabadi *et al.* 2011, Fleury *et al.* 2010). Yield is the most relevant trait to the breeder. So far, lots of QTL (quantitative trait loci) studies for drought tolerance are described. For example for wheat and barley, drought-QTL have been identified by using yield and yield components under water-limited conditions (Quarrie *et al.* 2006, McIntyre *et al.* 2009).

In hybrid rye breeding, the two gene pools Petkus and Carsten are used as base populations for developing seed and pollinator lines (Geiger and Miedaner 2009). The development of new parental lines for hybrid breeding comprises selection for combining ability based on testcross performance (Tomerius *et al.* 2008). Plant materials used in this study are intrapool crosses among elite material from the Petkus gene pool of current hybrid rye breeding programme. Crosses between the same gene pool result in a loss of genetic variation by strong selection pressure within these both pools. Additionally, the progenies were crossed with a tester from the same gene pool which reduces variation by half, not considering epistatic effects. In applied hybrid rye breeding programs, however, thousands of progenies have to be tested regarding their combining ability for agronomically important traits

(Tomerius *et al.* 2008). Therefore, a large-scale screening method is needed for selection of drought-resistant genotypes in the field.

### ***Molecular analyses of agronomic and quality traits in rye***

The combination of sandy locations where rye is mainly grown, and continuing climate changes force us to ensure yield stability, disease resistance and environmental adaption. Although, cross-pollinating winter rye shows highest tolerance against biotic and abiotic stresses among all small-grain cereals (Hoffmann 2008, Haseneyer *et al.* 2011). Compared to other field crops, winter rye was an unattended crop concerning marker development and linkage mapping. However, the realisation of effective molecular breeding programs in rye depends on the availability of high density molecular linkage maps (Bolibok-Brągoszewska *et al.* 2009). Several genetic maps have been published with various marker technologies, however, also for rye a few high density maps have been published (Table 1). In the beginning of marker development isozymes, restriction fragment length polymorphism (RFLP), and polymerase chain reaction-based marker, e.g. amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), randomly amplified polymorphic DNA (RAPD), were used for map construction. Today, these kinds of marker are obsolete due to the time-consuming handling and exorbitant prices. Instead about 5,000 diversity array technique (DArT) markers (Bolibok-Brągoszewska *et al.* 2009, Milczarski *et al.* 2011) and a 5,000 single nucleotide polymorphism (SNP) array (Haseneyer *et al.* 2011) are available.

**Table 1. Overview of genetic linkage maps, their number of marker, map length and average marker density for rye**

| References                                 | No. of marker | Map length<br>[cM] | Average marker<br>density [cM] |
|--|---------------|--------------------|--------------------------------|
| Milczarski <i>et al.</i> 2011 <sup>a</sup> | 7531          | 1593               | 1.1                            |
| Myśków <i>et al.</i> 2010                  | 1347          | 962                | 0.7                            |
| Bolibok-Brągoszewska <i>et al.</i> 2009    | 1818          | 3145               | 2.7                            |
| Hackauf <i>et al.</i> 2009                 | 248           | 724                | 2.9                            |
| Gustafson <i>et al.</i> 2008 <sup>a</sup>  | 501           | 780                | 1.6                            |

<sup>a</sup>Consensus map

Besides understanding the functional genetics of agronomic and quality traits, tolerances and resistances, rye provides beneficial traits for other crops (Ko *et al.* 2002). On genomic basis, rye is closely related to wheat, barley and triticale. As donor of translocated

chromosome segments rye has been widely used in wheat breeding programs (Lukaszewski 1990; Villareal *et al.* 1994).

The analysis of QTL by estimating their map position and effects has been conducted in detail for various crops. However, QTL mapping studies in rye are lagging far behind. First QTL mapping for several agronomic traits was done for a F<sub>2</sub> population by Börner *et al.* (1999). One QTL for plant height was found on chromosome 5R, and for several yield component traits prior on chromosome 2R and 5R. Several  $\alpha$ -amylase activity QTL were detected distributed over the whole genome in F<sub>2</sub>, recombinant inbred line and a doubled haploid populations (Masojć and Milczarski 2005, Myśków *et al.* 2011, Tenhola-Roininen *et al.* 2011, Masojć *et al.* 2007, Myśków *et al.* 2010), additionally preharvest sprouting QTL where estimated in the latter two studies. QTL for important agronomic traits were detected in two introgression libraries derived from an Iranian primitive rye (Falke *et al.* 2009a). For grain yield, plant height, test weight, 1000-kernel weight and protein content QTL were detected, no was found for falling number in this study. Additionally to the quality trait protein content, Falke *et al.* (2009a) found also QTL for starch and pentosan content distributed over the genome. Furthermore, two major genes for thousand-kernel weight on chromosomes 5R and 7R in a F<sub>2</sub> population were found using SSR and RFLP marker (Wricke *et al.* 2002). At that time, not many regions which are responsible for agronomic or quality traits in rye have been defined yet. Our study reports the first elaborate QTL mapping study of ten agronomic and quality traits across ten environments which are important for practical breeding.

In this study, testcross progenies inside the same gene pool (Petkus x Petkus) are used. Therefore, it has to be considered that for intrapool hybrids genetic variation is reduced due to alleles by descent, and additionally progenies are crossed with the same tester. It is known that testcross progenies show lower numbers of QTL than lines *per se* (Falke *et al.* 2010).

### **Objectives of this study**

In these present studies different kind of plant material were used. For analysing the biomass potential 59 entries were tested consisting of three groups of winter rye material: (i) germplasm resources of highly diverse origin, (ii) full-sib families selected for forage use and (iii) full-sib families selected for grain use. The biogas yield was tested particularly for 25

entries consisting of three groups of rye material: (i) experimental hybrids selected for grain use, (ii) population cultivars selected for grain use, and (iii) population cultivars selected for forage use. Analyzing the difference between irrigated and non-irrigated regimes, as well as performing a QTL analysis across ten environments, two seed-parent testcross populations were used.

The objectives of this study were to

**1. Biomass/biogas potential of rye (Publication 1 and 2)**

- (a) investigate the biomass potential of germplasm resources compared to adapted forage and grain rye,
- (b) estimate correlation between *per se* and testcross performance for biomass yield,
- (c) analyse the genetic variation for important biogas-related traits by the Hohenheim Biogas yield test,
- (d) examine the correlation between biomass and biogas yield,

**2. Drought resistance in rye (Publication 3)**

- (a) cluster environments by multivariate analyses in irrigated and rainfed environments,
- (b) analyse grain yield performance under irrigated and non-irrigated conditions,
- (c) partitioning of variance components and estimate heritabilities for grain yield of irrigated, rainfed and across both regimes
- (d) examine the relative efficiency for indirect selection in irrigated regime for drought tolerant genotypes

**3. Molecular analyses of agronomic and quality traits in rye (Publication 4)**

- (a) analyse phenotypic values across multiple environments for ten agronomic and quality traits,
- (b) map QTL for ten agronomic and quality traits.

For references please see chapter 7.

## **2. Publication 1: Biomass yield of self-incompatible germplasm resources and testcrosses in winter rye**

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Plant Breeding, 2010, 129(4):369-375

DOI: 10.1111/j.1439-0523.2010.01777.x

The original publication is available at

<http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0523.2010.01777.x/pdf>

## Abstract

Winter rye (*Secale cereale* L.) is an ideal crop for the energy production due to its vigorous growth, high nutrient- and water use efficiency, and low input in fertilizer and nitrogen. For the use in biogas plants, maximal biomass yield with dry matter contents of >30% is an essential breeding aim. The objectives were to analyse the potential of 25 germplasm resources of various geographic origin for the rapid improvement of biomass yield compared to 22 and 12 full-sib families selected for forage and grain use, respectively. Population *per se* and testcross performance with two testers were evaluated for early growth, heading, plant height, dry matter content, and dry matter yield across seven environments (location-year combinations) harvested as whole plants at late milk-ripening. Dry matter yield ranged, on average, from 130 to 141 dt ha<sup>-1</sup> for population *per se* performance and from 150 to 158 dt ha<sup>-1</sup> for testcross performance (0% water content). Genotypic variances were significant ( $P < 0.01$ ) throughout, entry-mean heritabilities for biomass yield were moderate to high (0.67-0.91). In both materials, germplasm resources and forage rye had on average the highest biomass yield. The best individual entry was a topcross hybrid with 'Florida Black' as pollinator averaging 173 dt ha<sup>-1</sup> dry matter yield. Three released hybrid cultivars selected for high grain yield were among the entries with the lowest biomass yield at milk ripening. Germplasm resources showed significant genotypic correlations between dry matter yield and early growth, heading date and dry matter content in the testcrosses. The genotypic correlations between populations *per se* and testcrosses were significant and high for germplasm resources and forage rye. In conclusion, germplasm resources have a high potential for maximal biomass yield when whole-plant harvest occurs at milk ripening.

### **3. Publication 2: Impact of genotype, harvest time and chemical composition on the methane yield of winter rye for biogas production**

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Biomass and Bioenergy, 2011 35(10):4316-4323

DOI: 10.1016/j.biombioe.2011.07.021

The original publication is available at

<http://www.sciencedirect.com/science/article/pii/S0961953411004296>

## Abstract

Rye (*Secale cereale* L.) is an ideal crop as supplier for the agricultural biogas production in regions with less fertile and sandy soils. Maximum methane yield per hectare is the main aim of the farmer. Objectives were to establish differences by the Hohenheim Biogas Test among (1) 25 genotypes (experiment 1) and (2) three harvest dates (early heading, early and late milk ripening) and three plant fractions (ears, leaves and stems, stubbles) for four genotypes including an analysis of their nutrient composition (experiment 2). Significant ( $P < 0.05$ ) genotypic variation was found for dry matter yield, specific gas yield and methane yield among the 25 genotypes, but no differences for methane content and specific methane yield. Broad ranges were achieved for dry matter yield (0% water content) and methane yield amounting to  $2.9 \text{ t ha}^{-1}$  and  $840 \text{ m}^3 \text{ ha}^{-1}$  respectively, combined with moderate to high heritabilities (0.71 to 0.98). Both traits were highly correlated ( $r = 0.95$ ,  $P < 0.01$ ). Compared to population and forage rye, hybrid rye achieved significantly higher methane yields. The latest harvest date at late milk ripening resulted in the highest dry matter yield on a whole plant level with an average of  $16.0 \text{ t ha}^{-1}$ . Accordingly, methane yield was reaching a mean of  $4,424 \text{ m}^3 \text{ ha}^{-1}$  and a maximum of  $4,812 \text{ m}^3 \text{ ha}^{-1}$ . No correlations between content of crude nutrients or cell-wall fractions and specific gas or methane yield were evident neither for the plant fractions nor for the whole plant. In conclusion, harvesting at late milk ripening was clearly superior in dry matter and methane yields although specific methane yield was higher at early heading. A selection for maximum dry matter yield in rye breeding should indirectly improve also methane yield.

#### **4. Publication 3: Hybrid rye performance under natural drought stress in Europe**

M. Hübner, P. Wilde, B. Schmiedchen, P. Dopierala, M. Gowda, J.C. Reif, T. Miedaner

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Theoretical Applied Genetics, 2013, 126(2):475-482

DOI: 10.1007/s00122-012-1994-4

The original publication is available at

<http://link.springer.com/article/10.1007%2Fs00122-012-1994-4>

## **Abstract**

Several rye growing regions of Central Europe suffered from severe drought periods in the last decade. Rye is typically grown on sandy soils with low water-holding capacity in areas with less rainfall, thus drought-resistant varieties are necessary. Our main objective was to test the drought-stress resistance of rye hybrids using large-scaled field experiments. Two biparental populations (Pop-A, Pop-B) with each of 220 F2:4 lines from the Petkus gene pool and their parents were evaluated for testcross performance under irrigated (I) and non-irrigated (NI) regime in six environments for grain yield. We observed for most environments severe drought stress leading to a maximum of 40.5% yield reduction for irrigated compared to non-irrigated regime. A decomposition of the variance revealed significant ( $P < 0.01$ ) genotypic and genotype x environment interaction variances but only a minor effect of drought stress on the ranking of the genotypes with regard to grain yield. In conclusion, separate breeding programs for drought-tolerant genotypes are not superior at present for hybrid rye breeding in Central Europe.

**5. Publication 4: Genetic architecture of complex agronomic traits in rye (*Secale cereale* L.)**

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BMC Genomics, 2012, 13:706

DOI:10.1186/1471-2164-13-706

The original publication is available at

<http://www.biomedcentral.com/1471-2164/13/706>

## **Abstract**

Winter rye is an important crop used for food, feed, and bioenergy. Several quality and yield-related traits are of agronomic importance for rye breeder. Profound knowledge of the genetic architecture of these traits is needed to successfully implement marker-assisted selection programs. However, little is known on quantitative loci underlying important agronomic traits in rye.

We developed 440 lines from two biparental populations. The progenies and their parents were genotyped by DArT, SSR, and SNP markers and evaluated for their testcross performance for ten agronomic traits in up to ten environments in Germany and Poland. The quality of the phenotypic data was high reflected by entry-mean heritability estimates (0.7-0.9). QTL analyses revealed one to ten QTL per trait. QTL x environment interactions were significant ( $P < 0.01$ ) in most cases but variance of QTL main effect was more prominent across environments.

QTL mapping were successfully applied based on two segregating rye populations. QTL underlying grain yield had mainly small effects. In contrast, yield components such as 1000-kernel was influenced by two major QTL. These QTL explaining large proportion of the genotypic variance can be exploited in marker-assisted selection programs and represent the first step towards map-based cloning of the underlying genes.

## **6. General discussion**

Agricultural crop improvement is a permanent challenge for breeders, either using crops as renewable energy resource or as food or for livestock feeding. The feasibility of utilizing rye as energy crop and its level of methane production through anaerobic digestion was evaluated in this thesis by the Hohenheim biogas yield test (HBT) for a wide range of rye materials. Biomass and methane yield potential of different germplasm have been explored.

During the last decade, Germany was touched by sporadically occurring drought periods in spring and autumn, seasons which are most relevant for winter cereals during plant development. When breeding winter rye in Germany, climate change and its effects come more and more into the focus. The improvement of cultivars suitable for drought tolerance as well as improving crop performance in general are to date most important for the rye breeder. We evaluated two segregating populations with one parent with improved drought tolerance under irrigated and rainfed conditions. Regarding molecular marker techniques, rye is lagging behind other crops but might catch up since the availability of a 9k SNP chip (Haseneyer et al. 2011) that is in the moment expanded to a 20k chip (Eva Bauer, Freising, pers. commun.). For both segregating populations, we developed a specific genetic map and the first comprehensive QTL mapping in rye. For each population ten most important agronomic and quality traits were analyzed in elite testcross progenies.

### **Rye as biomass supplier for biogas production**

The dominating crop supplying about 80% of biomass for biogas production in Germany is maize (FNR 2011). This emphasizes the urgent need for new crops adapted to different regions, as well as for more varied crop rotations to counteract the decreasing biodiversity in agricultural land (Mühlenhoff 2011, Vetter and Arnold 2010). Before propagating winter rye as biogas substrate for the farmer, several questions have to be answered in advance.

Rye as supplier for biogas plants can be used as both, whole plant silage and as forage rye (KWS LOCHOW 2010). The difference between them is the variety type and its harvest time. Forage rye is a population rye with an early growth in spring and strong biomass development before and shortly after winter. It is a winter catch crop and was specially bred for the production of green fodder early in the year. Due to its tendency to lodging forage

rye has to be harvested in the middle of May at the latest. Therefore, it is a well suited catch crop in a crop rotation with maize in regions with a sufficient water supply. As previous crop to maize forage rye has to be harvested end of April until beginning of May in order to ensure a sowing of maize on time.

Regions where rye is mainly grown are characterized by drought periods, less fertile and sandy soils. These are often regions where maize is not competitive due to limited water supply. In these areas rye might be used as whole crop silage as main supplier for biogas production with a harvest end of July (late milk ripeness/early wax ripeness). Principally, no agronomic differences between growing rye for whole plant silage and for grain usage exist (TerraVis 2012). This guarantees a flexibility of a use according to the market conditions. The decision which crop a farmer grows following whole crop silage depends on soil characteristics and water supply. In a crop rotation with rye whole plant silage with a harvest end of July and grasses as a catch crop followed by a winter cereal might be one possibility. Consequently, biogas farmers have the choice between rye population cultivars bred for forage use with an early harvest, population cultivars bred for grain use, as well as rye hybrid varieties bred for grain or silage use. **The question, however, arises which of these three types of varieties gain most success for the farmer. In other words: Do we need special variety types for biogas production?**

Maximizing methane yield produced per unit area is the key to the economic success of biogas producers, however, the energy yield per hectare is mainly affected by biomass yield and convertibility of the biomass to methane (Lethomäki 2008). Different methods for evaluation of biogas production in biogas fermenters with discontinuous batch methods exist (Hellfrich et al. 2003, Owen et al. 1979, Kaiser et al. 2007). Furthermore, differences between these methods and in practice applied biogas fermenters must be considered during the evaluation of the results. For example, the size of the sampled plant material used in HBT and in commercial biogas fermenters varied. In biogas plants larger fragments of plant material are used, whereas for the HBT plant material is grounded to 1 mm particle size (Hübner et al. 2011). Hence, the digestion time might be slower in a commercial biogas plant than in 100 ml retort samplers which are used in the HBT (Grieder et al. 2012b). Furthermore, the different inoculums used for the HBT and in biogas plants might be influencing the biogas production. Consequently, further research has to be done in the transferability of results from small batch methods to commercial biogas fermenters.

Additionally, in commercial breeding a high throughput of tested genotypes is necessary. The evaluation of biogas yield by laboratory methods is time consuming and expensive. **Concerning the different types of varieties, we did not find any significant differences between forage and grain varieties on the level of open-pollinated populations (Hübner et al. 2011). Hybrid cultivars had a slight advantage in methane yield.**

**A second question is whether genotypic differences for biogas or methane yield as well as methane concentrations exist among genotypes. In other words: Are some genotypes more suitable for biogas production than others?**

For maize, Lübken et al. (2010) recommend that breeders selecting for high methane yield should decrease the proportion of non-degradable substances like lignin and pay special attention to easy degradable substances like starch, sugar and fat. For diverse material of rye differences in cell wall fractions and crude nutrients could not lead to differences in biogas yield (Hübner et al. 2011). After about 20 days of fermentation, digestion reached a steady state for all harvest dates at similar levels indicating that all organic material has been fully metabolized. Grieder et al. (2012a) could not show any correlation between methane yield and starch concentration in maize. They concluded that this might be attributable to the long fermentation duration of 35 days in the biogas fermenter and, therefore, a complete degradation of all chemical substances occurred. In forage maize, breeding for digestibility traits play a major role beside biomass yield (Barrière et al. 1997). These traits might be of lower importance for biogas maize. This is substantiated due to the different conditions of a biogas fermenter compared to the rumen (Grieder et al. 2012b).

Rye breeders have to reply promptly to the demand of farmers using rye varieties for biogas production. On the short run a breeder can use varieties with high biomass yield because a close correlation between biomass yield and methane yield ( $r=0.95$ ; Hübner et al. 2011) exists, therefore each high biomass yielding variety is suitable. For maize, Grieder et al. (2012a) reported a narrow association between dry matter yield and methane yield ( $r=0.9$ ). When using either forage rye with an early harvest or rye as whole plant silage, existing varieties with high biomass yield can be used. **Consequently, our results indicate that breeding programs for biogas rye should concentrate on genotypes with high biomass yield and focus less on nutrient composition or biogas yield related traits.**

**As a third concern it should be discussed whether rye as a biogas substrate is capable to compete with the traditionally used maize?**

In rye, significant differences for dry matter yield exist for a wide range of breeding material currently available, including hybrids bred for grain use and population varieties selected for grain and forage use (Hübner *et al.* 2011). These genotypes yielded between 13 to 16 t ha<sup>-1</sup> biomass. Separating for harvest dates forage rye showed best results for biomass yield at early harvest (EC51). Between harvest at early milk ripeness (EC73) and at late milk ripeness/early wax ripeness (EC77/EC83) methane yield did not differ much. However, at latest harvest highest methane yield was reached. In conclusion, for rye breeders a wide range of rye elite varieties is available for a profitable economically usage as biogas rye with an early cut in April or as main crop with a late harvest at milk ripeness. With the best hybrid rye variety slightly more methane yield can be reached compared than with worst maize variety depending on soil fertility and water availability (Hübner *et al.* 2011, Amon *et al.* 2003; Oslaj *et al.* 2010). Furthermore, hybrid rye produced highest methane yield with the widest range for this trait.

Hybrid rye and maize might even been complementary in reaching maximal methane yields, because rye-maize mixtures increase the biogas production compared to pure maize or rye silage (KWS LOCHOW 2012a). Maximal biogas yields are reached with a mixture of 75% of maize silage and 25% of rye whole plant silage. This might be explained by the fact, that the use of different substrates for biogas plants is supporting the enhancement of different kinds of bacteria in the fermenter. Therefore, the diversity of bacteria is contributing to a stable process and ensures a stable gas production. Furthermore, different kinds of substrates might compensate yield differences of different crops. **With regard to sandy soils, maize would probably not reach dry matter yield and consequently methane yield levels like reported for rye under adverse conditions. Using hybrid rye as whole plant silage in those regions often suffering from drought in spring, would greatly contribute in stabilizing crop biomass yields and maximizing methane yields. It is, therefore, a promising alternative to maize as biogas supplier.**

**A last question concerning rye as biogas substrate is how to introduce efficiently selection for biomass yield in the hybrid breeding process.**

Hybrid rye cultivars have gained increasing importance because of their yield superiority and favorable trait combinations (Geiger and Miedaner 2009). Population varieties were partially replaced by hybrid varieties during the last 20 years (Geiger and Miedaner 1999). Today, hybrid rye varieties are devoted to more than the half of the German seed propagation area

(BSL 2012). Parental lines of new hybrid varieties are generated from crosses of elite breeding material developed from the two gene pools Petkus and Carsten (Miedaner 2007). Crossing two inbred lines (parental lines) out of these two gene pools causes heterosis and hybrid performance can be exploited (Hepting 1978). The Petkus gene pool serves as seed parent and the Carsten gene pool as pollen parent source (Miedaner 2007). The development of parental lines (seed- and pollen parent) for developing new hybrid varieties comprises two steps: (i) selection for line *per se* performance and (ii) selection for combining ability based on testcross performance. Evaluating lines regarding their testcross performance is important for the later performance of progenies by crossing a line out of the Petkus and a line out of the Carsten gene pool. The production and assessment of the testcrosses is time and cost intensive. Therefore, information on *per se* performance is necessary to preselect lines which are tested later against a tester from the opposite gene pool.

When improving rye varieties on a short run by breeding, using germplasm resources is one possibility (Miedaner *et al.* 2010). Non-adapted, but genetically diverse rye populations and landraces might serve as a genetic source for breeders, but are not fully exploited by practical breeding yet. Usually this material has low agronomic performance and no information about genetic distances to both heterotic pools is available (Hausmann *et al.* 2004). Nevertheless, it might be possible to exploit genetic resources and use their favorable alleles, e.g. their early start of growth in spring. This trait might be a benefit when breeding rye varieties for a crop rotation with maximum biomass yield at an early harvest and consequently an increased methane yield. Valuable QTL have already been found in an Iranian primitive rye population by Falke *et al.* (2009 a,b) and could be used in further rye breeding. The potential for biomass production using germplasm resources is shown by Miedaner *et al.* (2010). Variances for *per se* performance were larger for germplasm resources with highest biomass yield compared to variances of forage rye, grain rye and testcrosses. These results agree well with them of Grieder *et al.* (2012a), who could verify high genetic variation for maize including tropical germplasm. This is in part attributable to the fact that genetic resources are not preselected and therefore showing full genetic variance. In conclusion, broadening genetic variation for biomass yield using rye germplasm resources in breeding might be a successful opportunity. Using germplasm resources directly, however, is not recommendable, because they are highly susceptible for lodging

which in commercial growing is not acceptable (Miedaner *et al.* 2010). Subsequently, using them in a recurrent selection program or as pollinator in a hybrid combination with a superior seed parent might be good options for breeding for improved biogas rye.

Breeding rye parental lines for biogas usage in a hybrid breeding program on the seed and pollen parent side simultaneously, requires (i) effective indirect selection for *per se* performance to improve testcross performance and (ii) high genotypic correlation between both performances (Grieder *et al.* 2012c). Miedaner *et al.* (2010) could show a high correlation between *per se* and testcross performance for dry matter yield of forage rye, but low for germplasm resources and grain rye. This can be in part attributable to the small genetic variance within grain rye and to dominance effects of the tester in germplasm resources. In our study, high correlations between *per se* and testcross performance for dry matter content were achieved which is also reported for maize (Grieder *et al.* 2012c, Lübberstedt *et al.* 1997). We conclude, that dry matter yield is the most relevant trait for breeding biogas rye due to its high correlation between dry matter and biogas yield, and due to its high correlation between *per se* and testcross performance. However, these results indicate the need of an own breeding program for biogas rye using as substrate in biogas plants.

Within the last decade farmers in Germany were touched by yield losses through periodical water shortness mainly in spring and early summer. The effect of drought on rye is of large impact for both, biomass and grain yield (Hübner *et al.* 2013). In our study, considerable drought stress occurred in the rainfed variant at four out of six environments as concluded from significant yield reductions between rainfed and irrigated regime ranging up to 40.5%. Hübner *et al.* (2011) showed that the ears of rye plants contribute to a large extent to the complete biogas yield either harvesting at early milk ripeness or at late milk ripeness/early wax ripeness (Hübner *et al.* 2011). Therefore, not only grain yield should be in the focus of breeders, more frequently occurring drought periods in spring and early summer might also be taken into considerations when breeding rye for biogas usage because ears are touched by drought significantly and contribute at the same time to a large extent to biogas yield.

Taking all these aspects into account, biogas can be produced from winter rye either used as pre-crop in a crop rotation with maize and an early harvest in April or as main crop in regions with sandy soils and less rainfall. In both cases, we grant rye a great potential for a biogas farmer as supplier of raw material for biogas production, but also using rye in a mixture with

maize as substrate for biogas plants. According to our data, methane yield needs not to be analyzed routinely caused by the high correlation between biomass yield and biogas/methane yield. In course of climate change and drought periods during important physiological stages of crop plants the combination of general breeding aims with the improvement of drought tolerance should be brought into the focus of rye breeders.

### ***Improvement of grain yield and quality traits***

Grain yield is considered as most important trait in rye breeding. The largest proportion of the rye harvest (about 50%) in Germany is used for bread making and livestock feeding (Miedaner 2013a). For bread making, additionally to total grain yield itself, quality traits affecting milling and baking are in the main focus of rye breeders (Miedaner and Hübner 2010). For this, thousand-kernel weight and sprouting resistance are most important, whereas the latter being assessed as falling number. In contrast to wheat, in rye gluten plays no major role for baking, whereas crude protein content should be of low. Good baking quality is mainly determined by the amount of pentosans. These are complex carbohydrates increasing their volume by factor eight when adding water generating fluffy bread. In contrast, when using rye for livestock feeding, the content of pentosan has to be as low and the content of protein as high as possible (Miedaner and Hübner 2010). This becomes a dilemma for a rye breeder because different breeding programs are necessary for optimizing rye for feeding and rye for bread making. Nutrient content are contrary for both purposes. Using rye as supplier in biogas plants only dry matter yield plays the major and nutrient composition rather a minor role (Hübner *et al.* 2011). In our study, we could detect several QTL for agronomic and quality traits which are useful in breeding programs for rye either used for feeding or bread making (Miedaner *et al.* 2012).

The release of the first hybrid variety enables to an increase in grain yield performance (Miedaner 2007). When crossing two inbred lines from the rye gene pools Carsten and Petkus, full heterosis can be exploited. The two gene pools are serving as source for the development of seed and pollinator lines. The material used in our study belongs to the Petkus gene pool (seed parent, Miedaner *et al.* 2012). In hybrid rye breeding parental line development is done by continuous selfing, whereas during this phase inbreds are tested several times for line *per se* and for their testcross performance (Geiger and Miedaner,

2009), to obtain high performing seed parents. Selection for line *per* performance and selection for combining ability based on testcross performance has to be performed for producing hybrid rye varieties (Tomerius *et al.* 2008).

During these breeding cycles DNA markers for screening and selecting of plants or traits involves several advantages and therefore are considered a very useful tool for breeders (Brumlop and Finckh 2011). The implementation of marker-assisted selection (MAS) in plant breeding has advantages, *e.g.* (i) several traits which only become apparent in adult plants can already be selected in seedling stage, (ii) traits which are difficult, expensive and time consuming to assess phenotypically, can be selected with marker assistance, (iii) traits with low heritabilities can be easily selected by marker information, because analyses can be performed on a single plant, (iv) traits which are controlled by several genes can be selected, because every genetic component can be detected one by one through markers, (v) traits which are controlled by recessive genes or only appear in specific environments can be maintained and selected by markers, and subsequently (vi) multiple monogenic traits or various QTL for a trait with an complex inheritance, such as quality traits or drought resistance can be pyramided (Brumlop and Finckh, 2011; Young and Tanksley, 1989; Koebner 2004). MAS offers the advantage of fewer breeding cycles and compilation of desired traits in one genotype.

Application of MAS, as well as modern genomic approaches to improve breeding progress, such as association mapping, involve construction of a high-quality linkage map (Meuwissen *et al.* 2001, Jannink *et al.* 2010, Alheit *et al.* 2011). Rye is on a world-wide basis a neglected crop and therefore lagging far behind other crops in terms of genomic tools (Hackauf and Wehling 2002, Hackauf *et al.* 2009). Due to the availability of about 5,000 DArT markers (Bolibok-Bragoszewska *et al.* 2009, Milczarski *et al.* 2011) and a 5k-SNP array (Haseneyer *et al.* 2011) obsolete old marker techniques, which were still common and used in rye have been replaced. Miedaner *et al.* (2012) used both arrays in addition to SNP markers and created two high-density genetic linkage maps comprising 813 markers for Population A (Pop-A, including SNPs and SSRs) and 921 markers for population B (Pop-B, including DArTs and SSRs). The total map length of Pop-A counts 980 cM and of Pop-B 2,349 cM. Myśków *et al.* (2011) evaluated a genetic map comprising 1,285 DArT loci and 62 PCR-based markers with a map length of 962 cM. In our study, we created two genetic maps with about the same amount of markers but different map lengths. The map of Pop-B, wherein DArT and

SSR markers were combined is longer than that of Pop-A where SSR and SNP markers were combined. Comparable to our findings Bolibok-Bragoszewska *et al.* (2009) released a genetic rye map including 1,818 markers, combining DArT and SSR markers with a map length of about 3,150 cM. As well, Alheit *et al.* (2011) published a genetic map of triticale comprising of DArT marker, where genetic map lengths of the whole genome varied between 1,750 cM and 3,270 cM including 500 to 1,300 markers per population. In our study, chromosome 1R of Pop-A shows a length of 150 cM only, compared to 385 cM for 1R of Pop-B (Figure 3 and 4, Miedaner *et al.* 2012). We suppose either genetic mapping of DArT markers inflates genetic maps or mixing dominant and biallelic inherited markers might be contributing to long maps as suggested earlier by Liu (1998). It is, however, of greater importance that loci exhibit the right order than distances between markers (Korol *et al.* 2009). In our study, we attached great importance to this. We designed two genetic maps and used during the calculation of loci order of the individual maps several quality parameters which were provided by JoinMap®.4.0 (Van Ooijen 2006). Beside this, chromosome names and orientation were assigned to linkage groups for which the position were previously published (for DArTs: e.g. Akbari *et al.* 2006, Bolibok-Bragoszewska *et al.* 2009) or for SSR markers with known chromosomal location. We, therefore, anticipate that we designed high- quality maps as basis for QTL mapping.

Furthermore, it is obvious that for Pop-A consisting of SSR and SNP markers, markers clustered in some regions which is not seen for the map of Pop-B in where DArT and SSR markers were combined (Figure 3, Miedaner *et al.* 2012). This might again be in part attributable to the combination of dominant and biallelic inherited markers.

A QTL is a section of a chromosome that affects a phenotypic trait to some extent (Alonso-Blanco *et al.* 2006). Analyzing QTL across multiple traits and environments it is possible to construct a map of all contributing chromosomes and to analyze the genetic architecture of these traits. In our study of ten agronomic and quality traits, in total 31 QTL for Pop-A and 52 QTL for Pop-B were detected (Miedaner *et al.* 2012).

Different methods of QTL analysis exist, or to be more precise, multiple QTL models can be used for QTL detection (Alonso-Blanco *et al.* 2006). Generally, QTL analyses basically comprise testing of the effect of each marker of the genetic map with the trait of interest by an advanced analysis of variance. In our study, we used composite interval mapping (CIM) with cofactor selection (PLABQTL, Utz 2006). Using markers as cofactors that do not refer to

a detected QTL can negatively affect the power of those detected QTL (Cornforth and Long 2003). We followed the recommendation of Utz (2000) and performed a CIM run with automatic selection of cofactors followed by a permutation run to estimate adequate LOD thresholds for each trait. Furthermore, comparing cofactor selection in PLABQTL with that in other statistical packages, e.g. SAS (SAS Institute 2004), most packages eliminate the whole individual if a marker value is missing while PLABQTL estimates a missing value.

In literature, QTL mapping populations of about 100 to 150 progenies derived from crossing of two inbred lines have been reported (Bernardo 2008). Mapping populations should have a certain size, because populations of small sizes can only lead to detection of QTL with large effects and increase the rate of false positives (Wang *et al.* 2012). Also Utz (2000) remarks that problems may occur in QTL mapping using small populations ( $n=100$ ). A good quality of phenotypic data of the analyzed traits, marker spacing between 10 to 15 cM together with a suitable statistical analysis will usually result in identification of QTL (Bernardo 2008). In our study with 220 individuals per population, we found for both populations more than 90% of the genetic map distances between adjacent polymorphic markers being smaller than 10 cM (Miedaner *et al.* 2012). Piepho (2000) could show in a theoretical study that the power of QTL detection does not considerably increase if the distance between adjacent polymorphic markers is smaller than 10 cM. This indicates that marker density is not a major limiting factor for QTL detection. Therefore, we conclude that our maps are useful for QTL detection. Most important agronomic traits are quantitatively inherited, implicating that traits are controlled by a few to thousands of QTL, most of them possessing only small effects on the trait (Mackay 2001). Breeders most often select for several traits at the same time. Using only a few major QTL per trait can be processed more easily and is usually done. Therefore, the identification of closely linked markers to a QTL explaining a high amount of genetic variation for this trait and possessing a high recovery rate within the population are of great importance for using QTL in further breeding. In our study, we could find in both analyzed populations QTL with large effects for several agronomic and quality traits (Miedaner *et al.* 2012). One most prominent QTL was found for thousand-kernel weight on chromosome 7R which was probably already reported by Wricke (2002). Such QTL are of great importance for a breeder, especially for quantitatively inherited traits. Parallel screening of such large-effect QTL can be done with plant material by breeders besides executing their breeding programs.

Inconsistency of estimated QTL effects is forcing plant breeders to focus on major QTL with consistent effects (Bernardo 2008). Several explanations exist for inconsistencies when analyzing QTL, but most common reasons are (i) different QTL appearing in different mapping populations, (ii) QTL-by-genetic background interaction, and (iii) QTL-by-environment interaction. An exact evaluation of the latter can be executed when precision phenotyping in different locations was performed (Xu and Crouch 2008). For breeders only QTL with significant effects across environments are valuable. Miedaner *et al.* (2012) could identify QTL stable across ten diverse environments distributed across Germany and Poland. For grain yield in rye and other crops, presence of major QTL is not expected in populations of elite lines, although it is known that grain yield in cereal crops is controlled by many genes (Miedaner *et al.* 2012). It is suggested that through long breeding history major QTL became fixed, therefore QTL for grain yield are numerous but with small effects (Bernardo 2008). When the number of favorable QTL contributing to a trait is high, pyramidization of these QTL in a genotype is difficult.

Marker-assisted selection has been successfully applied in introgression and pyramidization of major-effect genes (Holland 2004). Before applying MAS for complex traits in conventional breeding, many challenges have to be resolved, e.g. costs and scalability of laboratory systems, the lasting and labor-intensive methods for identifying marker-trait associations, and the lack of freely available software which is designed for application by breeders and useful in molecular breeding programs (Holland 2004). Xu and Crouch (2008) state, that there is an increase in the usage of MAS in breeding companies but rather in transgenic approaches than in backcross programs. However, there are also clear signals from breeding companies that MAS is used for a range of simple and complex traits and provides an increase in selection gain resulting in time and cost advantages. Today, large-scale and low unit cost SNP marker, as well as DArT marker genotyping platforms are available for lots of cultivars (maize: Ganai *et al.* 2011, wheat: Akbari *et al.* 2006, barley: Wenzl *et al.* 2004; Close *et al.* 2009, rice: McCouch *et al.* 2010). We could provide the first comprehensive QTL analysis in rye based on a high-density map developed by such platforms (Miedaner *et al.* 2012). With the availability of a 5k-SNP array (Haseneyer *et al.* 2011) and about 5,000 DArT markers (Bolibok-Bragoszewska *et al.* 2009, Milczarski *et al.* 2011) large numbers of molecular markers can be provided with reduced costs. This opens also for rye new approaches which makes QTL mapping more efficient. Steinhoff *et al.*

(2011) demonstrated the potential of multiple-line cross QTL mapping in maize and could improve the power to detect QTL and the resolution to localize the QTL by switching from single population QTL mapping towards joint QTL analysis across several populations. Nevertheless, using complex genetic traits for marker-assisted selection is not that efficient (Buckler *et al.* 2009) compared to genomic selection approaches which might be more efficient (Moreau *et al.* 2004, Zhaou *et al.* 2012). Genomic selection is focusing on an efficient estimation of breeding values on the basis of a large number of molecular markers, ideally covering the full genome rather than mapping the effect of individual genes (Jannink *et al.* 2010). In simulation studies, it was proposed that genomic selection is promising for a rapid improvement of quantitative traits in plants and better suited compared to MAS (Bernardo and Yu 2007, Heffner *et al.* 2009). Based on this study, in rye MAS selection is possible, but further research is needed to implement these approaches. A part of this thesis could show the first results of genome-wide QTL analysis across two segregating rye populations for a comprehensive set of important traits (Miedaner *et al.* 2012). For quantitative traits inherited by many genes with small effects, like grain and biomass yield, genomic selection might be more efficient (Moreau *et al.* 2004, Zhaou *et al.* 2012), but this has still to be proven theoretically and experimentally in rye.

### ***Natural drought stress and QTL for drought tolerance***

Germany suffered from drought extremes in some regions in the last years. During plant development most critical for winter rye are the months April to June. April 2007, 2009, 2010, 2011 and 2012 were among the driest in the last twenty years in Germany (DWD 2011, 2012). Rye is clearly more drought tolerant than wheat, but about 75% of rye growing area are on light and sandy soils with less capacity to store water (Miedaner 2013b). As a consequence, grain yield of winter rye was considerably reduced in these regions. In 2010 and 2011, for example, rye grown in Lower Saxony, one of the main rye growing areas in Germany, suffered from an average yield reduction ranging from 14 to 27% compared to the mean of both previous years (LSV 2011). In Brandenburg the extreme dry year 2003 lead to a reduced yield of 35% in contrast to the following three years (Miedaner 2013b). Hübner *et al.* (2013) could show in a large scale screening of winter rye in Germany and Poland reduction effects of grain yield between irrigated and rainfed plots up to 40.5%.

Figure 1 clearly shows the drought stress at one location in Lower Saxony in 2010 of the study of Hübner *et al.* (2013). In a companion study of Haffke *et al.* (2012) reduction effects up to 27.2% between irrigated and rainfed variants were observed in winter rye 2011 and 2012.



**Figure1. Visible differences between irrigated and rainfed (non-irrigated) variant in Wohldede 2010.**

In temperate zones drought stress is often induced by rain-out shelters for creating well defined stress environments (Römer *et al.* 2012, LfL 2009, Friedlhuber *et al.* 2011). However, rain-out shelters are cost and labor intensive and limited in space and are, therefore, not useful for large scaled screening. Hübner *et al.* (2013), therefore, used naturally occurring drought stress in the field with an irrigated and a rainfed regime. Again, the two populations each consisting of 220 testcross progenies, were tested with two replications at three locations in two years. Altogether, 880 plots per location were set up which resulted in an area of about 1 ha per location.

Drought is a major environmental stress factor affecting growth and development of plants (Harb *et al.* 2010). Furthermore, drought or soil water deficit can occur regularly with low or random water availability and are unpredictable due to changes in weather conditions during the period of plant growth. Also in Central Europe drought stress occurs irregularly and drought and their effects are expected to increase with climate change. Therefore varieties are needed which perform high yielding in both conditions in stressed and non-stressed conditions. Rye will be affected more than other crops, because rye is typically

grown in regions with sandy soils of low water-holding capacity. Therefore, rye varieties are needed with improved drought tolerance for those regions.

Drought stress may vary from moderate, short periods to extremely severe summer drought of long duration that strongly influence development of plants and their whole life cycle (Pereira *et al.* 1993). The physiological responses of plants to drought stress and their influence for crop productivity vary with species, soil type, nutrients and climate (Austin 1989). Plants have several mechanisms to reply on drought: drought escape, drought avoidance or drought tolerance are mechanisms described in literature (Levitt, 1980), whereby a balance between those mechanisms has to be maintained to hold adequate productivity (Blum 2011). Plant processes, like membrane conformation, chloroplast organization and enzyme activities at a cellular level are modified through drought stress (Chevone *et al.* 1990). All these aspects influence the growth and yield reduction in the whole plant and make plants more susceptible to other stresses (for review see Akinci and Lösel 2012).

Haffke *et al.* (2012) could show in a different rye population than ours (pollen parent) for six out of eight environments significant differences between irrigated and rainfed variants. This confirmed our results where we observed for Pop-A five out of six, and for Pop-B four out of six environments significant differences between the irrigated and rainfed variant (Hübner *et al.* 2013). In this publication, we analyzed only grain yield. Additionally, in Table 2 all traits investigated in both regimes across six environments are shown. Plant height, all yield-related traits, and test weight showed significantly ( $P < 0.01$ ) different means between irrigated and rainfed variant ( $\Delta I$ ) in both populations. This was confirmed in a companion study in where also differences in thousand-kernel weight, heading date, and plant height were observed although to a lesser extent.

**Table 2. Means for ten agronomic and quality traits for irrigated (I) and rainfed (R) regime, their relative difference (Diff.), the comparison between both regimes ( $\Delta I$ ), and genotype x irrigation interaction variance ( $\sigma^2_{G \times I}$ ) across six environments.**

| Trait                             | Pop-A |       |           |            |                         | Pop-B |       |           |            |                         |
|-----------------------------------|-------|-------|-----------|------------|-------------------------|-------|-------|-----------|------------|-------------------------|
|                                   | I     | R     | Diff. (%) | $\Delta I$ | $\sigma^2_{G \times I}$ | I     | R     | Diff. (%) | $\Delta I$ | $\sigma^2_{G \times I}$ |
| Plant height [cm]                 | 114.4 | 97.2  | -15       | **         |                         | 112.4 | 102.4 | -8.9      | **         |                         |
| Yield-related traits:             |       |       |           |            |                         |       |       |           |            |                         |
| Grain yield [dt/ha]               | 77.8  | 63.2  | -19       | **         | *                       | 75.1  | 57.4  | -24       | **         | *                       |
| 1000-kernel weight [g]            | 35.2  | 33.0  | -6.3      | **         |                         | 34.4  | 31.3  | -9.6      | **         | *                       |
| Single ear weight [g]             | 1.99  | 1.80  | -9.5      | **         |                         | 1.93  | 1.71  | -11.4     | **         |                         |
| No. of ears [per m <sup>2</sup> ] | 483.3 | 458.0 | -5.2      | **         | *                       | 460.1 | 428.7 | -6.8      | **         |                         |
| Quality-related traits:           |       |       |           |            |                         |       |       |           |            |                         |
| Test weight [kg]                  | 69.3  | 68.2  | -1.6      | **         |                         | 71.0  | 69.4  | -2.3      | **         |                         |
| Protein [%]                       | 9.81  | 10.01 | +2        |            |                         | 10.08 | 10.61 | +5.3      |            |                         |
| Total pentosan [%]                | 10.18 | 10.14 | -0.4      |            |                         | 10.39 | 10.27 | -1.2      |            |                         |
| Soluble pentosan [%]              | 2.49  | 2.22  | -11       | +          | +                       | 2.46  | 2.21  | -10       |            |                         |
| Starch [%]                        | 61.50 | 61.20 | -0.5      |            |                         | 61.25 | 60.94 | -0.5      |            |                         |

+, \*, \*\* Significant at the 0.1, 0.05 and 0.01 probability level, respectively

For selection of drought-tolerant germplasm, genotype x irrigation interaction variance is crucial, because this indicates different reactions of genotypes to water stress. Hübner *et al.* (2013) could find significant genotype x irrigation interaction variance for grain yield across all environments in both populations, that was however considerably smaller than genotypic variance. Additionally, genotype x environment x irrigation interaction variance was for both populations larger than genotype x irrigation interaction variance indicating that water stress occurred at different plant stages in the individual environments (Hübner *et al.* 2013). In accordance Haffke *et al.* (2012) demonstrated for their population a much smaller genotype x irrigation variance relative to the genotypic variance across six environments. Both studies identified grain yield as the most sensitive trait to drought as also shown by Lafitte *et al.* (2003). In addition, for number of ears in Pop-A, and for 1000-kernel weight in Pop-B significant ( $P \leq 0.05$ ) genotype x irrigation interaction variance ( $\sigma^2_{G \times I}$ ) was observed in our study (see Table 2). Li *et al.* (2011) could verify significant genotype x irrigation

interaction variance for plant height, test weight and kernel weight in wheat. In conclusion, when selecting for drought tolerance breeders might search for other traits which are highly correlated with grain yield but are more susceptible to drought. Secondary traits are described in literature and could serve as possible traits in hybrid rye breeding. For example in maize and wheat secondary traits which are used for measuring physiological drought tolerance are leaf/canopy temperature, leaf rolling, relative water content and leaf colour (CROPSCIENCE 2012, Monneveux *et al.* 2012).

For the breeder efficient selection of large populations with low costs is most relevant. Following Harrer and Utz (1990) indirect selection of genotypes under optimal conditions will be superior to direct selection under suboptimal conditions when genotypic correlation is higher than 0.6 and/or heritability is considerably larger with indirect selection. In conclusion, the results of Hübner *et al.* (2013) indicate for the breeder selection under irrigated regime most useful. The error variance is then smaller, heritability higher, and this trait is tested anyway. For selection in later generations, when population size already is largely reduced, it might be useful to add a water-stress variant for verification.

In a common rye breeding program, thousand of plots have to be tested for evaluation of the performance of valuable genotypes. When measuring traits like plant height, heading and plant density a lot of human labour and time are needed. Especially, breeding programs as described in Hübner *et al.* (2013) are of huge size because many genotypes are tested in replications and additionally every genotype has an irrigated and rainfed variant. Grain yield is the primary trait for selection under stress conditions in drought-stress breeding programs. The absence of suitable high-throughput phenotyping platforms is a major factor limiting the development of improved crop varieties for drought tolerance (Myles *et al.* 2009, Xu and Crouch 2008). Several tests have been made on single measuring methods to record plant traits with one sensor (Busemeyer *et al.* 2010, Ehlert *et al.* 2010, Montes *et al.* 2011). Busemeyer *et al.* (2013) mentioned that the development of multiple-sensor technique platforms for measuring multiple traits at the same time is lagging far behind for small grain cereals.

In rye, KWS LOCHOW (2012b) reported such a platform to measure several plant traits at the same time. They connected a GPS (Global Positioning System), a spectrometer and an infrared thermometer at the sprayer boom of a tractor. It is possible to measure plant traits like plant density, leaf area, chlorophyll content, and diseases which could be show variation

for drought stress in one hour for 750 plots. However, the challenge is to handle the high data volume and using them later on for selection. Busemeyer *et al.* (2013) mentioned that the system architecture for data collection and generating plot-wise data is challenging and an important part in the development of such a platform. They developed an ultramodern sensor platform for triticale and mentioned to screen of about 250 plots per hour which results in a phenotyping capacity of multiple traits of more than 2000 plots per day. This phenotyping platform has the potential to measure several traits at the same time and can collect through repeated times of measurements the dynamic reactions of crops in response to abiotic or biotic stress. Consequently, those platforms offer the possibility to screen in a high throughput, and might be easily adapted to other varieties.

Besides improving the phenotyping analysis, as well as the throughput and precision, molecular approaches for improving plants in general and under stress environments might generate a higher progress from selection. Most QTL for drought tolerance in wheat and barley have been identified through yield and yield component measurements under water deficit (Quarrie *et al.* 2006, Matthews *et al.* 2008, Golabi *et al.* 2011). Breeding for drought tolerant rye in Central Europe is going along with analyzing genotypes which are good performing in both regimes, the irrigated and rainfed. Therefore two possible approaches for QTL detection usable for breeding for regions with unpredictable drought is possible: (i) analyzing QTL in the irrigated and rainfed regime separately and observing matching QTL in both regimes, or (ii) calculating the difference between the phenotypic values of both regimes of and performing QTL analysis with these data. The prerequisite in both cases is not only that the means of the investigated traits are significantly different in both regimes, but also that genotypes are reacting significantly different on stress.

We decided to follow method (i), because using the difference has the major limitation that those genotypes with a small difference between irrigated and rain-fed regimes are very often low yielding under non-stress conditions.

In addition to the phenotypic data of Hübner *et al.* (2013), here for the first time a QTL analysis is presented for this experiment calculated across those environments with significant genotype x irrigation interaction variance for the irrigated and rainfed variant separately (Table 3).

**Table 3. Differences between irrigated (I) and rainfed (R) regime for grain yield, number (No.) of detected quantitative trait loci (QTL) in each variant, and number of common QTL ( $\cap$ ) for both regimes for Pop B across four environments.**

| Trait                                 | Diff. (%) | QTL I |             | QTL R |             | $\cap$ |
|---------------------------------------|-----------|-------|-------------|-------|-------------|--------|
|                                       |           | No.*  | Total $R^2$ | No.*  | Total $R^2$ | No.    |
| Grain yield<br>[dt ha <sup>-1</sup> ] | -24.0     | 7     | 49.4        | 4     | 40.4        | 2      |

\*According to critical LOD score after 1000 permutations ( $\alpha=10\%$ ).

QTL analysis yielded seven and four grain yield QTL for irrigated and rainfed variants, respectively. In both variants, cross-validated phenotypic variances ( $R^2_{cv}$ ) were of similar order than the original  $R^2$  indicating a high quality mapping (data not shown). Also, some QTL revealed significant QTL x environment interaction variance as expected for quantitative traits, however, this variance was in all cases smaller than QTL variance. Furthermore, the identified QTL had a high stability across locations. Comparing QTL of both variants, only two QTL with similar positions were detected. The amount of explained phenotypic variance ( $R^2$ ) for these two QTL together was for the irrigated, as well as for the rainfed variant 26%. This is not astonishing, because grain yield follows an infinitesimal model (Fisher 1918). But these two QTL could be defined as “must-have alleles” that should be introduced into the breeding populations by MAS and subjected afterwards to field experiments under managed-drought stress, where all relevant stress-related loci that have not been detected by QTL mapping are selected phenotypically.

Agronomically desirable drought-specific alleles present as quantitative trait loci (QTL) were found under water-limited conditions in several crops. For maize, QTL for several agronomic traits were found in different water regimes (Sari-Gorla *et al.* 1999). Furthermore, QTL found for male flowering time and plant height were the same under well-watered and water-stressed conditions, whereas for the female-flowering and anthesis-silking-interval the expression of several QTL was different between both regimes (Sari-Goral *et al.* 1999). This can be in part attributable to quantitative nature of several of these traits together with different physiologic reactions caused by different environments (Yue *et al.* 2005). As mentioned in the Introduction chapter, the physiological responses of plants to drought stress and their influence on crop productivity largely vary with species, soil type, nutrients and climate (Austin 1989), furthermore plants have several mechanisms to reply on drought, drought escape, drought avoidance or drought tolerance (Levitt 1980). Taking all these

aspects into account this implies that common QTL between both irrigated and rainfed variant are rare. For rye further analyses are necessary, also for yield related and secondary traits for making a valid conclusion.

Although, we analyzed only environments with heavy drought stress leading to yield reductions between non-irrigated and irrigated regimes till 41%, only a few drought QTL were found. This can be in part attributed to a limited differentiation in drought-resistance among testcrosses. Accordingly, high genotypic correlations between both regimes were found for both populations (0.86 and 0.84 for Pop-A and Pop-B, respectively). Furthermore, we consider an everlasting indirect selection for drought stress in hybrid rye breeding as the most probable reason for low crossover interaction (Hübner *et al.* 2013). In hybrid rye breeding, parental lines are routinely selected on less fertile and sandy soils, wherefore a selection on those 'normal' locations goes along with higher yield under drought stress (Cattivelli *et al.* 2008).

It is reported about a number of drought resistant cultivars/lines are reported in other crops, e.g., maize (Bänzinger *et al.* 2004), rice (Mac Lean *et al.* 2002), and in wheat (Valkoun 2001). These cultivars have been identified through conventional breeding, although this is very time-consuming, cost- and labor-intensive (Ashraf *et al.* 2010). However, it is reported that limited success in improving drought tolerance is attributable to (i) transferring desired genes from one plant to another by crossing implies transferring undesirable genes and makes breeding complicated, and (ii) drought tolerance is controlled by multiple genes having additive effects and strong interactions with genes involved in yield potential (Ashraf *et al.* 2010).

Nevertheless, drought tolerant maize varieties can be purchased from the seed market. Monsanto/BASF developed drought tolerant maize varieties by genetic engineering (Miedaner 2013b). In cases of stress (drought, cold, and heat) these varieties are able to maintain important cell functions. Furthermore, Pinooer/Syngenta is selling drought tolerant maize varieties which are developed by MAS. Until it is possible using such modern methods in practise for rye, only 'normal' phenotypic selection of the best lines under the respective stress in the field can be done, as CIMMYT has done it in maize (Miedaner 2013b).

### ***Consequences for hybrid rye breeding***

Biogas can be produced from winter rye either used as main crop in a crop rotation in regions with sandy soils and less rainfall, or using winter rye as a pre-crop in a crop rotation with maize and an early harvest in April. Our research demonstrates that breeding rye varieties used as supplier in biogas plants biomass yield is the most important trait. This is substantiated by the high correlation between biomass yield and methane yield found in this study for rye. Rye breeders have to reply promptly to the demand of farmers using rye varieties for biogas production. On the short run a breeder can use varieties with high a biomass production, therefore high biomass yielding varieties are suitable. Broadening the genetic variation the use of germplasm resources is useful. Compared to forage rye and grain rye, germplasm resources show a large genetic variation for biomass yield. Therefore germplasm resources provide a large potential for breeding biogas rye and we recommend further breeding using such germplasm resources by recurrent selection is useful.

Biogas related traits show genotypic variation but are labor and cost intensive. A normal hybrid rye breeding program involves assessing of thousands of genotypes. Therefore, the main breeding goal for rye genotypes used as supplier in biogas plants should be maximum biomass yield. Biomass yield and methane yield show such a high correlation that breeders only have to select for biomass yield.

In course of climate change and drought periods during important physiological stages of crop plants the combination of general breeding aims with the improvement of drought tolerance should be brought into the focus of rye breeders. A grain yield reduction of up to 40.5% was observed. We defined those environments exhibiting a significant difference between rainfed and irrigated variant as 'drought environments'. For the breeder only environments that show additionally a significant genotype by irrigation interaction variance are valuable and were found in three environments for Pop-A and four populations for Pop-B. A high interaction variance between genotype and environment was observed, therefore no cluster revealed between environments of irrigated and rainfed variant. This indicates that every environment suffered from a different drought stress. The period and intensity of drought was highly variable. Low heritability was observed in the rainfed variant, therefore selection gain for stress tolerance is reduced. To improve drought tolerance of hybrid rye, breeder should include into their field trial system stress environments, i.e. in later breeding steps testing advance candidate lines under both water variants.

For the breeder there is a dilemma, because different breeding programs are necessary for optimizing rye for feeding and rye for bread making. We could detect several QTL for agronomic and quality traits which are useful in breeding programs for rye either used for feeding or bread making. These QTL can be used in MAS and would offer the advantage of fewer breeding cycles and compilation of desired traits in one genotype. Parallel screening of such large effect QTL can be done with plant material by breeders besides executing their breeding programs. For breeders only QTL with significant effects across environments are valuable. We could identify QTL stable across ten diverse environments distributed across Germany and Poland. We conclude, that they should be valuable for the breeder because they have been occurred in all tested diverse environments in this study and therefore may appear in other environments not tested here. A new field of genetics and QTL analysis approaches which makes mapping QTL more efficient are already in several crops applied, even for complex traits which should also be used in rye. For quantitative traits inherited by many genes with small effects genomic selection might be more efficient (Moreau *et al.* 2004, Zhaou *et al.* 2012) but has to be promoted in rye. When breeding winter rye in Germany and Eastern Europe breeders have to deal with lots of breeding aims based on the different usage of rye. Furthermore, the ongoing climate change holds new challenges for the breeder. This study could show that rye plant material is already preselected. Grain yield shows differences between irrigated and rainfed variants but the usable part for the breeder for breeding is small. Therefore, another option would be the search for secondary traits showing larger effects to drought and less variance to the environment. For handling the measurements of those traits in a high-throughput screening, suitable phenotyping platforms should be the next step.

## 7. References

- Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor Appl Genet* 113:1409-1420
- Akıncı Ş, Lösel DM (2012) Plant Water-Stress Response Mechanisms. In: Rahman IMM, Hasegawa H (eds) *Water Stress*. Internet resource: <http://www.intechopen.com/books/water-stress/plant-water-stress-response-mechanisms>. Accessed June 2013
- Alaru M, Olt J, Kukk L, Luna-delRisco M, Lauk R, Noormets M (2011) Methane yield of different energy crops grown in Estonian conditions. *Biosyst Eng* 1:13-22
- Alonso-Blanco C (2006) QTL Analysis. In: Salinas I and Sanchez-Serrano JJ (eds) *Molecular Biology Vol 323: Arabidopsis Protocols*, 2nd edn. Humana Press Inc., Totowa, NJ, pp.79-99
- Alheit KV, Reif JC, Maurer HP, Hahn V, Weissmann E, Miedaner T, Würschum T (2011) Detection of segregation distortion loci in triticale (*x Triticosecale* Wittmack) based on a high-density DArT marker consensus genetic linkage map. *BMC Genomics* 12:380
- Amon T, Kryvoruchko V, Amon B, Zollitsch W, Mayer K, Buga S, Amid A (2003) Biogaserzeugung aus Mais-Einfluss der Inhaltsstoffe auf das spezifische Methanbildungsvermögen von früh- bis spätreifen Maissorten. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (eds) 54. Tagung der Vereinigung der Pflanzenzüchter Saatgutkaufleute Österreichs. Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft Raumberg-Gumpenstein, Irdning, pp. 1-10
- Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V (2007) Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresour Technol* 98:3204-3212
- ANONYMOUS (2010) Der Roggenmarkt. Internet resource: <http://www.ryebelt.de/de/startseite/roggen/markt.html>. Accessed June 2013
- Ashraf M (2010) Inducing drought tolerance in plants: Recent advances. *Biotechnol Adv* 28:169-183
- Austin RB (1989) Prospect for improving crop production in stressful environments. In: Jones

- HG, Flowers TJ, Jones MB (eds) Plants under stress. Cambridge University Press, Cambridge, pp. 235-248
- Awise JC (2004) Molecular markers, natural history, and evolution. 2nd edn. Sinauer Associates, Sunderland, MA
- Bänzinger M, Setimela PS, Hodson D, Vivek B (2004) Breeding for improved drought tolerance in maize adapted to southern Africa. In: Fischer T et al. (eds) New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress. The Regional Institute Ltd, Brisbane
- Bernardo R (2008) Molecular Markers and selection for complex traits in plants: Learning from the last 20 Years. *Crop Sci* 48:1649-1664
- Bernardo R, Yu J (2007) Prospects for genome-wide selection for quantitative traits in maize. *Crop Sci* 47:1082-1090
- Blum A (2005) Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive?. *Aust J Agr Res* 56:1159-1168
- Blum A (2011) Drought resistance is it really a complex trait? *Funct Plant Biol* 38(10):753-757
- BMELV (2011a) Pressemitteilung Nr. 045 des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz. Internet resource: <http://www.bmelv.de/SharedDocs/Pressemitteilungen/2011/045-AI-EEG-Kongress.html>. Accessed June 2013
- BMELV (2011b) Besondere Ernte- und Qualitätsermittlung 2011. Internet resource: <http://berichte.bmelv-statistik.de/EQB-1002000-2011.pdf>. Accessed June 2013
- Barrière Y, Argillier O, Michalet-Doreau B, Hèbert Y, Guingo E, Giauffret C, Émile JC (1997) Relevant traits, genetic variation and breeding strategies in early silage maize. *Agronomie* 17:395-411
- Bolibok-Brągoszewska H, Heller-Uszyńska K, Wenzl P, Uszyński G, Kilian A, Rakoczy-Trojanowska M (2009) DArT markers for the rye genome-genetic diversity and mapping. *BMC Genomics* 10:578
- Börner A, Korzun V, Voylokov AV, Weber WE (1999) Detection of quantitative trait loci on chromosome 5R of rye (*Secale cereale* L.). *Theor Appl Genet* 98:1087-1090
- Boros D (2007) Quality aspects of rye for feed purposes. *Vorträge für Pflanzenzüchtung* 71:80-85

- Böse (2007) Höchste Methanausbeuten mit Hybridroggen-GPS. Internet source:  
[http://www.praxisnah.de/data/documents/praxisnah.de/2007-03/Praxisnah\\_3-07-ES.pdf](http://www.praxisnah.de/data/documents/praxisnah.de/2007-03/Praxisnah_3-07-ES.pdf). Accessed June 2013
- Brumlop S, Finckh MR (2011) Applications and potentials of marker assisted selection (MAS) in plant breeding. Federal Agency of Nature Conservation, Federal Ministry of Environment, Nature Conservation and Nuclear Safety, Bonn
- Bruni E, Jensen AP, Pedersen ES, Angelidaki I (2010) Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl Energy* 87(7):2212-17
- BSL (Beschreibende Sortenliste, 2012) Beschreibende Sortenliste Getreide Mais Öl- und Faserpflanzen Leguminosen Rüben Zwischenfrüchte. Bundessortenamt, Hannover
- Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ et al (2009) The genetic architecture of maize flowering time. *Science* 680 325:714-718
- Bunce JA (2010) Leaf Transpiration Efficiency of Some Drought-Resistant Maize Lines. *Crop Sci* 50(4):1409-1413
- Busemeyer L, Klose R, Linz A, Thiel M, Wunder E, Ruckelshausen A (2010) Agro-sensor systems for outdoor plant phenotyping in low and high density crop field plots. In *Proceedings of Landtechnik 2010 - Partnerschaften für neue Innovationspotentiale*, Düsseldorf, Germany, pp. 213-218
- Busemeyer L, Mentrup D, Möller K, Wunder E, Alheit K, Hahn V, Maurer HP, Reif JC, Würschum T, Müller J, Rahe F, Ruckelshausen A. BreedVision - A Multi-Sensor Platform for Non-Destructive Field-Based Phenotyping in Plant Breeding. *Sensors* 13:2830-2847
- Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mast-Rangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: An integrated view from breeding genomics. *Field Crop Res* 105:1-14
- Changhai S, Baodi D, Yunzhou Q, Yuxin L, Lei S, Mengyu L, Haipei L (2010) Physiological regulation of high transpiration efficiency in winter wheat under drought conditions. *Plant Soil Environ* 56:340-347
- Chevone BL, Seiler JR, Melkonian J, Amundson RG (1990) Ozone - water stress intersctions. In: Alscher RG, Cumming JR (eds) *Stress response in plants: Adaption and Acclimation Mechanisms*. Wiley Liss, New York
- Chynoweth DP, Turick CE, Owens JM, Jerger DE, Peck MW (1993) Biochemical methane

- potential of biomass and waste feedstocks. *Biomass Bioenerg* 5(1):95-11
- Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, Druka A, Stein N, Svensson JT, Wanamaker S, Bozdog S, Roose ML, Moscou MJ, Chao S, Varshney RK, Szűcs P, Sato K, Hayes PM, Matthews DE, Kleinhofs A, Muehlbauer GJ, DeYoung J, Marshall DF, Madishetty K, Fenton RD, Condamine P, Graner A, Waugh R (2009) Development and implementation of high-throughput SNP genotyping in barley. *BMC Genomics* 10:582
- Cornforth TW and Long AD (2003) Inferences regarding the numbers and locations of QTLs under multiple-QTL models using interval mapping and composite interval mapping. *Genet Res* 82:139-149
- CROPSCIENCE (2012) Secondary traits for maize grain yield under drought stress conditions. <http://cropsscience.ch/?p=707>. Accessed June 2013
- DWD (Deutscher Wetterdienst, 2011) Trockenheit in Europa 2011. Internet resource: [http://www.dwd.de/bvbw/generator/DWDWWW/Content/Oeffentlichkeit/KU/KU2/KU23/rcc-cm/products/BesondereWetterereignisse/European/20110510\\_\\_dryness2011\\_\\_statement\\_\\_for\\_\\_WMO,templateId=raw,property=publicationFile.pdf/20110510\\_dryness2011\\_statement\\_for\\_WMO.pdf](http://www.dwd.de/bvbw/generator/DWDWWW/Content/Oeffentlichkeit/KU/KU2/KU23/rcc-cm/products/BesondereWetterereignisse/European/20110510__dryness2011__statement__for__WMO,templateId=raw,property=publicationFile.pdf/20110510_dryness2011_statement_for_WMO.pdf). Accessed June 2013
- DWD (Deutscher Wetterdienst, 2012) Trockenheit in Europa im Frühjahr 2012. Internet resource: [http://www.dwd.de/bvbw/generator/DWDWWW/Content/Landwirtschaft/Interessantes/2012/Trockenheit\\_\\_Europa\\_\\_21012,templateId=raw,property=publicationFile.pdf/Trockenheit\\_Europa\\_21012.pdf](http://www.dwd.de/bvbw/generator/DWDWWW/Content/Landwirtschaft/Interessantes/2012/Trockenheit__Europa__21012,templateId=raw,property=publicationFile.pdf/Trockenheit_Europa_21012.pdf). Accessed June 2013
- Ehlert D, Horn HJ, Adamek R (2008) Measuring crop biomass density by laser triangulation. *Comput Electr Agr* 61:117-125
- FAOSTAT (2011) Production crops. Internet resource: <http://faostat.fao.org/site/567/default.aspx#ancor>. Accessed March 2013
- Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. *J Exp Bot* 61(12):3211-3222
- FVB (Fachverband Biogas e.V., 2012) Entwicklung der Anzahl Biogasanlagen und der gesamten installierten elektrischen Leistung in Megawatt [MW]. Internet resource: [http://www.biogas.org/edcom/webfwb.nsf/id/DE\\_Branchenzahlen/\\$file/12-11-29\\_Biogas%20Branchenzahlen%202011-2012-2013.pdf](http://www.biogas.org/edcom/webfwb.nsf/id/DE_Branchenzahlen/$file/12-11-29_Biogas%20Branchenzahlen%202011-2012-2013.pdf). Accessed June 2013

- FNR (Fachagentur Nachwachsende Rohstoffe, 2011) Anbau nachwachsender Rohstoffe in Deutschland. Internet resource:  
[http://www.nachwachsenderohstoffe.de/uploads/media/Anbau\\_Grafik\\_Kurve\\_2012.jpg](http://www.nachwachsenderohstoffe.de/uploads/media/Anbau_Grafik_Kurve_2012.jpg). Accessed June 2013
- Falke KC, Wilde P, Wortmann H, Geiger HH, Miedaner T (2009a) Identification of genomic regions carrying QTL for agronomic and quality traits in rye (*Secale cereale*) introgression libraries. *Plant Breed* 128:615-623
- Falke KC, Sušić Z, Wilde P, Wortmann H, Möhring J, Piepho H-P, Geiger HH, Miedaner T (2009b) Testcross performance of rye introgression lines developed by marker-assisted backcrossing using an Iranian accession as donor. *Theor Appl Genet* 118:1225-1238.
- Falke KC, Wilde P, Wortmann H, Müller B, Möhring J, Piepho HP, Miedaner T (2010) Correlation between per se and testcross performance in rye (*Secale cereale* L.) introgression lines estimated with a bivariate mixed linear model. *Crop Sci* 50:1863-1873
- Fisher RA (1918) The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh* 52:399-433
- Friedelhuber R, Schmidhalter U, Hartl L (2011) Einfluss von Trockenstress auf die Bestandestemperatur und den Ertrag bei Weizen (*Triticum aestivum*). In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (eds) 61. Tagung der Vereinigung der Pflanzenzüchter Saatgutkaufleute Österreichs. Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft Raumberg-Gumpenstein, Irdning, pp. 155-158
- Ganal MW, Durstewitz G, Polley A, Bérard A, Buckler ES, Charcosset A, Clarke JD, Graner E-M, Hansen M, Joets J, Le Paslier M-C, McMullen MD, Montalent P, Rose M, Schön C-C, Sun Q, Walter H, Martin OC, Falque M (2011) A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. *PLoS ONE* 6(12): e28334. doi:10.1371/journal.pone.0028334
- Geiger HH, Miedaner T (1999) Hybrid rye and heterosis. In: Coors JG, Pandey S (eds) *Genetics and exploitation of heterosis in crops*. Crop Science Society of America, Madison
- Geiger HH, Miedaner T (2009) Rye Breeding. In: Carena MJ (ed) *Cereals Handbook of Plant*

- Breeding, Vol. 3, Springer, New York
- Golabadi M, Arzani A, Mirmohammadi Maibody SAM, Sayed Tabatabaei BE, Mohammadi SA (2011) Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. *Euphytica* 177:207-221
- Grieder C, Dhillon BS, Schipprack W, Melchinger AE (2012a) Breeding maize as biogas substrate in Central Europe: I. Quantitative-genetic parameters for testcross performance. *Theor Appl Genet* 124:981-988
- Grieder C, Mittweg G, Dhillon BS, Montes JM, Orsini E, Melchinger AE (2012b) Kinetics of methane fermentation yield in biogas reactors: Genetic variation and association with chemical composition in maize. *Biomass Bioenerg* 37:132-141
- Grieder C, Dhillon BS, Schipprack W, Melchinger AE (2012c) Breeding maize as biogas substrate in Central Europe: II. Quantitative-genetic parameters for inbred lines and correlations with testcross performance. *Theor Appl Genet* 124:971-980
- Gustafson JP, Ma XF, Korzun V, Snape JW (2009) A consensus map of rye integrating mapping data from five mapping populations. *Theor Appl Genet* 118:793-800
- Hackauf B, Wehling P (2002) Identification of microsatellite polymorphisms in an expressed portion of the rye genome. *Plant Breed* 121:17-25.
- Hackauf B, Rudd S, Van der Voort JR, Miedaner T, Wehling P (2009) Comparative mapping of DNA sequences in rye (*Secale cereale* L.) in relation to the rice genome. *Theor Appl Genet* 118:371-384
- Haffke S, Hübner M, Wilde P, Schmiedchen B, Kusterer B, Hackauf B, Miedaner T (2012) Field studies on the inheritance of drought tolerance of hybrid rye in Central Europe. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (eds) 61. Tagung der Vereinigung der Pflanzenzüchter Saatgutkaufleute Österreichs. Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft Raumberg-Gumpenstein, Irdning, pp. 73-75
- Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010) Molecular and Physiological Analysis for Drought Stress in *Arabidopsis* Reveals Early Responses Leading to Acclimation in Plant Growth. *Plant Physiol* 154:1254-1271
- Harrer S, Utz HF (1990) A model study for breeding low-input varieties using maize as an

- example. pp. 9-20. In: Bericht über die Arbeitstagung der “Arbeitsgemeinschaft der Saatzüchtleiter” im Rahmen der “Vereinigung österreichischer Pflanzenzüchter”. Bundesanstalt für alpenländische Landwirtschaft, Gumpenstein, Austria, pp. 20-22
- Haseneyer G, Schmutzer T, Seidel M, Zhou R, Mascher M, Schön CC, Taudien S, Scholz U, Stein N, Mayer KFX, Bauer E (2011) From RNA-seq to large-scale genotyping - genomics resources for rye (*Secale cereale* L.). *BMC Plant Biol* 11:131
- Hausmann BIG, Parzies HK, Prestler T, Sušić Z, Miedaner T (2004) Plant genetic resources in crop improvement. *Plant Genet Resour* 2:3-21
- Heffner EL, Sorrells ME, Jannink JL (2009) Genomic selection for crop improvement. *Crop Sci* 49:1-12
- Heiermann M, Plöchl M, Linke B, Schelle H, Herrmann C (2009) Biogas Crops - Part I: Specifications and Suitability of Field Crops for Anaerobic Digestion. *Agricultural Engineering International: The CIGR Ejournal*. Internet resource: <http://www.cigrjournal.org/index.php/Ejournal/article/view/1087> (accessed March 2013)
- Helffrich D, Oechsner H (2003) Hohenheimer Biogasertragstest – Vergleich verschiedener Laborverfahren zur Vergärung von Biomasse. *Landtechnik* 58:149-149
- Hepting L (1978) Analyse eines 7x7–Sortendiallels zur Ermittlung geeigneten Ausgangsmaterials für die Hybridzüchtung bei Roggen. *Z Pflanzenzüchtung* 80:188-197
- Hoffmann B (2008) Alteration of drought tolerance of winter wheat caused by translocation of rye chromosome segment 1RS. *Cereal Res Commun* 36:269-278
- Holland JB (2004) Implementation of molecular markers for quantitative traits in breeding programs-Challenges and opportunities. In: Fischer T (ed) *New directions for a diverse planet. Protocols of the 4th International Crop Science Congress*, Brisbane, Australia. The Regional Institute Ltd., Gosford, Australia
- Hübner M, Oechsner H, Koch S, Seggl A, Hrenn H, Schmiedchen B, Wilde P, Miedaner T (2011) Impact of genotype, harvest time and chemical composition on the methane yield of winter rye for biogas production. *Biomass Bioenerg* 35:4316-4323
- Hübner M, Wilde P, Schmiedchen B, Dopierala P, Gowda M, Reif J, Miedaner T (2013) Hybrid Rye Performance under Natural Drought Stress in Europe. *Theor Appl Genet* 126(2):475-482

- Jannink J-L, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: From theory to practice. *Brief Funct Genomic Proteomic* 9(2):166-177
- Kaiser F, Gronau A (2007) Methanproduktivität nachwachsender Rohstoffe in Biogasanlagen. Lerchl Druck, Freising
- Ko JM, Do GS, Suh DY, Seo BB, Shin DC, Moon HP (2002) Identification and chromosomal organization of two rye genome-specific RAPD products useful as introgression markers in wheat. *Genome* 45: 157–164
- Koebner RMD (2004) Marker-Assisted Selection in Cereals: The Dream and Reality. In: Gupta PK, Varshney RK (eds) *Cereal Genomics*. Kluwer Academic Publisher, Dordrecht, pp.317-330
- Korol AB, Mester D, Frenkel Z, Ronin Y (2009) Methods for genetic analysis in the Triticeae In: Feuillet C, Muehlbauer GJ (eds) *Genetics and Genomics of the Triticeae*. Springer Science+Business Media, New York
- KWS LOCHOW (2010) Roggen als Biogas – Alternativen für den Einsatz. Internet resource: [http://www.kws-lochow.de/news-heinze.html?&tx\\_ttnews\[tt\\_news\]=25&cHash=56b8f30633a5ea057ae7ca77db3c6f9c](http://www.kws-lochow.de/news-heinze.html?&tx_ttnews[tt_news]=25&cHash=56b8f30633a5ea057ae7ca77db3c6f9c). Accessed June 2013
- KWS LOCHOW (2012a) Roggen als Biogaslieferant. Internet resource: [http://www.kws-lochow.de/fileadmin/Redakteure/Downloads/Dossier\\_Biogas.pdf](http://www.kws-lochow.de/fileadmin/Redakteure/Downloads/Dossier_Biogas.pdf). Accessed June 2013
- KWS LOCHOW (2012b) Trockenstresstoleranz - Fortschritt durch modernste Selektionsmethoden. Internet resource: [http://www.youtube.com/watch?v=\\_OYILwLB6Rw](http://www.youtube.com/watch?v=_OYILwLB6Rw). Accessed June 2013
- Lafitte R, Blum A, Atlin G (2003) Using secondary traits to help identify drought-tolerant genotypes. In: Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B (eds) *Breeding Rice for Drought-Prone Envrionments*. Los Banos (Philippines): International Rice Research Institute, pp 37-49
- Lehtomäki A, Viinikainen TA, Rintala JA (2008) Screening boreal energy crops and crop residues for methane biofuel production. *Biomass Bioenergy* 32:541-550
- Levitt J (1972) Responses of plants to environmental stresses. Academic Press, New York
- LfL (Bayrische Landesanstalt für Landwirtschaft, 2009) "Züchtung für den Klimawandel - Rain-

- Out-Shelter setzt Pflanzen unter künstlichen Trockenstress". Internet resource: <http://www.lfl.bayern.de/ipz/zuechtungsforschung/36099/>. Accessed June 2013
- Li P, Chen J, Wu P (2011) Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and non-stress conditions. *Agron J* 103:1619- 1628
- Liu (1998) *Statistical Genomics: Linkage, Mapping, and QTL Analysis*. CRC Press, Boca Raton
- LSV (Landessortenversuche 2011) *Ergebnisse der Landessortenversuche. Bericht zum Versuchswesen*. Landwirtschaftskammer Niedersachsen, Germany
- Lübberstedt T, Melchinger AE, Schön CC, Utz HF, Klein D (1997) QTL mapping of testcrosses of European flint lines of maize: I. Comparison of different testers for forage yield traits. *Crop Sci* 37:921-931
- 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
- Lukaszewski AJ (1990) Frequency of 1RS·1AL and 1RS·1BL translocations in United States wheats. *Crop Sci* 30: 1151-1153
- Mackay TFC (2001) The genetic architecture of quantitative traits. *Annu Rev Genet* 35:303-339
- MacLean JL, Dawe DC, Hardy B, Hettel GP (2002) *Rice Almanac: sourcebook for the most important economic activity on Earth*, 3rd edn. CABI Publishing, Wallingford
- Masojć P, Milczarski P (2005) Mapping QTLs for  $\alpha$ -amylase activity in rye grain. *J Appl Genet* 46(2):115-123
- Masojć P, Banek-Tabor A, Milczarski P, Twardowska M (2007) QTLs for resistance to preharvest sprouting in rye (*Secale cereale* L.). *J Appl Genet* 48(3):211-217
- Matthews KL, Malosetti M, Chapman S, McIntyre L, Reynolds M, Shorter R, Van Eeuwijk F (2008) Multi-environment QTL mixed models for drought stress adaption in wheat. *Theor Appl Genet* 117:1077-1091
- McCouch SR, Zhao K, Wright M, Tung C-W, Ebana K, Thomson M, Reynolds A, Wang D, DeClerck G, Ali L, McClung A, Eizenga G, Bustamante C (2010) Development of genome-wide SNP assays for rice. *Breeding Science* 60(5):524-535
- McIntyre CL, Matthews KL, Rattey A, Chapman SC, Drenth J, Ghaderi M, Reynolds M, Shorter

- R (2009) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. *Theor Appl Genet* 120:527-541
- Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(1):1819-1829
- Miedaner T (2007) Roggenzüchtung. In: Roggenforum e.V. (ed) Roggen - Getreide mit Zukunft!. DLG Verlag, Frankfurt am Main, pp 27-51
- Miedaner T, Hübner M, Koch S, Seggl A, Wilde P (2010) Biomass yield of self-incompatible germplasm resources and their testcrosses in winter rye. *Plant Breed* 129:369-375
- Miedaner T, Hübner M (2010) Qualitätsansprüche für unterschiedliche Verwendungszwecke bei Hybridroggen. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (eds) 61. Tagung der Vereinigung der Pflanzenzüchter Saatgutkaufleute Österreichs. Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft Raumberg-Gumpenstein, Irnding, pp. 45-49
- Miedaner T, Hübner M, Korzun V, Schmiedchen B, Bauer E, Haseneyer G, Wilde P, Reif JC (2012) Genetic architecture of complex agronomic traits examined in two testcross populations of rye (*Secale cereale* L.). *BMC Genomics* 13:706
- Miedaner (2013a) Roggenanbau - Eine erfolgreiche Alternative. DLG Verlag, Frankfurt am Main
- Miedaner (2013b) Trockentoleranz - Schneller zum Erfolg. DLG-Mitteilungen 7/13, Saatgut Magazin, S.28-29.
- Milczarski P, Bolibok-Bragoszewska H, Myśków B, Stojalowski S, Heller-Uszyńska K, Góralska M, Bragoszewski P, Uszyński G, Kilian A, Rakoczy-Trojanowska M (2011) A high density consensus map of Rye (*Secale cereale* L.) based on dart markers. *PLoS ONE* 6(12):28495
- Mitra J (2001) Genetics and genetic improvement of drought resistance in crop plants. *Curr Sci* 80:758-763
- Mohammadi M, Taleei A, Zeinali H, Naghavi MR, Ceccarelli S, Grandtner M (2005) QTL analysis for phenologic traits in doubled haploid population of barley. *Int J of Agr Biol* 7(5):820-823
- Monneveux P, Jing R, Misra SC (2012) Phenotyping for drought adaptation in wheat using physiological traits. *Front Physiol* 3:429

- Montes JM, Melchinger AE, Reif JC (2007) Novel throughput phenotyping platforms in plant genetic studies. *Trends Plant Sci* 12:433-436
- Moreau I, Charcosset A, Gallais A (2004) Use of trial clustering to study QTL x environment effects for grain yield and related traits in maize. *Theor Appl Genet* 110:92-105
- Mühlenhoff J (2011) Anbau von Energiepflanzen - Umweltauswirkungen, Nutzungskonkurrenzen und Potenziale. Agentur für Erneuerbare Energien e.V. [http://www.unendlich-viel-energie.de/uploads/media/34\\_Renews\\_Spezial\\_Energiepflanzen\\_jul11\\_online.pdf](http://www.unendlich-viel-energie.de/uploads/media/34_Renews_Spezial_Energiepflanzen_jul11_online.pdf). Accessed March 2013
- Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang Z, Costich DE, Buckler ES (2009) Association mapping: Critical considerations shift from genotyping to experimental design. *Plant Cell* 21:2194-2202
- Myśków B, Stojalowski S, Milczarski P, Masojć P (2010) Mapping of sequence-specific markers and loci controlling preharvest sprouting and alpha-amylase activity in rye (*Secale cereale* L.) on the genetic map of an F2 (S120xS76) population. *J Appl Genet* 51(3):283-287
- Myśków B, Stojalowski S, Łań A, Bolibok-Bragoszewska H, Rakoczy-Trojanowska M, Kilian A (2012) Detection of the quantitative trait loci for  $\alpha$ -amylase activity on a high-density genetic map of rye and comparison of their localization to loci controlling preharvest sprouting and earliness. *Mol Breed* 30(1): 367-376
- Osaj M, Mursec B, Vindis P (2010) Biogas production from maize hybrids. *Biomass Bioenerg* 34:1538-1545
- Owen WF, Stuckey DC, Healy Jr JB (1979) Bioassay monitoring biochemical methane potential and anaerobic toxicity. *Water Res* 13(6):485-492
- Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander EC, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: Comparison across species, generation, and environments. *Genetics* 127:181-197
- Pereira JS, Chaves MM (1993) Plant responses to drought under climate change in mediterranean-type ecosystems. In: Moreno JM, Oechel WC (eds) *Global change and Mediterranean-type ecosystems. Ecological studies*. Vol. 117, Springer Verlag, New York, pp. 140-160
- Petersson A, Thomsen MH, Hauggaard-Nielsen H, Thomsen AB (2007) Potential bioethanol

- and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. *Biomass Bioenerg* 31:812-819
- Piepho HP (2000) Optimal marker density for interval mapping in a backcross population. *Heredity* 84:437-440
- Quarrie SA, Pekic Quarrie S, Radosevic R, Rancic D, Kaminska A, Barnes JD, Leverington M, Ceoloni C, Dodig D (2006) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. *J Exp Bot* 57(11): 2627-2637
- Rajaram S (2001) Prospects and promise of wheat breeding in twenty-first century. *Euphytica* 119:3-15
- Rebaï A, Goffinet B (1993) Power of tests for QTL detection using replicated progenies derived from a diallel cross. *Theor Appl Genet* 86:1014-1022
- Rincón B, Banks CJ, Heaven S (2010) Biochemical methane potential of winter wheat (*Triticum aestivum* L.): Influence of growth stage and storage practice. *Bioresour Technol* 101:8179-8184
- Römer C, Wahabzada M, Ballvora A, Pinto F, Rossini M, Panigada C, Behmann J, León J, Thureau C, Bauckhage C, Kersting K, Rascher U, Plümer L (2012) Early drought stress detection in cereals: simplex volume maximisation for hyperspectral image analysis. *Funct Plant Biol* 39:878-890
- Sari-Gorla M, Krajewski P, Di Fonzo N, Villa M, Frova C (1999) Genetic analyses of drought tolerance in maize by molecular markers. II. Plant height and flowering. *Theor Appl Genet* 99:289-295
- SAS Institute (2004) 9.13. SAS Institute, Cary, USA
- Schittenhelm S (2008) Chemical composition and methane yield of maize hybrids with contrasting maturity. *Eur J Agron* 29:72-79
- Steinhoff J, Liu W, Maurer HP, Würschum T, Longin CFH, Ranc N, Reif JC (2011) Multiple-Line Cross Quantitative Trait Locus Mapping in European Elite Maize. *Crop Sci.* 51:2505-2516
- Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander EC (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132:823-839
- Tenhola-Roininen T, Kalendar R, Schulman AH, Tanhuanpää P (2011) A doubled haploid rye linkage map with a QTL affecting  $\alpha$ -amylase activity. *J Appl Genet* 52(3):299-304

- TerraVis (2012) Infoblock GPS & Grünschnittroggen. Internet resource:  
[http://www.terravis.info/mainnav/leistungen\\_8/biomasseproduktion\\_2/getreideganzpflanzensilage/getreideganzpflanzensilage\\_1.html](http://www.terravis.info/mainnav/leistungen_8/biomasseproduktion_2/getreideganzpflanzensilage/getreideganzpflanzensilage_1.html). Accessed June 2013
- Thomas S, Fukai S (1995) Growth and yield response of barley and chickpea to water stress under three environments in southeast Queensland. III. Water use efficiency, transpiration efficiency and soil evaporation. *Aust J Agric Res* 46(1):49-60
- Tomerius AM, Miedaner T, Geiger HH (2008) A model calculation approach towards the optimization of a standard scheme of seed-parent line development in hybrid rye breeding. *Plant Breed* 127:433-440
- Turner NC (1979) Drought resistance and adaptation to water deficits in crop plants. In: Mussel H and Staples RC (eds) *Stress physiology in Crop Plants*. Wiley, New York, pp 181-194
- Utz (2000) PlabQTL Frequently asked questions. Internet source: [https://www.uni-hohenheim.de/fileadmin/einrichtungen/plant-breeding/software/pq\\_faq.txt](https://www.uni-hohenheim.de/fileadmin/einrichtungen/plant-breeding/software/pq_faq.txt). Accessed June 2013
- Utz HF, Melchinger AE (2006) PLABQTL: A computer program to map QTL. Version 1.2. Universität Hohenheim, Germany
- Valkoun JJ (2001) Wheat pre-breeding using wild progenitors. *Euphytica* 119:17-23
- Van Ooijen JW (2006) JoinMap®4 In: Kyazama BV (ed) *Software for the calculation of genetic linkage maps in experimental populations*, Wageningen, Netherlands
- Vetter A and Arnold K (2010) Klima- und Umwelteffekte von Biomethan: Anlagentechnik und Substratauswahl. [http://wupperinst.org/uploads/tx\\_wupperinst/WP182.pdf](http://wupperinst.org/uploads/tx_wupperinst/WP182.pdf). Accessed June 2013
- Villareal RL, Mujeeb-Kazi A, Rajaram S, Toro ED (1994) Morphological variability in some synthetic hexaploid wheats derived from *Triticum turgidum* x *T. tauschii*. *J Genet Breed* 48:7-16
- Walhardt R, Otte A, Simmering D, Ginzler O (2011) Biogas gegen Biodiversität?. *DLG-Mitteilungen* 3:20-23
- Walla C, Schneeberger W (2008) The optimal size for biogas plants. *Biomass Bioenerg* 32(6):551-57
- Wang H, Smith KP, Combs E, Blake T, Horsley RD et al (2012) Effect of population size and

- unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. *Theor Appl Genet.* 124(1):111-24
- Wegenast T, Longin CFH, Utz HF, Melchinger AE, Maurer HP, Reif JC (2008) Hybrid maize breeding with doubled haploids IV. Number versus size of crosses and importance of parental selection in two-stage selection for testcross performance. *Theor Appl Genet* 117:251-260
- Weiland P (2006) Biomass Digestion in Agriculture: A Successful Pathway for the Energy Production and Waste Treatment in Germany. *Eng Life Science* 6(3):302-309
- Weiland P (2010) Biogas production: Current state and perspectives. *Appl Microbiol Biotechnol* 85:849-860.
- Weipert D (1983) Brotroggen - Qualität und Beurteilung. *Die Mühle + Mischfuttertechnik* 39:517-521
- Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *PNAS* 101(26):9915-9920
- Wricke G (2002) Two major genes for kernel weight in rye. *Plant Breed* 121:26-28
- Xu Y and Crouch H (2008) Marker-Assisted Selection in Plant Breeding: From Publications to Practice. *Crop Sci* 48:391-407
- Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. *Theor Appl Genet* 77:353-359
- Yue B, Xiong L, Xue W, Xing Y, Luo L, Xu C (2005) Genetic analysis for drought resistance of rice at reproductive stage in field with different types of soil. *Theor Appl Genet* 111(6):1127-1136
- Zhao J (2002) QTLs for oil content and their relationship to other agronomic traits in an European x Chinese oilseed rape population. Dissertation, Georg-August University of Göttingen
- Zhao Y, Gowda M, Liu W, Würschum T, Maurer HP, Longin FH, Ranc N, Reif JC (2012) Accuracy of genomic selection in European maize elite breeding populations. *Theor Appl Genet* 124:769-776

## 8. Summary

Rye (*Secale cereale* L.) is a small grain cereal used for bread making, livestock feeding and as renewable energy source. These types of usages are leading to different breeding goals. Rye growing regions are affected by climate change and consequently by drought. Germany is touched by rainless periods in spring and early summer in the last years. Again, in spring 2012 farmers in Brandenburg and Lower Saxony were affected by drought periods. Yield losses in those regions, especially in combination with sandy soils are expected. Therefore much attention is paid for breeding of drought resistant germplasm.

Briefly, our objectives of this study were to (1) estimate the biomass and biogas potential of different plant materials, their quantitative genetic parameters and biogas-related traits, (2) analyze two recombinant inbred lines and differences in their yield potential between irrigated and rainfed regime, as well as the relative efficiency for indirect selection for drought resistance in irrigated regime, and (3) investigate the phenotypic performance for ten agronomic and quality traits across multiple environments and estimated the number and effects underlying QTL.

For the biomass-/ biogas analyses a wide range of plant material was analysed. Germplasm resources, full-sib families selected for grain and forage use were tested for their *per se* and testcross performance and experimental hybrids selected for grain use and population cultivars selected for grain and forage use were analyzed.

Dry matter yields varying across environments from 106 to 177 dt ha<sup>-1</sup> for *per se* and testcross performance, respectively. For testcross performance, germplasm resources showed similar values to forage rye. The later the maturity stage, the more dry matter yield on the whole plant level was achieved. Estimates of genotypic variances for biomass yield were significant for all rye materials, whereas the variances *per se* and for testcrosses were for germplasm resources exorbitant higher than for forage and grain rye.

Typical cumulative methane production curves were obtained for the whole plant material from the Hohenheim biogas yield test. Methane yield showed large differences between second and third harvest date for individual plant fractions. Differences between genotypes were not substantial for methane yield although significant in some instances. At EC77/83 hybrids and forage rye reached similar methane yield of about 5000 m<sup>3</sup>/ha. A high correlation between dry matter yield and methane yield was observed (r=0.95). Concerning

high cost and time consuming analysis of biogas tests, for breeders the main breeding goal should be maximum dry matter yield. Direct selection on dry matter yield should indirectly improve methane yield.

Two biparental populations were used for the analysis of drought tolerance. The analysis was performed in duplicate. Both populations were grown under irrigated and rainfed regimes. Striking less rainfall compared to long-term precipitation occurred between April and July, during critical phases of plant development. Grain yield reduction between irrigated and non-irrigated regime ranged from 2% to 29.6% for population A and 2% to 40% for population B, whereas differences between both regimes were significant ( $P < 0.05$ ) for five and four environments, respectively.

Genotypic variances of grain yield were significant in all instances, whereas genotype by irrigation interaction ( $\sigma^2_{G \times I}$ ) variance between both regimes being significant only in three and four environments for population A and B, respectively. Analysis across those environments revealed significant difference for  $\sigma^2_{G \times I}$  and the three-way interaction variance ( $\sigma^2_{G \times E \times I}$ ) in both populations. Heritability estimates were higher for the irrigated than for the rainfed regime. High interaction variance with environment and no clustering of the two regimes in a multi-dimensional analysis were found. This illustrates the different soil and weather conditions between locations and additionally every location suffered from a different drought stress.

The correlation between both regimes was significant but moderate, but genotypic coefficients considerably higher (Pop-A: 0.86, Pop-B: 0.84), which could be substantiated that testcrosses differed not substantially in drought-resistance. Indirect selection for drought in the irrigated regime was predicted to be equally or more efficient than direct selection in the non-irrigated regime.

Phenotypic and genotypic analysis was done across ten environments for both biparental populations for the general improvement of agronomic and quality traits in rye. Population A were genotyped with a Rye5K SNP array and for population B DArT genotyping was done with a 3K rye array. Additionally both populations were genotyped with about 150 SSRs. The genetic linkage maps comprised 1,819 and 1,265 markers for population A and B, respectively and were used for the QTL analysis for ten agronomic and quality traits.

Phenotyping revealed large genetic variation for ten agronomic and quality traits. Intensive phenotyping at up to ten environments led to moderate to high heritabilities. Across

environments explained genotypic variance of the individual QTL ranged from 5 to 55%. For 1000-kernel weight, test weight, falling number, and starch content, several QTL with high effects and a frequency of recovery of about 90% were identified in both population.

Rye suffered from drought stress in the last decade. Focusing on general improvement of rye regarding yield and quality, as well as improving rye regarding drought-resistance is important. Future research should be done in fine mapping and validation of the detected QTLs, for exploiting their potential in marker assisted breeding.

## 9. Zusammenfassung

Roggen (*Secale cereale* L.) ist eine kleinkörnige Getreideart, die zum Backen, in der Tierfütterung und seit ungefähr zehn Jahren auch als Nachwachsender Rohstoff für die Ethanol- und Methanproduktion genutzt wird. Als Fremdbefruchter ist er für Roggenzüchter besonders interessant und demzufolge auch für die Entwicklung von Hybridsorten. Hybridroggen wird in Deutschland auf ca. 60% der gesamten Roggenanbaufläche kultiviert. Traditionell sind die wichtigsten Zuchtziele der Kornertrag, die Wuchshöhe, die Lagerresistenz, das Tausendkorngewicht und die Auswuchsfestigkeit. Roggen ist gegenüber Weizen und Triticale wegen seiner höheren Leistungsfähigkeit in Deutschland und Polen vor allem auf leichten und sandigen Böden zu finden. In der Zeit zwischen April und Juni regnete es in diesen Regionen weniger als im Vergleich zum langjährigen Mittel. Dieser dadurch entstandene Trockenstress führte in den letzten Jahren zu Ertragsverlusten von bis zu 40%. Die Nutzung von Roggen als Substrat für die Methanproduktion und das wachsende Verlangen nach trockentoleranten Sorten stellen Züchter immer wieder vor neue Herausforderungen. Molekulare Methoden können helfen, kurzfristiger auf diese Ansprüche zu reagieren.

Die Hauptziele der vorliegenden Studie waren (1) die Schätzung des Biomassepotentials von verschiedenem Roggenmaterial und die der quantitativ genetischen Parameter, welche wichtig für die Selektion sind, (2) die Untersuchung genetischer Unterschiede für Biogas-bezogene Merkmale, (3) Analyse von zwei Testkreuzungspopulationen bezüglich des Kornertrages unter bewässerten und unbewässerten (regenabhängigen) Bedingungen, um Trockentoleranz zu ermitteln und (4) Untersuchung der genetischen Architektur von insgesamt 10 agronomischen Merkmalen und Qualitätsmerkmalen. Alle Experimente beinhalteten Elitematerial und wurden über mehrere Orte getestet (3 bis 5 Orte, 2 Jahre).

In der Biomasse- und Biogasanalyse wurden Genetische Ressourcen, Vollgeschwisterfamilien, Populationssorten und Experimentalhybriden auf ihre Eigen- und Testkreuzungsleistung untersucht. Für die Eigenleistung lag der durchschnittliche Trockenmasseertrag bei 110 dt ha<sup>-1</sup> und für die Testkreuzungsleistung bei 180 dt ha<sup>-1</sup>, wobei für letzteres die genetischen Ressourcen die gleichen Trockenmasseerträge wie der Grünschnittroggen erzielte. Die genotypische Varianz für den Biomasseertrag variiert signifikant für das komplette Roggenmaterial mit mittleren bis hohen Heritabilitäten.

Diese Ergebnisse weisen gute Perspektiven für eine weitere Selektion auf. Signifikante Unterschiede wurden für den Methanertrag, welcher im Hohenheimer Biogas-Ertragstest

gemessen wurde, bezogen auf Erntetermin und Pflanzenfraktionen ermittelt. Die Genotypen zeigen nur geringe Unterschiede in diesem Merkmal. Die beste Hybride erreichte ein Methanertrag von 5000 Nm<sup>3</sup>/ha bei der Ernte zur späten Milchreife. Eine hohe Korrelation zwischen Trockenmasseertrag und Methanertrag ( $r=0.95$ ,  $P<0.01$ ) wurde ermittelt. Eine direkte Selektion auf Trockenmasseertrag sollte eine indirekte Selektion auf Methanertrag ergeben.

Zwei biparentale Testkreuzungspopulationen (A, B) mit je 220 Nachkommen wurden bezüglich Trockentoleranz analysiert. Dabei wurden die Genotypen in einer bewässerten und in einer unbewässerten (regenabhängig) Variante auf sechs Umwelten angebaut. Verglichen mit dem langjährigen Mittel ist auffallend wenig Niederschlag zwischen April und Juni auf den vier Standorten gefallen. Aufgrund dieser Tatsache ist der Kornertrag durchschnittlich um rd. 26% niedriger ausgefallen. Die Heritabilitäten waren für die bewässerte Variante höher als für die Unbewässerte. Geringe, aber signifikante Genotyp x Bewässerungs-Interaktionsvarianz wurde in drei Umwelten für Population A und in vier Umwelten für Population B ermittelt. Die Korrelation zwischen den beiden Varianten war hoch (Pop-A: 0.86, Pop-B: 0.84). Die Genotyp x Umwelt x Bewässerungs-Interaktionsvarianz war ähnlich hoch wie die Genotyp x Bewässerungs-Interaktionsvarianz, was bedeutet, dass an jedem Ort ein unterschiedlicher Trockenstress vorherrschte. Zusammenfassend kann man sagen, dass sich Testkreuzungen nicht substantiell in ihrer Trockenstress Eigenschaft unterschieden. Für eine Vorhersage indirekter Selektion innerhalb der bewässerten Variante wurde eine gleiche oder sogar höhere Effizienz ermittelt, als bei der direkten Selektion für beide Varianten.

Um die genetische Architektur von 10 agronomischen Merkmalen und Qualitätsmerkmalen zu analysieren, wurden beiden Populationen mit 800 bis 900 molekularen Markern genotypisiert und in zehn Umwelten phänotypisiert. Signifikante genotypische Variation wurde für alle Merkmale mit einer moderaten bis hohen Heritabilität (0.33-0.92) ermittelt. Für die Population A wurden 31 und für die Population B 52 *quantitative trait loci* (QTL) detektiert, welche 4% bis 74% der gesamten genotypischen Varianz pro Merkmal erklärten. Für die Merkmale Tausendkorngewicht, Hektolitergewicht, Fallzahl und Stärkegehalt konnten einzelne QTL mit großen Effekten detektiert werden, welche mögliche Kandidaten für markergestützte Selektion sind.

Zusammenfassend sollte Hybridroggen als Substrat für die Produktion von Biogas möglichst hohe Biomasseerträge erbringen. Diese können durch die Selektion von genetisch breiten Züchtungspopulationen oder durch die Kreuzung von Genetischen Ressourcen als Bestäuber erreicht werden. Um die Ertragsstabilität und Trockentoleranz zu erhöhen, sollte man unbewässerte Orte auf leichten und sandigen Böden in das Panel der Orte für die Selektion mit einbeziehen. Experimentalhybriden, welche unter diesen Bedingungen vorselektiert sind, könnten direkt auf ihren Biomasseertrag und Trockentoleranz in speziellen Versuchen getestet werden. Einige QTL mit großen Effekten könnte der Züchter in Zukunft nutzen, um wechselnde Züchtungsziele in einem verkürzten Zeitraum zu erreichen.

## **10. Acknowledgements**

One of the joys of completion is to look over the journey past my time at the State Plant Breeding Institute in Hohenheim and remember all the friends and family who have helped and supported me along this long road.

Foremost, I would like to thank my advisor Prof. Dr. Miedaner for providing me the opportunity to complete my PhD thesis in his working group. Many thanks for encouraging me in your personal way, with passion and constructive feedback.

This project was financially supported by the Federal Ministry of Food, Agriculture and Consumers Protection (BMELV, Bonn) via the Agency of Renewable Resources (FNR). I would like to thank for their generous support. I also wish to extend a big thank to Peer Wilde, as well as to the teams at KWS Lochow and Universität Hohenheim for their technical assistance in data collection and help at the field. To name them, I would like to thank Silvia, Bärbel, Heike, Siggi, Mark, Marlene, Britta, Jenny and Jörn.

I want to express a sincere thank you to present and past members and friends of the whole Institute: Christoph, Alex, Matthias, Sebastian, Stefan, Diana, Rasha, Firas, Maren, Peter, Hans-Henning, Christiane, Delphine, Vanessa, Manje, Franciele, Christian, Simon as well as all the colleagues of Institute 350 and 720 – I always felt very welcome in Hohenheim, and I met some very kind and friendly people!

I would like to express my special thanks to Jana Steinhoff and Katharina Alheit:

Kaddi, I want to say my deepest gratitude for your endless help in reading my papers and thesis, providing critical questioning scientifically, but rather personally. Thank you for your friendship!

Jani, thank you for your time being my house mate in Plieningen, and our endless hours talking about life on the balcony. I would like to express my gratitude thanks for being my friend.

I would also like to thank my friends and family in Hofheim. My special thanks goes to my HIFFT friends, helping me to survive the last year with great parties, weekends, trips and concerts. As well, I want to thank Matt for providing me the time to finish my PhD and Klaus for setting up the action plan and supporting me.

Finally, I would like to thank my husband Michi for his sincere love, being on my side and supporting me in every situation. The last year was a strenuous time. I like to thank you from the bottom of my heart!

## 11. Curriculum vitae

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## **12.**

### **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.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Stuttgart, März 2014

Marlen Gottwald