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Phenotypic, Genetic, and Genomic Assessment of Triticale Lines and Hybrids

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ABBREVIATIONS

BM	biomass
CHAs	chemical hybridizing agents
CMS	cytoplasmic male sterility
DH	doubled haploid
DON	trichothecene deoxynivalenol
F ₂	second filial generation
FAO	Food and Agriculture Organization of the United Nations
FHB	<i>Fusarium</i> head blight
GCA	general combining ability
GS	genomic selection
GWAS	genome-wide association study
GY	grain yield
h^2	heritability
kg ha ⁻¹	kilogram per hectare
kg hL ⁻¹	kilogram per hectoliter
LM	linkage mapping
MAS	marker assisted selection
Mg ha ⁻¹	megagram (ton) per hectare
PIC	polymorphism information content
p_G	proportion of explained genotypic variance
QTL	quantitative trait locus
r	(Pearson product-moment) correlation coefficient
R ²	coefficient of determination
<i>Rht</i>	reduced height
SCA	specific combining ability
TKW	thousand kernel weight
YR	yellow rust

1 GENERAL INTRODUCTION

The first man-made cereal crop triticale (\times *Triticosecale* Wittmack) was initially described by the Scottish botanist Stephan Wilson in 1875 (WILSON 1876), resulting from an intergeneric hybridization of the genus *Triticum* as female and rye (*Secale cereale*) as male parent. However, he obtained only sterile plants. The first fertile offspring was reported by the German plant breeder Wilhelm Rimpau in 1888 (RIMPAU 1891). Over time, triticale was produced from different species of wheat, but hexaploid triticale ($2n = 6\times = 42$; AABBRR) arose from a cross between durum (*Triticum durum*) and rye and has become the most effective way to produce primary triticale because of its crossability, superior vitality and performance. The hybridization is followed by an embryo culture and a colchicine treatment, which results in a doubling of the chromosome set. The first commercial varieties were released in the late 1960s in Hungary (No. 57, 64), Mexico ('Armadillo'), Spain ('Cachirulo'), and Canada ('Rosner') with secondary triticale being produced by combining two primary triticales or by crossing primary triticale with bread wheat (*Triticum aestivum*) or rye (OETTLER 2005; MIEDANDER 2014). The primary goal was to combine the yield potential and nutritional quality of wheat with the disease resistance and environmental tolerance of rye. Nowadays, newly approved triticale varieties are mainly crosses between elite triticale lines. To enlarge the gene pool, wheat and rye were sometimes used as crossing partners followed by backcrosses, since there are no wild ancestors or landraces available as source of genetic variation. Today, molecular markers provide a powerful tool to investigate the genetic diversity on a genome-wide level.

Triticale is cultivated on a global scale with main production areas in Central and Eastern Europe, where Poland, Germany, France, Belarus, and Russia are the largest producers. The current global production quantity reaches about 17 million tons and 90% are produced in Europe (FAO 2015). DOGAN ET AL. (2009) report that 80% of worldwide grown triticale are winter types. Primarily, triticale is used as feed grain especially for pigs, but it is also well recognized as an energy crop, e.g. in the field of bioethanol production. In the last decades, new market segments were discovered by using the whole crop as a renewable energy source for the production of biogas (MIEDANER 2014).

TRITICALE HYBRID BREEDING

Ever since the beginnings of triticale breeding, the crop was treated as a classical self-pollinating species and the applied breeding methods are similar to the breeding schemes of barley (*Hordeum vulgare*), oat (*Avena sativa*), and wheat (*Triticum aestivum*) (BECKER 2011). Current homozygous cultivars are developed by line breeding methods, both for winter and spring types. Triticale possesses a larger degree of cross-pollination than wheat, with an estimated mean outcrossing rate of about 10% (OETTLER 2005). The crop is therefore well suited for hybrid breeding (GÓRLAN ET AL. 2015).

In Europe, two different systems for hybrid seed production in cereal crops are mainly used: (i) chemical hybridizing agents (CHAs), and (ii) cytoplasmic male sterility (CMS) (LONGIN ET AL. 2012). The different systems for hybrid seed production have been reviewed in detail by WHITFORD ET AL. (2013). Serious interest in triticale hybrids by public and private research has started in the 1980s (NALEPA 1990). This applied research resulted in the worldwide first released triticale hybrid variety ‘HYT Prime’ of the German plant breeder Elmar Weissmann in the year 2010 (MIEDANER 2014), developed by a CMS-system based on the cytoplasm of Zanduri wheat (*Triticum timopheevii*).

A main benefit of hybrids is the maximum exploitation and systematic use of heterosis, which may result in a higher yield potential in comparison with line varieties (LONGIN ET AL. 2014). Yield stability of hybrids is an additional important point for hybrid breeding, especially in marginal environments (HALLAUER ET AL. 1998). Different studies have shown that the amount of heterosis may depend on the used hybridization system. For instance, heterosis for grain yield was reported to be about 10% in the context of CHA based systems (OETTLER ET AL. 2001; FISCHER ET AL. 2010), whereas GOWDA ET AL. (2013) and MÜHLEISEN ET AL. (2015) observed a lower level of mid-parent heterosis in CMS-based triticale hybrids of about 2% or 3%, respectively.

TRITICALE BIOMASS BREEDING

Crop biomass can be used as a renewable source of energy and the world-wide future demand of this bioenergy from agricultural-derived substrates is increasing continuously. It offers considerable environmental benefits such as a significant net carbon emission reduction, which may be realized if fossil resources were replaced with increased agricultural biomass production (CHYNOWTH 2004; WIT AND FAAIJ 2010; POUDEL ET AL. 2011). Moreover, in the last decade the extension of the cultivation area was also driven by governmental policies. The increase of area under bioenergy crops results in a competition for land used for food and feed production or for biomass production (PIMENTEL ET AL. 2009). Consequently, energy crops should have a high biomass yield potential, along with minimal use of fertilizer and pesticides (BOEHMEL ET AL. 2008).

The most dominant crop for biomass production from agricultural energy plants in Central Europe is maize (*Zea mays*) grown for silage (AMON ET AL. 2007). Other plants, such as alfalfa (*Medicago sativa*), rye (*Secale cereale*), sugar beet (*Beta vulgaris*), triticale (\times *Triticosecale*), and wheat (*Triticum aestivum*) were also used as biomass crops in Central Europe. Although the biomass yield is lower compared to maize (WEILAND 2010), triticale yielded a higher whole-plant silage than other small grain cereals (CHRISTEN 2009). Triticale is therefore ideally suited to diversify narrow crop rotation for biomass production areas with maize as main crop, as it utilizes winter soil moisture and provides an opportunity for intercropping (BAUBÖCK ET AL. 2014).

Since only a niche market for whole-plant biomass existed previously, most triticale studies focused on grain yield. So far, little research was conducted in the context of biomass yield potential of triticale in both lines and hybrids.

In a recent study, large genetic variation for biomass yield was observed which ensures future selection gain (GOWDA ET AL. 2011). Nevertheless, a detailed investigation about the biomass yield potential and differences between hybrids, their parents and line varieties was missing so far.

TRITICALE DIVERSITY ASSESSMENT AND BREEDING PROGRESS

A fundamental prerequisite for the design of efficient breeding programs in triticale is detailed knowledge about phenotypic (Figure 1) and genetic diversity. In case of small grain cereals, many phenotypic studies have been conducted in wheat and rye, but relatively few attempts have been made in triticale (OETTLER ET AL. 2003; GOWDA ET AL. 2011; LIU ET AL. 2014; BOEVEN ET AL. 2016). There is no doubt that yield is the most important agronomic trait, but other plant traits such as plant height, heading time, lodging, disease resistance and other grain characteristics are crucial factors that influence yield (OETTLER 2005).



Figure 1: Phenotypic variation in triticale yield trials.

Important aspects for a continued and long-lasting breeding progress are the availability of a broad genetic variation and a wide range of the evaluated traits combined with a high mean (BERNARDO 2010). For the design of breeding programs and the comparison of different breeding schemes, robust estimates of relevant quantitative genetic parameters (first and second degree statistics and the pattern of phenotypic trait correlations) are of utmost importance for every plant breeder. Phenotypic trait correlations indicate how direct selection for one trait will affect another trait (LUBY AND SHAW 2009). So far, only few studies on phenotypic trait correlations are available in triticale and no study examined the influence of the current yellow rust (*Puccinia striiformis*) epidemic, which is spreading over Europe since 2011.

Analysis of genetic diversity assists the selection of adequate genetic material for various breeding schemes and has a significant effect on the improvement of new cultivars. Molecular markers provide a useful tool to measure genetic characteristics such as familial relatedness, allelic diversity and population structure on a genome-wide scale. Apart from these aspects, analysis of genetic diversity based on molecular markers is necessary for the construction of genetic maps and approaches such as association mapping or genomic selection become feasible (WÜRSCHUM AND KRAFT 2015).

Suitable approaches for the analysis of genetic relationships among different triticale cultivars are principal coordinate analysis (GOWER 1966) and phylogenetic trees (PAVLOPOULOS ET AL. 2010). Polymorphism information content (PIC) can be used as a measure of allelic diversity generally as well as within clusters such as time periods in which cultivars were registered. So far, only a limited number of markers have been used in triticale (TAMS ET AL. 2004, 2005). Recent advances in genomics offer new tools that allow analyzing the whole genome with high marker coverage also in triticale (BADEA ET AL. 2011).

The increased agricultural productivity in the past decades can be explained by the following key reasons: (i) improved agronomic cultivation methods, (ii) changes in environmental conditions, and (iii) breeding progress. Yield trends can be estimated from historical or joint trials, where cultivars to be tested are grown side-by-side. Testing of cultivars bred in different periods, but grown side-by-side, displays purely the genetic long-term trend based on breeding efforts (PIEPHO ET AL. 2014). LAIDIG ET AL. (2014) showed triticale results from historical German official variety performance trials and additionally provided a good summary of published studies on yield trends. While long-term trend analyses of yield and other agronomical relevant traits from side-by-side cultivation are available for small grain cereals such as wheat (AHLEMEYER AND FRIEDT 2010; LOPES ET AL. 2012), joint variety trials in triticale were lacking so far.

MAPPING QUANTITATIVE TRAIT LOCI

Many phenotypic traits with agronomic importance and a continuous trait distribution are usually inherited by many genes. Single gene positions are designated as quantitative trait loci (QTL) and each QTL has a different individual contribution on the expression of the complex trait depending on the genetic architecture (MIEDANER 2010; WÜRSCHUM 2012). During the past quarter of a century, classical linkage mapping (LM) analysis employing bi-parental populations detected many QTL and LM has become a routine tool in plant breeding (BERNARDO 2008). However, LM has three fundamental limitations: (i) only the allelic diversity segregating between the crossing parents of the F₂ progeny can be examined, (ii) the allele frequencies will differ from total existing population (KORTE AND FARLOW 2013), and (iii) the direct transfer of QTL, detected by LM, to different populations and genetic backgrounds is difficult due to an overestimation of the proportion of explained genotypic variance (MELCHINGER ET AL. 1998). Moreover, the power to detect QTL is greatly dependent on the employed population size (SCHÖN ET AL. 2004). On the other hand, multiple-line cross QTL mapping is a more recent mapping approach based on different genetic backgrounds by multiple families that are combined in a mapping population for a joint analysis (BLANC ET AL. 2006) and promises an increased power of QTL detection.

Genome-wide association mapping studies (GWAS) are a relatively new method to identify genes involved in the expression of plant traits. It is a powerful tool to dissect the genetic basis of simple or complex traits, i.e. to investigate their genetic architecture, and to overcome the limitations of classical QTL mapping (YU ET AL. 2006). GWAS have been developed by human geneticists (HIRSCHHORN AND DALY 2005) and are nowadays also widely used in plant genetics (ZHU 2008; WÜRSCHUM AND KRAFT 2014). The strength of association mapping lies in the detection of QTL by examination of marker-trait associations across a set of diverse germplasm (BERNARDO 2010). The availability of new methods for high-throughput genotyping makes it possible to use GWAS more efficiently. In small grain cereals, GWAS has been successfully applied in studies with several plant species revealing the genetic control of many different traits (e.g. REIF ET AL. 2011; PASAM ET AL. 2012). In triticale, however, the number of GWAS is limited (e.g. ALHEIT ET AL. 2012, 2014; WÜRSCHUM ET AL. 2014a).

TRITICALE DISEASE RESISTANCE BREEDING

Resistance breeding is an ongoing dynamic process depending on the host, the environment, and the pathogen. An increasing growing area and the prevalence of few cultivars with very high market share might favor the quick spread and evolution of pathogens. A few decades ago, triticale had the image of a healthy crop, but biotic stress, in particular fungal pathogens, became a serious problem in triticale production during recent years (SCHINKEL 2002; MENARDO ET AL. 2016). Triticale can be affected by the same fungal diseases known in wheat and rye, which can lead to significant losses in yield and reduced grain quality. Additionally, a host-range expansion from wheat to triticale was observed (WALKER ET AL. 2011; TROCH ET AL. 2012). Today, triticale is affected by several fungal pathogens such as brown rust (*Puccinia recondite*), *Fusarium* head blight (*F. graminearum*, *F. culmorum*), powdery mildew (*Blumeria graminis*), Septoria leaf blotch (*Septoria tritici*) and yellow rust (*Puccinia striiformis*) (OETTLER 2005; AUDENAERT ET AL. 2014).

A broad disease resistance is very important for the often applied low-input farming strategy in triticale. In general, it can be distinguished between two categories of host resistance in crops. Qualitative resistance is conferred by a single resistance gene and quantitative resistance is passed on by multiple genes (e.g. MIEDANER ET AL. 2011, 2012). Although quantitative resistance genes transmit only small resistance effects, the combination of many non-race-specific genes can considerably improve plant health (MIEDANER AND KORZUN 2012) and is more durable than race-specific qualitative resistance genes (BROWN AND TELLIER 2011).

The detection of durable disease resistances is a major challenge in triticale breeding. Molecular markers offer a powerful means to unravel the genetic architecture of resistance traits. This knowledge might then be used for marker-assisted selection (MAS) and genomic selection (GS). MAS uses molecular markers in linkage disequilibrium with QTL of interest. GS is a new form of MAS that estimates all marker effects across the whole genome to calculate genome estimated breeding values. GS uses two types of datasets, a training population and a validation set. The training population is genotyped and phenotyped, whereas the validation set is only genotyped and predicted based on marker effects estimated in the training set (BERNARDO 2016).

OBJECTIVES OF THIS STUDY

The main goal of this thesis was the phenotypic, genetic and genomic assessment of important traits with relevance for triticale line and hybrid breeding.

More specifically, the objectives of this study were to

1. evaluate agronomic traits, trait correlations, and the amount of heterosis in triticale hybrids,
2. examine the potential of line and hybrid cultivars for biomass production,
3. assess the phenotypic and genotypic diversity of triticale germplasm,
4. investigate long-term trends in phenotypic triticale performance based on varieties registered in the past three decades, and
5. unravel the genetic control of important agronomical traits in triticale.

2 PUBLICATION I: HYBRID BREEDING FOR BIOMASS YIELD IN WINTER TRITICALE: I. HYBRID PERFORMANCE, TRAIT CORRELATIONS AND HETEROSIS

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Abstract

Triticale (\times *Triticosecale* Wittmack) holds great potential as a source for biomass production for industrial applications in Central Europe and hybrid breeding in particular appears promising owing to the higher vigor of hybrids compared to lines. In this study, a set of 178 winter triticale genotypes, including 91 hybrids, their 10 male and 23 female parental lines, as well as 54 varieties were evaluated for biomass yield and other agronomically relevant traits in two years at five locations in Germany. We observed a wide range of dry biomass yield as well as significant genotypic variances and high heritabilities for all traits. For the hybrids, a moderate correlation was observed between biomass and plant height and between biomass and grain yield. Mid-parent heterosis of biomass yield ranged from -13.6 to 16.5% with an average of 4.8% and the maximum commercial heterosis was 9.1%. Taken together, our results illustrate the potential of hybrid breeding of triticale for biomass yield in order to diversify our portfolio of crops for biomass production.

3 PUBLICATION II: PHENOTYPIC AND GENOTYPIC ANALYSES OF DIVERSITY AND BREEDING PROGRESS IN EUROPEAN TRITICALE (\times TRITICOSECALE WITTMACK)

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Abstract

Knowledge of the extent of phenotypic variability, genetic diversity and the realized breeding progress is central for the optimum design of breeding programs but little information is available for triticale (\times Triticosecale Wittmack). In this study, a collection of 885 diverse European triticale lines was evaluated in multi-location field trials in two years. We observed significant genotypic variances and high heritabilities for several agronomic and morphological traits and significant correlations among different traits including grain yield. Based on a subset of the population of 121 varieties registered in Europe between 1983 and 2014, we observed a substantial breeding progress for grain yield with a significant rate of increase of 53 kg ha⁻¹ or 0.67 % per year. All lines were genotyped by a genotyping-by-sequencing approach yielding 58,888 polymorphic markers. Our analyses revealed the absence of major population structure but a certain grouping of lines according their origin. Taken together, our results on triticale germplasm and its breeding history provide important information for breeding programs and future selection gain in this crop.

4 PUBLICATION III: MULTIPLE-LINE CROSS QTL MAPPING FOR GRAIN YIELD AND THOUSAND KERNEL WEIGHT IN TRITICALE

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Abstract

Grain yield and its component trait thousand kernel weight are important traits in triticale breeding programmes. Here, we used a large mapping population of 647 doubled haploid lines derived from four families to dissect the genetic architecture underlying grain yield and thousand kernel weight by multiple-line cross QTL mapping. We identified 3 QTL for grain yield and 13 for thousand kernel weight which cross-validated explained 5.2% and 48.2% of the genotypic variance, respectively. Both traits showed a positive phenotypic correlation, and we found two QTL overlapping between them. Full two-dimensional epistasis scans revealed epistatic QTL for both traits, suggesting that epistatic interactions contribute to their genetic architecture. Based on QTL identified in our results, we conclude that the potential for marker-assisted selection is limited for grain yield but more promising for thousand kernel weight.

5 PUBLICATION IV: DEFEATING THE WARRIOR: GENETIC ARCHITECTURE OF TRITICALE RESISTANCE AGAINST A NOVEL AGGRESSIVE YELLOW RUST RACE

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Abstract

Yellow rust is an important destructive fungal disease in small grain cereals and the exotic ‘Warrior’ race has recently conquered Europe. The aim of this study was to investigate the genetic architecture of yellow rust resistance in hexaploid winter triticale as the basis for a successful resistance breeding. To this end, a diverse panel of 919 genotypes was evaluated for yellow rust infection on leaves and ears in multi-location field trials and genotyped by genotyping-by-sequencing as well as for known *Yr* resistance loci. Genome-wide association mapping identified ten QTL for yellow rust resistance on the leaves and seven of these also for ear resistance. The total genotypic variance explained by the QTL amounted to 44% for leaf and 26% for ear resistance. The same three medium-effect QTL were identified for both traits on chromosomes 1B, 2B, and 7B. Interestingly, plants pyramiding the resistance allele of all three medium-effect QTL were generally most resistant, but constitute less than 5% of the investigated triticale breeding material. Nevertheless, a genome-wide prediction yielded a higher predictive ability than prediction based on these three QTL. Taken together, our results show that yellow rust resistance in winter triticale is genetically complex, including both medium-effect QTL as well as a quantitative resistance component. Resistance to the novel ‘Warrior’ race of this fungal pathogen is consequently best achieved by recurrent selection in the field based on identified resistant lines and can potentially be assisted by genomic approaches.

6 GENERAL DISCUSSION

The main objective of this thesis was to identify the phenotypic, genetic, and genomic basis of important traits with significance for triticale line and hybrid breeding. Breeding for agronomic traits including grain and biomass yield, primary yield components, plant height, lodging tolerance, and disease resistance are the most important breeding goals. As part of this thesis, various experiments were performed including about 23.000 evaluated field plots and their interpretation in terms of applied triticale plant breeding (Table 1).

Table 1: Summary of integrated projects

Project	BreedVision	HyEnergy	Predbreed	
			Diversity	Biomass
Genotypes	647	178	885 – 919	25
Plant material	647 DH lines (4 families)	10 male lines, 23 female lines, 91 hybrids, 54 listed varieties	142 listed varieties, 777 breeding lines	20 listed varieties, 5 listed hybrids
Environments (year x location)	4	8	11	6
Trial plots	3,840	1,424	16,880	900
Evaluated traits	GY, TKW	BM, HT, PH	GY, HLW, HT, PC, PH, PM, ST, TKW, YRE, YRL	BM, FHB, GY, HI, HT, PH
Methods	Phenotypic analysis, genotypic analysis (multiple-line cross QTL mapping)	Phenotypic analysis	Phenotypic analysis, genotypic analysis (allelic diversity, GWAS, genomic prediction)	Phenotypic analysis

Evaluated Traits: biomass (BM), Fusarium head blight (FHB), grain yield (GY), harvest index (HI), hectoliter weight (HLW), heading time (HT), protein content (PC), plant height (PH), powdery mildew (PM), starch (ST), thousand kernel weight (TKW), yellow rust leaf (YRL), yellow rust ear (YRE)

TRITICALE HYBRID BREEDING

Hybrid breeding was one of the most important and lasting inventions in cross-fertilized (allogamous) plants such as maize (*Zea mays*) and rye. Until now, the success of hybrids in small grain self-fertilized (autogamous) cereals such as wheat, barley, and triticale is still limited, but the success in outcrossing crops has motivated the interest for the selfing species. Despite the moderate success until now, large public and private projects investigating hybrid breeding in autogamous cereals have recently been launched. Expected benefits of hybrids versus lines are increased grain or biomass yield, which are still the main goals in breeding programs (LONGIN ET AL. 2012), but the major advantage of hybrids versus lines is the exploitation of heterosis (SHULL 1908).

To breed new hybrid varieties that are superior to their parents and currently existing commercial line varieties, it is necessary for triticale breeders to have a detailed knowledge of the genetic relationships and diversity on a whole genome level. Genetic diversity in triticale plays a key role for future breeding progress and can be accessed on the genomic level by molecular markers (MELCHINGER AND GUMBER 1998). However, genetic diversity in triticale is rated as rather narrow (KULEUNG ET AL. 2006). Knowledge of germplasm diversity has a significant impact on triticale breeding, as it is the basis for the selection of crossing parents and the establishment of heterotic groups (TAMS ET AL. 2005). A heterotic group defines a set of genotypes which create a similar hybrid performance when crossed with another genetically different set of genotypes, while a heterotic pattern refers to a specific pair of heterotic groups showing an optimum exploitation of heterosis (MELCHINGER AND GUMBER 1998). Crosses between varieties from different heterotic groups result in vigorous hybrids with significantly more heterosis than hybrids from lines within the same heterotic group or pattern.

In accordance with previous studies on genetic diversity of European triticale (TAMS ET AL. 2004, 2005; ALHEIT ET AL. 2012), no major population structure was observed in a set of 885 triticale varieties. However, sets of genotypes from the same breeding companies tended to cluster (Publication II). The absence of a major population structure might be explained by the short breeding history of triticale, the predominant line breeding method, and a constant exchange of germplasm between geographical

origins as well as breeding companies. For instance, the same holds true for wheat (WÜRSCHUM ET AL. 2013; BOEVEN ET AL. 2016).

Furthermore, the genotypes from one breeding company showed a certain grouping into lines from the maternal and paternal pool, which may reflect the efforts of HegeSaat GmbH & Co. KG to install a hybrid breeding program and create heterotic groups for the identification of a high-yielding heterotic pattern. The presence of such groups can lay a solid foundation of an efficient hybrid breeding program. However, in triticale, the evaluation of such heterotic groups is only at the very initial stage (FISCHER ET AL. 2010). For the selection of the best inbred line as parental component for hybrid production, line per se performance followed by general combining ability (GCA) has to be tested. Due to the moderate correlation between line per se and hybrid performance, only good performing lines should be tested for their GCA. For this purpose, lines are crossed with one or more testers from the opposite gene pool. The primary target of triticale hybrids is high performance in the trait of interest. Hybrid performance can successfully be predicted by the relation between GCA and specific combining ability (SCA) (MELCHINGER ET AL. 1987), for which the ratio is lower for established heterotic groups (REIF ET AL. 2007).

Midparent heterosis, being a component of hybrid performance, is also relevant for a successful hybrid breeding (FISCHER ET AL. 2010). In this thesis a multi-location study with a set of 91 hybrids, their ten male and 23 female parental lines were evaluated for yield and other agronomical relevant traits (Publication I). The hybrids showed a marginal grain yield advantage compared to their parents of 2.3%, which is considerably smaller than the average mid-parent heterosis of 4.8% observed for biomass yield. Mid-parent heterosis of biomass and grain yield correlated significantly as well as both traits showed a high correlation. This indicates that a program targeting grain yield can profit from the progress made in programs aimed at increasing biomass yield also for hybrid breeding. Several studies agree that grain yield increases in hybrids are often associated with high heterosis for biomass (MORGAN ET AL. 1989; OURY ET AL. 1993; PICKETT 1993). If breeders of line varieties fail to increase biomass production by fixation of heterosis in inbred lines, then hybrids might become a practicable solution to enhance triticale grain yield.

TRITICALE BIOMASS BREEDING

Previous enhancement in grain yield potential of line and hybrid varieties was mainly attributed to an increased harvest index, resulting from shorter plants, larger ears, and higher number of grains (AUSTIN ET AL. 1989). The term harvest index refers to the ratio between grain yield and the total amount of above-ground biomass. So far, the increase of total biomass production has not been taken into account and only few efforts were undertaken to increase biomass yield by a specific breeding program for biomass triticale.

HAY (1995) indicates a progressive increase in harvest index from 0.36 to 0.48 for wheat, combined with an increasing grain yield and a constant total biomass yield between 1900 and 1980. In addition, he reported for triticale comparable harvest indices between 0.45 and 0.47 in the 1980s. Our analysis including 25 recent triticale cultivars showed a mean harvest index of 0.56 with a maximum of 0.62 (Figure 2), a value that has been widely accepted as the upper limit (AUSTIN 1980; CROWLEY ET AL. 1993). The harvest index of modern cultivars is probably nearing its upper theoretical limit. Consequently, selection gain for grain yield can be achieved only by simultaneously increasing biomass yield, which has been observed in maize (TOLLENAAR ET AL. 1994).

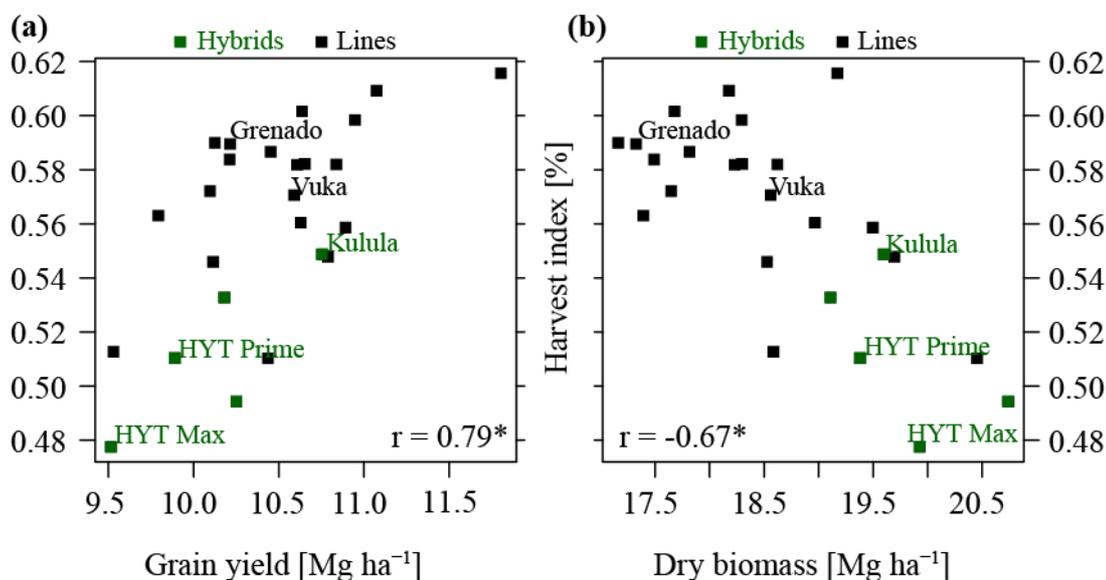


Figure 2: Scatter plots (a) between harvest index and grain yield and (b) between harvest index and dry biomass for five hybrids (green squares) and 20 commercial lines (black squares).

In the present thesis, multi-environmental field trials in split-plot designs were investigated, involving in total 25 genotypes, five hybrids and 20 commercial lines, each replicated six times per environment. Two replications per environment were chopped for direct evaluation of biomass, four replications were threshed to determine the grain yield. To evaluate resistance to *Fusarium* head blight (FHB), artificial inoculation (MIEDANER ET AL. 1996) was performed in two of the threshed replications. In the fungicide treated replication, hybrids possessed a similar amount of grain yield, more above-ground dry biomass, and a distinctly lower harvest index compared to commercial lines (Figure 2). In a previous study, KINDRED AND GOODING (2005) found that greater biomass in hybrids might be due to a combination of greater light interception and radiation use efficiency.

In addition, hybrid performance was investigated within this study, as well as correlations among agronomic relevant traits and heterosis in 91 single-cross triticale hybrids, derived from crosses between 23 female and ten male lines (Publication I). Phenotypic variability was evaluated for heading time, plant height, fresh biomass, dry matter content, and dry biomass. Phenotypic data for grain yield was taken from a parallel experiment including the same hybrids (MÜHLEISEN ET AL. 2015). Biomass yield in the hybrids was substantially higher than that of the parental lines. Hybrids showed the same range for dry biomass than the current registered varieties, although the parental lines of the hybrids were very old and not up-to-date cultivars with comparably low yield performance (HANS PETER MAURER, personal communication). Nevertheless, one third of the hybrids outperformed the three reference varieties for biomass of the German Federal Plant Variety Office (Bundessortenamt), which shows the potential of hybrid breeding of triticale for biomass utilization.

Dry biomass showed a moderate-strong correlation with plant height for the hybrids and a strong correlation for the line varieties, which agrees well with the results from GOWDA ET AL. (2011) and ALHEIT ET AL. (2014). Plant height can therefore be referred to as a key contributor to biomass yield in triticale. To sum up, these study results underscore the potential of a triticale breeding for biomass yield. However, an essential paradigm shift is necessary, namely the change from shorter plants with high grain yield to taller cultivars, with additional breeding efforts for lodging resistance.

TRITICALE BREEDING PROGRESS

Early released triticale cultivars were characterized by low grain yield, poor physical grain quality, high protein content, exorbitant height, lodging problems and a good disease resistance. Efforts in triticale breeding resulted in considerable improvements, particularly in grain yield and lodging tolerance (OETTLER 2005; RANDHAWA ET AL. 2015).

Recent triticale varieties very often surpass the grain yield potential of wheat varieties especially grown under mediocre conditions (BASSU ET AL. 2011; ESTRADA-CAMPUZANO ET AL. 2012; BLUM 2014). Average grain yield of triticale increased from 2.7 Mg ha⁻¹ in 1968 (ZILLINSKY AND BORLAUG 1971), up to 8.2 Mg ha⁻¹ in 1983 and 9.9 Mg ha⁻¹ in 2015 (Publication II and Figure 3).

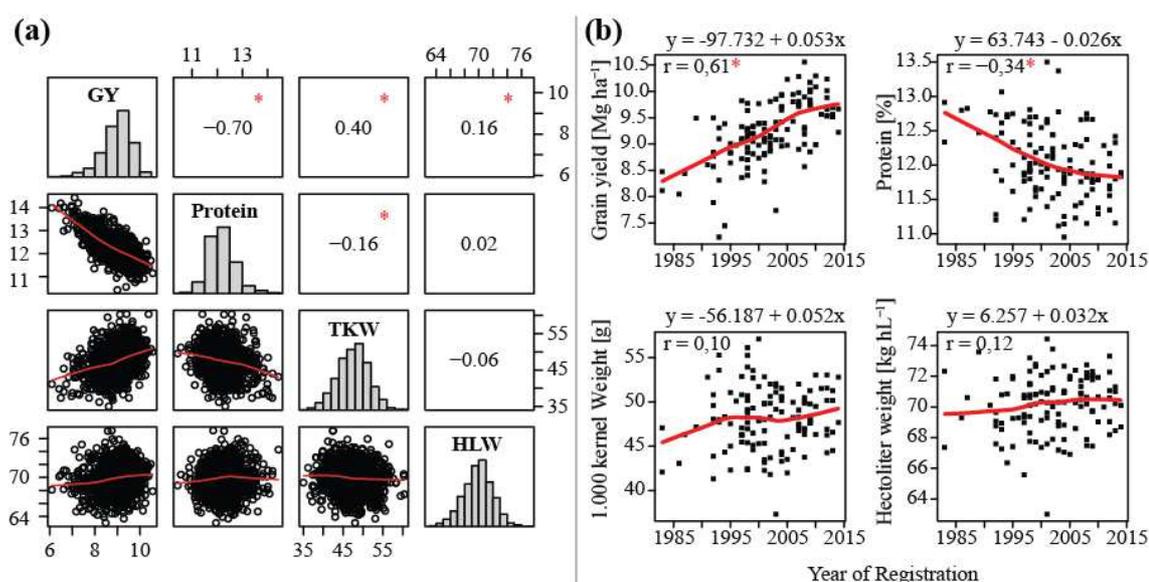


Figure 3: Correlation plots (a) for agronomical relevant traits (grain yield 'GY' in Mg ha⁻¹, Protein in %, thousand kernel weight 'TKW' in g, hectoliter weight 'HLW' in kg hL⁻¹) of 885 triticale genotypes and (b) among traits and year of release of 121 triticale cultivars. * significantly different from zero at $P < 0.001$ level of probability.

One of the most significant improvements in triticale breeding is a fuller or plumper grain determined by test or hectoliter weight (kg hL⁻¹) (OETTLER 2005). As can be seen within Figure 3a, a very weak positive correlation between grain yield and hectoliter weight could be found. DOGAN ET AL. (2009, 2016) also reported positive and

significant correlations between these two traits, while FURAN ET AL. (2005) found a negative correlation. OETTLER (2005) emphasizes the role of wrinkled, shriveled grains with abnormal endosperm formation as one of the most serious problems of the early triticale varieties. The test weight of the best line in 1968 at CIMMYT was 65.8 kg hL^{-1} (ZILLINSKY AND BORLAUG 1971) compared to 73.0 kg hL^{-1} of the best line in the 1990s (HERRMANN AND SCHINKEL 2000). The best variety in the present experiment, which comprised of 885 triticale genotypes, reached an even higher hectoliter weight of 76.8 kg hL^{-1} . Although slowly, constant progress in improved test weight has been achieved, which underscores the potential of selection progress to improve this former critical trait that is significantly associated with grain yield.

Early triticale genotypes with wrinkled kernels had higher protein concentrations ranging between 14 and 20% compared to 11 to 13% of currently released cultivars. Protein content and quality are important properties whereby improvements are desirable for animal feed as well as for human consumption. OETTLER (2005) concluded that protein concentration decreased as a consequence of increased plumpness. Although this study also observed a significant reduction of protein content over the years and a strong correlation between grain yield and protein concentration, no trait association between protein content and hectoliter weight could be identified (Figure 3a).

Grain yield in cereals is a product of the three primary yield components: (i) thousand kernel weight (TKW), (ii) grains per ear, and (iii) ears per square meter (AHLEMEYER AND FRIEDT 2011). The primary yield component TKW is a measure of seed size in grams of 1,000 seeds and is routinely measured by plant breeders. UKALSKA AND KOCIUBA (2013) reported similar mean values between 1982 and 2008 of winter triticale genotypes with a multi-year mean of 50.1 g. The highly heritable trait, $h^2 = 0.92$ in publication III and $h^2 = 0.95$ in publication II (data not shown), showed no significant breeding progress but strong variations between 36 and 61 grams (Figure 3a) during the last three decades. Seed size is moderately positively correlated with grain yield in triticale (Publication III and Figure 3a) and shows a higher correlation between the two traits than parental components in wheat (e.g. MENGISTU ET AL. 2012) or rye (e.g. MIEDANER ET AL. 2012). Therefore, it might be concluded that an indirect selection for enhanced TKW could support the breeding for increased grain yield in triticale.

Traditionally, triticale is used as feed, but its usage in biomass production became more important during the last decade. For biomass accumulation in triticale, plant height is a major contributor (Publication I), whereas other agronomic traits have lower impact (GOWDA ET AL. 2011; ALHEIT ET AL. 2014). Triticale is relatively tall compared with other small grain cereals except for rye. The first commercial varieties, ‘No. 57’ and ‘No. 64’ released in Hungary, ranged between 140 to 160 cm plant height and were very susceptible to lodging (OETTLER 2005). The reduction of plant height and the identification of variants that reduce height without negative yield effects is still an important breeding target to achieve improved grain yield (GRIFFITHS ET AL. 2012; WÜRSCHUM ET AL. 2014b). Average plant heights of modern lines, released between the 1980s and the 2010s, were measured among 90 and 120 cm (Publication II, Figure 2b). For biomass production, this selection for short-statured plants should be reconsidered due to the strong correlation between biomass yield and plant height (Publication I, Figure 2). At the same time, it must be taken into account that increased plant height is accompanied with lodging (Publication II, Figure 1).

In general, the results about breeding efforts in the past three decades allocate key findings for breeding programs and also provide a basis for potential future yield increases in triticale. Molecular tools can be helpful to analyze the genetic control underlying agronomical relevant traits. To attain a deeper understanding and to improve important agronomic traits it is necessary to enlarge the knowledge about the genetic architecture of these traits.

MAPPING QUANTITATIVE TRAIT LOCI

In order to analyze the genetic control underlying agronomical relevant traits, this study investigated thousand kernel weight (Publication III), resistance to yellow rust (Publication IV), and plant height (Figure 4) by several QTL mapping approaches.

GWAS with a panel of 919 diverse triticale genotypes identified four QTL for plant height, which explained 40.6% of the total genotypic variance. A major QTL, with a proportion of explained genotypic variance (p_G) of 35.9%, was identified on

chromosome 4B which likely corresponds to the semi-dwarfing gene *Rht-B1* (GALE AND YOUSSEFIAN 1985).

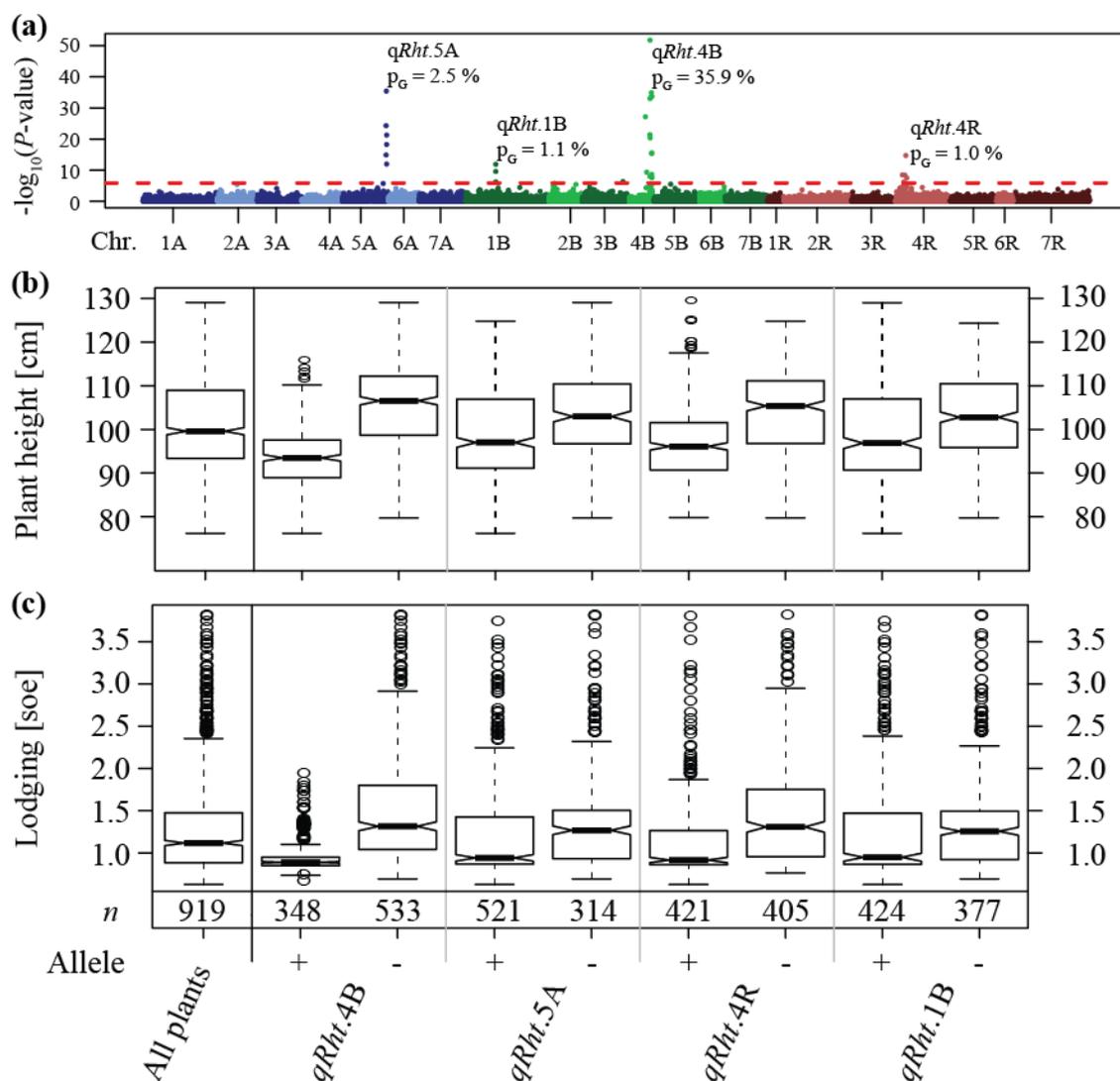


Figure 4: (a) Genome-wide scan for markers associated with plant height. p_G refers to the proportion of explained genotypic variance. The dashed horizontal line indicates the significance threshold. Boxplots showing characteristic values for genotypes carrying different alleles of the detected *Rht*-QTL explaining more than one percent of the genotypic variance, shown for both, (b) plant height and (c) lodging. The numbers underneath the boxplots indicate the number of genotypes in each group. Non-overlapping notches indicate statistically significant differences between the medians.

There is a distinct difference between our GWAS-based results and findings of KALIH ET AL. (2014) based on a bi-parental mapping population. They discovered a major QTL on chromosome 5R closely linked to the rye dwarfing gene *Ddw1*, inherited by the parental triticale cultivar ‘Pigmej’. *Ddw1* might have pleiotropic effects on plant height, heading time, and *Fusarium* head blight (FHB) severity. ‘Pigmej’ was also included in the diversity panel of this study, but the dwarfing gene inherent in ‘Pigmej’ was not widespread in the total set and thus probably lacked enough statistical power to be detected as QTL. The results showed that marker-assisted selection has potential for knowledge-based fine-tuning of plant height. Biomass yield showed a moderate-high correlation with plant height for hybrid varieties and a high correlation for line varieties (Publication I). Plant height is therefore well suitable as an indirect selection criterion to improve biomass yield, potentially assisted by marker-based approaches.

The old Polish variety ‘Purdy’ (year of registration: 1991) is a good example for a genotype combining excellent lodging resistance with high plant height. The availability of tall, lodging resistant triticale genotypes, ideally combined with high yield potential, is a good starting point to breed new varieties targeting biomass yield.

TRITICALE DISEASE RESISTANCE BREEDING

The theoretical genetic yield potential of crops can never be realized due to many environmental stress factors. The natural resistance to diseases can be enhanced by multiple breeding methods. For this purpose, a prerequisite is the evaluation of the existing genetic diversity in triticale. For instance, this knowledge might then be used to combat fungal diseases in triticale breeding programs.

Fusarium head blight (FHB) is a major problem in all small grain cereals including triticale. FHB leads to reduced grain yield and to a contamination of feed and food by harmful to health mycotoxins. The most important mycotoxin is the trichothecene deoxynivalenol (DON) due to its ubiquity and negative effects on pigs (MIEDANER ET AL. 2015). Triticale is less susceptible to FHB compared to wheat (GÓRAL ET AL. 2013). Similar to OETTLER AND WAHLE (2001) this work observed a strong reduction of grain yield under high infection pressure by artificial inoculation (Figure 5).

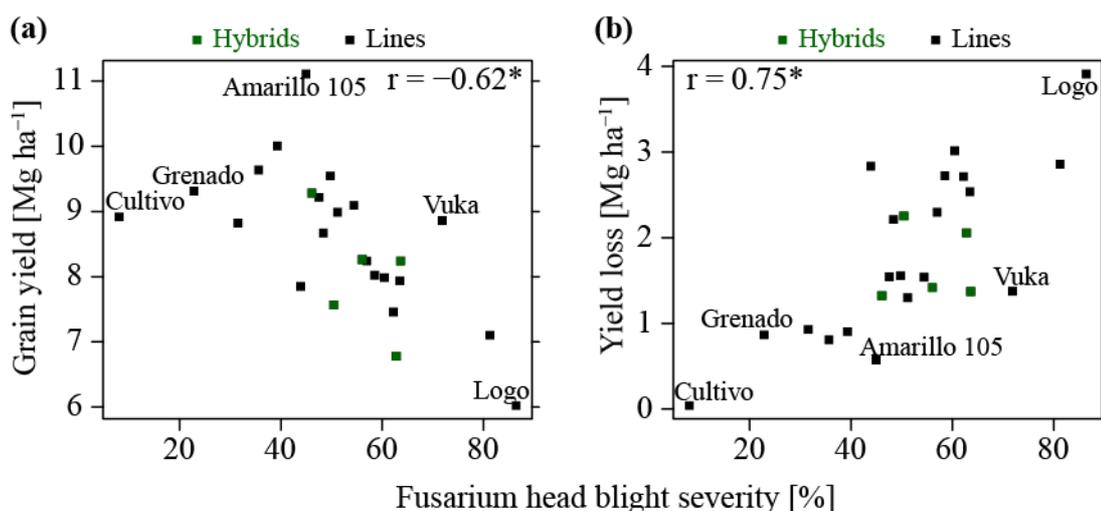


Figure 5: Scatter plots (a) between *Fusarium* head blight severity and grain yield of infected plants and (b) between *Fusarium* head blight severity and yield loss (grain yield healthy minus grain yield infected) for five hybrids (green squares) and 20 commercial lines (black squares). * significantly different from zero at $P < 0.001$ level of probability.

Breeding for *Fusarium* resistance is the most effective way to control for FHB and should reduce blighting symptoms but also mycotxin contents in the grain (BECHER ET AL. 2013). FHB is well suited to display the negative influence of fungal diseases on grain yield. In a recent study, SHARMA ET AL. (2016) found that also the fungal pathogen yellow rust reduced grain yield of winter wheat by 24 to 39%.

Yellow (stripe) rust is one of the most destructive diseases in small grain cereals (CHEN ET AL. 2014). Changes in virulence and population shifts of *Puccinia striiformis* f. sp. *tritici*, the causal plant pathogen of yellow rust, are an enormous challenge in resistance breeding. In 2011, a new virulent race, termed ‘Warrior’, was found also in triticale (HOVMØLLER ET AL. 2015). Since then, natural disease pressure in Central Europe increased strongly if untreated with fungicides, and formerly resistant varieties are partly heavily infested. The whole range of expression from non-infected to collapsed plants caused by the new stripe rust race was observed (Publication IV, Figure 1). Plant infections with yellow rust both on leaves and ears were significantly negatively correlated with grain yield (Publication II, Figure 2).

Using GWAS employing high resolution markers in a set of 919 diverse triticale genotypes made it possible to investigate the genetic architecture underlying the yellow rust resistance mechanism in triticale (Publication IV). We found several QTL, some of them new and undescribed, some located nearby already known and still effective resistance genes (MACCAFERRI ET AL. 2015). In Figure 6, the pyramiding of several QTL and the effect on the resistance level of single genotypes is displayed.

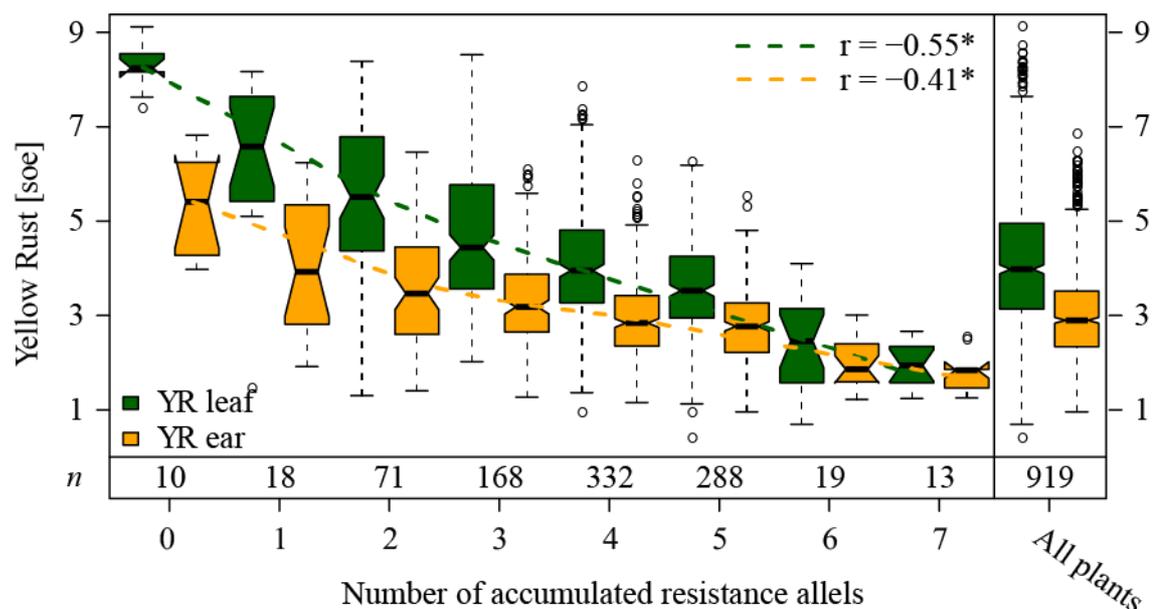


Figure 6: Boxplots showing the resistance level of genotypes carrying different numbers of yellow rust resistance alleles. The numbers underneath the boxplots indicate the number of genotypes in each group. * significantly different from zero at $P < 0.001$ level of probability.

In the long term, developing triticale varieties with durable resistance by the combination of several QTL (gene pyramiding) in a single plant (YOUNG 1996; JOSHI AND NAYAK 2010) could provide a labor intensive but promising approach, which can be realized by marker-based selection. The availability of still non-infected triticale genotypes is a good starting point to breed new resistant cultivars, ideally combined with high yield potential. The epidemic dimension triggered by a pathogen shift shows how quickly breeding goals can be changed when old but neglected diseases come back to the fore, and selection of crossing parents for line and hybrid breeding vary dramatically.

FUTURE TRENDS AND CONCLUSIONS

The constantly rising demand for renewable resources triggered by an ever-growing human world population requires ambitious efforts by plant breeders to increase the agricultural production ideally by knowledge-based breeding methods. Nowadays, the collection of phenotypic data in a fast, cost-effective and objective way is a major bottleneck in plant breeding. To tackle this problem, the development of a high-precision phenotyping platform has been started in a previous project (BUSEMEYER ET AL. 2013a, 2013b) and is still continued (LOSERT ET AL. 2015, 2016). The newly developed phenotyping platform collects phenotypic data non-invasively, which can currently be analyzed to predict plant height and biomass yield in triticale, wheat and rye. In addition, it can measure traits of interest dynamically during different developmental stages. Increasing grain yield is still the main goal in triticale breeding programs, but the question arises, whether an additional breeding program for biomass triticale is necessary (GOWDA ET AL. 2011). BUSEMEYER ET AL. (2013a) obtained very promising prediction accuracies for biomass with R^2 values of 0.92 for the years 2011 through 2012 in several environments. On the basis of these results, precision phenotyping platforms may become the method of choice to save space of cost-intensive field trials and combine breeding programs for grain and biomass utilization. Efforts are being expanded to collect other plant traits like tiller density (SAEYS ET AL. 2009) and plant disease (SANKARAN ET AL. 2010) by sensors. A statistically verified fusion of platform-based phenotyping and marker-based genotyping allows an ideal case of knowledge-based breeding.

Various experiments were performed as part of this thesis, including 23,044 evaluated trial plots, to tackle questions which are useful in triticale line breeding as well as in hybrid breeding. This research suggests that both breeding methods have potential to increase the yield level in triticale. Hybrids can compete with line cultivars especially for biomass use, triggered by lower harvest index and increased plant height. The results underline the potential of winter triticale to diversify the portfolio of crops for biomass production. The analyses revealed the absence of major population structure but a certain grouping of lines according to their breeding companies. The results of this study imply that triticale shows a large phenotypic variation for diverse agronomic relevant traits, which should guarantee further breeding progress. An analysis of the

breeding progress in the past three decades showed a continuous improvement of grain yield, reduction of protein content and plant height, and a simultaneous improvement of lodging tolerance. A major QTL for plant height and several QTL for thousand kernel weight and yellow rust resistance (on the leaves and on the ears) were identified by quantitative trait locus mapping. Based on the QTL identified in these studies, it can be concluded that the potential for marker-assisted selection is limited for grain yield but appears promising for plant height, thousand kernel weight, and yellow rust resistance. Taken together, these results point out the current performance of elite triticale breeding material but also suggest room for improvements in both, line and hybrid cultivars, to continue the success story of this first man-made cereal.

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8 SUMMARY

Triticale (\times *Triticosecale* Wittmack) is a small grain cereal used for livestock feeding and as renewable energy source. These diverse types of usage lead to different breeding strategies, ideally resulting in continued increase of both, grain and biomass yield.

Briefly, the objectives of this thesis were to explore aspects with relevance for line and hybrid breeding in triticale by phenotypic, genetic and genomic assessment of important traits. More specifically, the objectives of this study were to (i) evaluate agronomic traits, assess trait correlations, and investigate the amount of heterosis in triticale hybrids, (ii) examine the potential of line and hybrid cultivars for production of biomass, (iii) assess the phenotypic and genotypic variability in triticale germplasm, (iv) investigate long-term phenotypic trends based on cultivars registered in the past three decades, and (v) identify QTL for agronomical relevant traits.

Hybrid breeding in triticale has recently received increased interest, especially in Germany and France. This research observed large variation, significant genotypic variances, and high heritabilities in 91 evaluated triticale hybrids for heading time, plant height, fresh biomass yield, dry mass content, and dry biomass yield (Publication I). A significant positive correlation was observed between biomass yield, plant height and grain yield within the group of hybrids. Average mid-parent heterosis was positive for all traits, except dry matter content. Thus, hybrids averaged a higher fresh and dry biomass, increased plant height, and reached earlier heading compared with their mid-parent values. The average mid-parent heterosis for dry biomass (4.8%) was twice as large as that for grain yield (2.3%). One third of the tested hybrids outyielded the three reference cultivars for biomass yield of the German Federal Plant Variety Office (Bundessortenamt) and reached a maximum commercial heterosis of 9.1%. In a set of 25 genotypes, including five hybrids and 20 commercial lines, the evaluated hybrids clearly showed a smaller harvest index and consequently higher biomass yield compared to the line cultivars (Figure 2). Taken together, the results of the present work demonstrate the potential of triticale lines and specially hybrids to diversify the portfolio of crops available for biomass production. Knowledge of the phenotypic and genetic diversity within triticale germplasm is the basis for selecting crossing parents, establishing heterotic groups, and is also pivotal for the improvement of this crop. We

observed large variation, significant genotypic variances and high heritabilities in 885 evaluated triticale genotypes for heading time, plant height, grain yield, yellow rust affecting the ears and leaves, powdery mildew, lodging (Publication II), protein content, thousand kernel weight, and hectoliter weight (Figure 3). In order to determine the influence of the breeding efforts of the last decades, a set of 121 triticale cultivars, registered between 1983 and 2014 was grown in side-by-side multi-environmental field trials. The analyses of the cultivars bred in different periods showed a continuous improvement of grain yield as well as a concomitant reduction of protein content (Figure 3), a reduction of plant height and a resulting improvement of lodging tolerance. These findings strongly support the view that the current breeding material provides a solid basis for a continuation of the selection gain achieved during the past three decades.

Quantitative trait locus (QTL) mapping has become a routine tool for the identification of loci affecting breeding-relevant traits. In a mapping population of 647 double haploid lines derived from four families, 13 QTL were identified by multiple-line cross QTL mapping for thousand kernel weight, which explained 48.2% of the genotypic variance (Publication III). By using a genome-wide association study (GWAS), with a set of 919 diverse triticale genotypes, four QTL for plant height were found, which explained 40.6% of the total genotypic variance (Figure 4). A major QTL for plant height was located on chromosome 4B which likely corresponds to the semi-dwarfing gene *Rht-B1*. The GWAS approach additionally identified ten QTL for yellow rust resistance on the leaves and seven of these also for ear resistance (Publication IV). The total genotypic variance explained by these QTL was 44.0% for leaf and 26.0% for ear resistance. Taken together, the promising proportion of explained genotypic variance shows that marker-assisted selection has great potential to improve certain traits like thousand kernel weight, plant height, and yellow rust resistance affecting the leaves.

In conclusion, hybrids of triticale possess an increased biomass yield potential compared with their mid-parent values as well as compared with commercial reference cultivars. The findings on triticale germplasm and its breeding history provide important information for breeding programs. Furthermore, based on the obtained results, genomic approaches like marker-assisted or genomic selection appear promising to assist triticale breeding in the future.

9 ZUSAMMENFASSUNG

Triticale (\times *Triticosecale* Wittmack) ist eine kleinkörnige Getreideart welche in der Nutztierfütterung und als erneuerbare Energieressource eingesetzt wird. Diese unterschiedlichen Nutzungsformen führen zu diversen Züchtungsstrategien, welche im Idealfall in einem fortschreitenden Ansteigen sowohl des Korn- als auch des Biomasseertrags münden.

Die Ziele dieser Arbeit in Kürze dargestellt sind es, Aspekte mit Relevanz für die Triticale-Linien- und die Triticale-Hybridzüchtung durch die phänotypische, genetische und genomische Auswertung von wichtigen Pflanzenmerkmalen zu untersuchen. Im Detail sind die Ziele dieser Studie (I) die Evaluierung agronomischer Merkmale, das Aufzeigen von Zusammenhängen bei wichtigen Pflanzenmerkmalen und die Untersuchung des Umfanges der Heterosis, (II) das Potential von Linien- und Hybridsorten für die Biomasseproduktion festzustellen, (III) die phänotypische und genetische Variabilität des genetischen Materials von Triticale zu bewerten, (IV) die Untersuchung der langfristigen Entwicklungen von phänotypischen Merkmalen in registrierten Sorten der letzten drei Jahrzehnte und (V) die Identifizierung von QTL für agronomisch wichtige Pflanzenmerkmale.

Speziell in Deutschland und Frankreich erfuhr die Triticale-Hybridzüchtung in jüngster Zeit zunehmende Beachtung. Bei 91 evaluierten Hybridtricalen wurde große Variationen, signifikante genotypische Varianzen und hohe Heritabilitäten für Ährenschieben, Wuchshöhe, Trockenmassegehalt, Frisch- und Trockenbiomasseertrag festgestellt (Veröffentlichung I). Bei den Hybriden wurde ein signifikanter positiver Zusammenhang zwischen Biomasseertrag und Wuchshöhe bzw. Kornertrag beobachtet. Die durchschnittliche Leistungsüberlegenheit der Hybriden über das Mittel ihrer Eltern (mid-parent heterosis) war positiv für alle Merkmale, außer für den Trockenmassegehalt. Zusammenfassend erzielten Hybriden, verglichen mit den Mittelwerten ihrer Eltern, eine höher Frisch- und Trockenbiomasse, die Pflanzen waren höher und erreichten schneller die phänologische Phase des Ährenschiebens. Die durchschnittliche Heterosis für Trockenbiomasse (4,8%) war doppelt so groß wie für den Kornertrag (2,3%). Ein Drittel der getesteten Hybriden war leistungsstärker als die drei Referenzsorten des deutschen Bundessortenamtes für Biomasseertrag und sie

erreichten eine maximale kommerzielle Heterosis von 9,1%. In einem Set von 25 Genotypen zeigen die fünf enthaltenen Hybriden, verglichen mit den 20 zum Set gehörenden kommerziellen Liniensorten, eindeutig einen geringeren Harvest Index und folglich einen höheren Biomassertrag (Abbildung 2). Als Fazit daraus, demonstrieren die Resultate das Potential von Triticale-Liniensorten und im speziellen von Triticale-Hybriden, unseren Bestand von geeigneten Pflanzen für die Biomasseproduktion zu diversifizieren. Kenntnisse der phänotypischen und genetischen Diversität innerhalb des genetischen Materials sind die Grundlage für die Selektion von Kreuzungseltern, die Etablierung von heterotischen Gruppen und haben einen maßgeblichen Einfluss auf die Verbesserung von Triticale. Es wurde eine große Variation, signifikante genotypische Varianzen und hohe Heritabilitäten in 885 evaluierten Triticale-Genotypen für die Merkmale Ährenschieben, Wuchshöhe, Kornertrag, Befall von Gelbrost auf den Ähren und Blättern, Mehltau, Lager (Veröffentlichung II), Proteingehalt, Tausendkorngewicht und Hektolitergewicht (Abbildung 3) beobachtet. Um den Einfluss der Züchtungsarbeit der vergangenen Jahrzehnte zu ermitteln, wurde ein Set von 121 Triticale-Sorten, deren Registrierung zwischen 1983 und 2014 lag, nebeneinander in mehrortigen Feldversuchen angebaut. Die Analyse der Sorten, gezüchtet in unterschiedlichen Zeiträumen, zeigte sowohl eine konstante Verbesserung des Kornertrages als auch eine gleichzeitige Reduktion des Proteingehaltes (Abbildung 3), einen Rückgang der Pflanzenhöhe und infolgedessen eine Verbesserung der Lagerneigung. Die Ergebnisse bekräftigen nachhaltig den Standpunkt, dass das aktuelle Zuchtmaterial ein solides Fundament für die Fortschreitung des Selektionsgewinns aus den vergangenen drei Jahrzehnten darstellt.

Die Kartierung von Quantitativen *Trait Loci* (QTL) ist ein Routinewerkzeug für die Identifikation von züchtungsrelevanten Merkmalen geworden. In einer aus vier Familien abgeleiteten Kartierungspopulation bestehend aus 647 doppelhaploiden Linien, konnten über *multiple-line cross* QTL-Kartierung, 13 QTL für das Tausendkorngewicht identifiziert werden, welche 48,2% der genotypischen Varianz erklärten (Veröffentlichung III). Durch eine genomweite Assoziationsstudie (GWAS), mit einem diversen Set von 919 Triticale-Genotypen, konnten vier QTL für Wuchshöhe gefunden werden, welche 40,6% der gesamten genotypischen Varianz erklärten (Abbildung 4). Ein Haupt-QTL für die Wuchshöhe war auf dem Chromosom 4B lokalisiert, welches eventuell mit dem Halbverzweigungsgen *Rht-B1* übereinstimmt.

Zusätzlich konnten durch GWAS Ansätze zehn QTL für Gelbrostresistenz auf den Blättern und sieben deckungsgleiche auf den Ähren identifiziert werden (Veröffentlichung IV). Die gesamte erklärte genotypische Varianz der QTL belief sich auf 44,0% für die Blatt- und 26,0% für die Ährenresistenz. Der vielversprechende Anteil an erklärter genotypischer Varianz zeigt als Fazit, dass marker-basierte Selektion ein großes Potential bietet, um die Merkmale Tausendkorngewicht, Wuchshöhe und Gelbrostresistenz der Blätter zu verbessern.

Zusammengefasst betrachtet besitzen Triticale-Hybriden, verglichen mit dem Leistungsmittel ihrer Eltern, ein erhöhtes Ertragspotential für Biomasse. Die Untersuchungsergebnisse des genetischen Materials von Triticale und seiner Züchtungshistorie stellen beträchtliche Informationen für Zuchtprogramme zur Verfügung. Auf Grund der gewonnenen Ergebnisse, sind wir darüber hinaus zur Ansicht gekommen, dass genomische Ansätze wie die Präzisionszucht (*Marker Assisted Selection*) und die genomische Selektion vielversprechend erscheinen, um die Triticale-Züchtung in der Zukunft zu fördern.

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