Genomic and phenotypic improvement of triticale (×*Triticosecale* Wittmack) line and hybrid breeding programs

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Abbreviations

BLUE	Best linear unbiased estimate
CMS	Cytoplasmic male sterility
Ddw1	Dominant height reducing gene from rye
Ddw3	Dominant height reducing gene from rye
Ddw4	Dominant height reducing gene from rye
gBLUP	Genomic best linear unbiased prediction
GCA	General combining ability
GWAS	Genome-wide association study
QTL	*Quantitative trait loci*
Rht-B1	Semi-dominant height reducing gene from wheat
Rht12	Dominant height reducing gene from wheat
Rht24	Dominant height reducing gene from wheat
Rht25	Semi-dominant height reducing gene from wheat
SCA	Specific combining ability

1 General introduction

Triticale (×*Triticosecale* Wittmack) is an artificially produced allopolyploid small-grain cereal (Mergoum et al. 2009) and first artificial but sterile crosses were reported in 1875 (Wilson 1876; Mergoum et al. 2009). The first fertile triticale genotypes (Rimpau 1891; Lindschau and Oehler 1935) were octoploid crosses (2n = 8x = 56; AABBDDRR) between bread wheat (*Triticum aestivum* L., 2n = 6x = 42; AABBDD) and rye (*Secale cereale*, L., 2n = 2x = 14; RR), but were characterized by a low level of fertility (Mergoum et al. 2009; Oettler 2005). Large numbers of fertile triticale genotypes could only be obtained with the establishment of appropriate *in vitro* techniques (Laibach 1929; Wang et al. 1973) and efficient chromosome doubling through the discovery of colchicine (Blakeslee and Avery 1937; Maluszynski et al. 2003). Today, only hexaploid triticales (2n = 6x = 42; AABBRR), initially achieved by crosses between durum wheat (*Triticum durum* L., 2n = 28 = AABB) and rye, are grown due to their increased fertility, reproductive stability, and superior vigor (Mergoum et al. 2009; Oettler 2005). In recent years, crosses between wheat and rye to generate triticale, so-called *primaries* (Kiss 1966), are mainly performed to introduce new genetic variation into triticale germplasm (Oettler 2005). The main improvement of triticale focuses on *secondary* triticales comprising crosses between two or more primaries or secondary triticales as well as crosses between secondary triticales with either primaries, wheat, rye, or triticale (Oettler 2005).

Triticale's importance has grown steadily despite all the challenges in early triticale breeding and its relatively low attention on a global scale. Since the release of the first registered triticale cultivars in the late 1970s (Mergoum et al. 2009; Oettler 2005), global triticale acreage grew to approximately 4 million ha

until 2019, of which the main percentage is grown in Europe (FAOSTAT 2021). This success story of triticale is also reflected by the fact that 48 triticale cultivars are currently listed in the German Plant Variety Catalogue (Bundessortenamt 2020) and that more than 2,600 triticale cultivars or genotypes are registered or in the application processes on a global scale (CPVO 2021).

Triticale – its uses and breeding goals

Today, triticale grains are mainly used as animal fodder, especially in pig and poultry farming due to their high-quality protein composition (Myer 2002; Mergoum et al. 2009; Boros 2002; Myer and Del Lozano Río 2004), for bioethanol production (McGoverin et al. 2011; Zechner et al. 2011), and to a lesser extent for human consumption (Peña 2004; Zhu 2018) since triticale shows a low baking quality (Mergoum et al. 2019). Triticale whole-plant biomass has been employed as fodder, *e.g.*, grazing, silage, straw, or hay for several years (Myer and Del Lozano Río 2004) and more recently, it is also used as an energy crop for biogas production (McGoverin et al. 2011; Cantale et al. 2016; Weiland 2010).

These uses clearly point to the most important breeding goals in triticale breeding programs. The main efforts to improve triticale are grain (Neuweiler et al. 2020) and biomass yield (Gowda et al. 2011; Losert et al. 2016), and to enhancement against disease resistances, *e.g.*, Fusarium head blight (caused by *Fusarium* spp.), powdery mildew (*Blumeria graminis*), *Septoria tritici* blotch (*Zymoseptoria tritici*), and yellow rust (*Puccinia striiformis*) which can substantially reduce grain yield especially in triticale crop production systems with low-input strategies (Troch et al. 2012; Losert et al. 2017b; Walker et al. 2011; Miedaner et al. 2016). Additional breeding goals are, *e.g.*, baking quality (Mergoum et al. 2019; Wrigley and Bushuk 2017), protein content (Neuweiler et al. 2021) or morphological characteristics such as plant height, lodging tolerance, or flowering time (Losert et al. 2017b; Kalih et al. 2014; Alheit et al. 2014).

Genetic basis of plant height in triticale

Plant height is usually examined in triticale breeding programs because it is one of the criteria for the evaluation of distinctness, uniformity, and stability to register a newly developed cultivar (CPVO 2011). It influences lodging tolerance, which often leads to increased drying costs at harvest, reduces grain quality, and results in significant grain yield losses (Losert et al. 2017b; Rajkumara 2008; Liu et al. 2015). Furthermore, resistance against Fusarium head blight has been reported to be associated with plant height (Miedaner and Voss 2008; Kalih et al. 2014; Yan et al. 2011). In recent years, the interest in plant height increased as it has been shown to be one of the main contributors to biomass and straw yield in triticale (Losert et al. 2016; Alheit et al. 2014; Gowda et al. 2011). In addition, it is of relevance to increase hybrid seed set by fine-tuning plant height of the parental pools in hybrid breeding programs, with male parents being taller than females (Longin et al. 2012).

On a genetic basis, plant height is a quantitatively inherited trait in small-grain cereals controlled by several minor and a couple of major quantitative trait loci (QTL; Würschum et al. 2014; Alheit et al. 2014; Griffiths et al. 2012; Börner et al. 1996; Würschum et al. 2017a; Würschum et al. 2015). Moreover, plant height shows undesirable and in wheat well evaluated pleiotropic effects on other developmental processes, such as root elongation, coleoptile length, or early seedling vigor (Ellis et al. 2004; Botwright et al. 2001; Rebetzke et al. 2001; Bai et al. 2013).

Many height-reducing genes have been studied in the parental species of triticale, wheat and rye (Börner et al. 1996; McIntosh et al. 2017; McIntosh et al. 2013; Kantarek et al. 2018; Stojałowski et al. 2015). To date, the use of *Rht-B1* in spring (Chernook et al. 2019) and *Ddw1* in winter triticale (Alheit et al. 2014; Kalih et al. 2014) has been reported. However, there are still only few studies focusing on the genetic control of plant height with several small-effect QTLs being detected in triticale (Kalih et al. 2014; Alheit et al. 2014; Würschum et al. 2014). So far only *Ddw1* could be identified in QTL studies (Kalih et al. 2014; Alheit et al. 2014).

Breeding for biomass yield in triticale breeding programs

Grain yield is the most important breeding goal in triticale breeding programs (Aydoğan et al. 2010). However, in recent years triticale biomass yield became more and more important due to its use as, *e.g.*, forage crop (Ayalew et al. 2018) and the incorporation of biomass into energy production strategies established by a European promoting policy (EU 2001, 2009, 2018; Monforti et al. 2013). The predominant crop concerning biogas production is silage maize (*Zea mays*; Oslaj et al. 2010), although crops such as fodder and sugar beet (*Beta vulgaris*), clover (*Trifolium*), or small-grain cereals, *e.g.*, wheat (*Triticum aestivum*), rye (*Secale cereale*), and triticale (×*Triticosecale* Wittmack) are well suited to produce biogas and can contribute to widen crop rotations (Weiland 2010).

These trends together with the great potential of triticale regarding biogas production (Weiland 2010; LTZ 2013) shifted the interest of triticale breeding programs into the direction of biomass production and the release of cultivars solely developed for these purposes (Gowda et al. 2011; Liu et al. 2017; Bundessortenamt 2020). As part of this development, simultaneous improvement of triticale germplasm for grain and biomass yield has been proposed for triticale breeding programs (Lekgari et al. 2008; Gowda et al. 2011; Liu et al. 2017; Losert et al. 2016). Considering the large genetic variation and the medium to high heritabilities of biomass yield in triticale (Gowda et al. 2011; Losert et al. 2016; Alheit et al. 2014; Liu et al. 2017), great potential to improve biomass yield in line (Gowda et al. 2011) and hybrid breeding programs (Losert et al. 2016) has been identified. However, efficient strategies to select the most promising genotypes in hybrid breeding programs are still missing and are urgently needed, due to a quadratically increasing number of putative experimental hybrids with an increasing number of parents (Bernardo 2010).

Molecular markers, related technologies, and their use in triticale breeding

Most traits observed in plants, including triticale, are quantitatively inherited and are therefore not subject to Mendelian genetics with discrete phenotypic distributions (Tanksley 1993; Würschum 2012; Falconer and Mackay 1996). Such traits are controlled by many QTL with small effects (Falconer and Mackay 1996; Mackay et al. 2009). Consequently, the development and application of markers, especially of molecular markers since the 1980's (Collard et al. 2005; Rafalski and Tingey 1993; Mammadov et al. 2012; Bernardo 2010), led to effective methods in plant breeding unraveling the genetic control of various agronomically important traits and to identify putative QTL contributing to the traits plant breeders are interested in (Buerstmayr et al. 2009; Collard et al. 2005; Würschum 2012). This trend is expected to continue as the costs of genotyping plants steadily decrease (Würschum 2012).

One method which has been used throughout the last decades is linkage mapping based on, *e.g.*, the utilization of bi-parental populations (Tanksley 1993; Würschum 2012) and molecular markers (Collard et al. 2005). However, linkage mapping is highly population specific and strongly depends on the genetic constitution of the parents of the underlying population and shows only a low mapping resolution (Würschum 2012). The more recently used method of linkage disequilibrium mapping, also known as genome-wide association study (GWAS), has originally been developed by human geneticists (Hästbacka et al. 1992; Hirschhorn and Daly 2005) and has then been applied for research in the plant breeding context (Gupta et al. 2005; Zhu et al. 2008).

The effects of detected QTL are often overestimated (Melchinger et al. 1998) and the power to identify a QTL is highly dependent on the type of population and the underlying population size (Tanksley, 1998; Würschum 2012; Vales et al. 2005). Besides these points, in GWAS the allele frequency influences the detection of a QTL (Korte and Farlow 2013; Würschum and Kraft 2014; Zhu et al. 2008). One of the big advantages of GWAS over linkage mapping is that the allelic variation is not restricted to the parental lines and the potentially high mapping resolution resulting from the historical recombination events in the

studied population (Myles et al. 2009; Korte and Farlow 2013). Furthermore, the time-consuming production of mapping populations, the big disadvantage of linkage mapping, is eliminated (Zhu et al. 2008). Nevertheless, the population structure always must be accounted for in GWAS to avoid false-positive results (Bernardo 2010; Würschum 2012). Furthermore, a high marker density and a large mapping population are required for a sufficiently high mapping resolution and QTL detection power in GWAS, especially for small or medium effect size and rare QTL (Zhu et al. 2008; Würschum 2012). In particular, the need for many markers prevented GWAS from becoming a routinely used method in various crops, also in triticale (Zhu et al. 2008). Continuously decreasing costs for genotyping has and will make high marker densities affordable in several crops in the future (Würschum 2012). Despite these obstacles, numerous QTL mapping studies have already been published in triticale investigating a wide range of traits (Kalih et al. 2014; Miedaner et al. 2016; Kalih et al. 2015; Neuweiler et al. 2020; Alheit et al. 2014; Würschum et al. 2014; Galiano-Carneiro et al. 2019; Wajdzik et al. 2019; Szechyńska-Hebda et al. 2011; Dhariwal et al. 2018; Niedziela et al. 2014; Ollier et al. 2020).

Well characterized QTL of agronomically important traits can routinely be accumulated in breeding populations using marker-assisted selection and therefore increase the genetic gain of a breeding program (Xu and Crouch 2008; Collard and Mackill 2008; Mohan et al. 1997). A further advancement of marker-assisted selection is genomic selection. Here the information of a great number of molecular markers is exploited at once to predict the performance of untested genotypes (Crossa et al. 2011; Meuwissen et al. 2001). This approach has only been evaluated in a limited number of studies in triticale (Liu et al. 2015; Würschum et al. 2017b). Fine-mapping of the genetic control of important traits was not feasible in triticale until recently, as high marker densities were not available and a reference genome of triticale is not available until today. However, physical map positions can nowadays be used since recently from wheat and rye (Alaux et al. 2018; Rabanus-Wallace et al. 2021) to, *e.g.*, physically fine-map identified genes or QTL, perform positional candidate gene identification and positional cloning.

Hybrid breeding in the autogamous crop triticale

Hybrid breeding has always been associated with the utilization of heterosis and hybrids have shown higher resistance to biotic and abiotic stress as well as an increased yield stability (Mette et al. 2015). Therefore, hybrid breeding methodology has been routinely introduced in many allogamous species such as maize, rye, sugar beet, or sunflower (Coors and Pandey 1999; Carena 2009; Cooke and Scott 1993). It also has been proposed recently for autogamous small-grain cereals, such as barley (*Hordeum* vulgare), wheat (*Triticum aestivum*), and triticale (×*Triticosecale* Wittmack) to further improve trait performances (Longin et al. 2012). Triticale is a partly outcrossing small-grain cereal and therefore well suited for hybrid breeding (Herrmann 2002; Kiss 1970; Sowa and Krysiak 1996). Furthermore, suitable hybrid mechanisms based on chemical hybridization agents and cytoplasmic male sterility (CMS) are available in triticale breeding, facilitating the use of hybrid methodology in triticale (Longin et al. 2012; Warzecha and Salak-Warzecha 2002).

In addition, favorable heterosis and its magnitude have been widely evaluated in triticale germplasm (Oettler et al. 2001; Oettler et al. 2003; Oettler et al. 2005; Losert et al. 2016; Tams et al. 2006; Mühleisen et al. 2015; Fischer et al. 2010; Gowda et al. 2013; Herrmann 2007) and triticale hybrids showed an increased yield stability compared to lines for many traits such as plant height, grain and biomass yield, or various yield and quality traits (Oettler et al. 2005; Mühleisen et al. 2014). However, for an effective exploitation of heterosis and an efficient hybrid breeding program heterotic pools are needed (Fischer et al. 2010; Reif et al. 2007). To date no heterotic pools were identified in triticale breeding programs (Fischer et al. 2010; Gowda et al. 2013; Losert et al. 2017b; Tams et al. 2004; Tams et al. 2006). This is mainly due to the lively exchange among breeders and generally common in autogamous small-grain cereals. Nevertheless, first CMS-based triticale hybrids were released in 2012 in France and Germany showing the great potential of triticale hybrid breeding (Longin et al. 2012).

Prediction of biomass yield in triticale hybrids

One of the major challenges in hybrid breeding programs is the quadratically increasing number of possible experimental hybrids as the number of parental lines is increasing (Bernardo 2010). This makes it impossible to evaluate all possible hybrid combinations and implies that a pre-selection must be implemented prior to the evaluation of experimental hybrids in the field. Therefore, appropriate methods are needed and have been extensively evaluated for typical hybrid crops like maize or sunflower (Reif et al. 2013; Smith 1986; Jenkins 1934; Melchinger et al. 1987; Schrag et al. 2006; Nyaga et al. 2020), but also for small-grain cereals such as barley, rye, and wheat (Miedaner et al. 2013; Zhao et al. 2014; Mühleisen et al. 2013; Philipp et al. 2016; Bernal-Vasquez et al. 2017; Wang et al. 2014).

Currently, a diverse range of phenotypic and genotypic approaches to predict hybrid performance has been reported with different advantages and disadvantages. Predicting the performance of hybrids based on their mid-parent values is very desirable as no experimental hybrids must be produced and therefore a lot of resources can be saved in a breeding program. However, practicable results have only been reported for less complex and highly heritable traits such as plant height or ear emergence in triticale breeding programs (Boeven et al. 2016; Gowda et al. 2013; Oettler et al. 2005), whereas more complexly inherited traits like grain yield or thousand-kernel weight showed medium to low prediction accuracies (Gowda et al. 2013; Oettler et al. 2005) owed to non-additive masking effects (Bernardo 2010; Smith 1986). For biomass yield the same is suspected due to its quantitative inheritance (Alheit et al. 2014) but has not been evaluated in further detail so far.

The standard procedure predicting the performance of experimental hybrids in many of today's hybrid breeding programs is based on general combining ability (GCA) effects (Guimarães 2009; Hallauer et al. 2010; Henzell and Jordan 2009). This has also shown very good results compared to predictions based on the parental *per* se values predicting the performance of quantitatively inherited traits in triticale, such as Fusarium head blight and grain yield (Boeven et al. 2016; Fischer et al. 2010; Gowda et al. 2013). To predict hybrids based on GCA effects,

however, at least a subset of experimental hybrids must be produced and tested in the field charging a breeder's budget. Furthermore, prediction accuracies are presumed to be less accurate with an increasing ratio of specific combining ability (SCA) in comparison to the total genetic variance (Melchinger 1999; Hallauer et al. 2010) what in turn is strongly dependent on the examined trait as shown in recent publications in triticale (Boeven et al. 2016; Gowda et al. 2013; Oettler 2005).

Latest developments gave triticale breeding programs access to molecular markers (Badea et al. 2011; Kuleung et al. 2004) and therefore the possibility, to avoid resource-intensive field trials at least partly. Genomic prediction approaches have been broadly evaluated in both line (Alemu et al. 2021; Rapp et al. 2019; Sapkota et al. 2020; Duhnen et al. 2017) and hybrid breeding programs (Werner et al. 2018; Zhao et al. 2013; Gowda et al. 2013; Technow et al. 2012; Windhausen et al. 2012; Technow et al. 2014; Philipp et al. 2016; Li et al. 2017; Wang et al. 2017; Reif et al. 2013; Wang et al. 2014; Gaikpa et al. 2020; Zhao et al. 2014) and have proven their usefulness in triticale breeding programs due to the observed high prediction accuracies for traits such as ear emergence, flowering time, Fusarium head blight, grain yield, as well as plant height (Boeven et al. 2016; Gowda et al. 2013). However, genomic prediction approaches have not been evaluated for biomass related traits in triticale so far, especially in the context of hybrid breeding.

Phenotypic widening of the female pool in triticale hybrid breeding

Evaluating novel female candidate lines is a major challenge in CMS-based hybrid breeding programs. Though, genomic prediction has provided promising results in many crops (Wang et al. 2014; Mette et al. 2015; Werner et al. 2018; Technow et al. 2012; Philipp et al. 2016), it is still expensive in triticale due to high genotyping cost and large training populations required to achieve high prediction accuracies (Gupta et al. 2019; Liu et al. 2016).

Furthermore, for the evaluation of experimental hybrids solely based on

phenotypic parameters, an efficient hybrid mechanism is needed to evaluate the parental GCA effects. In small-grain cereals mainly sterility systems based on CMS and chemical hybridization agents are used (Longin et al. 2012; Adugna et al. 2004). Besides the high cost, the use of chemical hybridization agents comes hand in hand with difficulties such as environmental toxicity and phytotoxicity leading to a reduced seed set and hybrid vigor, as well as a narrow time-window for its application (Adugna et al. 2004; Cisar and Cooper 2002; Gupta et al. 2019) what ultimately makes them financially inferior compared to CMS systems (Hede 2001). On the contrary, using a hybridization mechanism based on CMS requires among others the time- and resource-intensive introgression of female lines into a sterile cytoplasm.

Objectives

The goal of this thesis was to evaluate potentials to further improve triticale line and hybrid breeding programs with a special attention on plant height, biomass traits, and the evaluation of different hybrid prediction approaches. In detail, the objectives were to:

(i) Unravel the genetic control and evaluate long-term genetic trends of plant height in Central European winter triticale

(ii) Evaluate the potential of triticale hybrid breeding and hybrid prediction approaches in triticale with a focus on biomass yield

(iii) Introduce and examine a concept bypassing the time- and resource-consuming evaluation of female candidate lines in CMS-based hybrid breeding

(iv) Draw conclusions for the future improvement of triticale line and hybrid breeding programs

2 Identification and fine-mapping of quantitative trait loci controlling plant height in Central European winter triticale (×*Triticosecale* Wittmack)

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Identification and Fine-Mapping of Quantitative Trait Loci Controlling Plant Height in Central European Winter Triticale (×*Triticosecale* Wittmack)

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Abstract: The quantitatively inherited trait plant height is routinely evaluated in triticale breeding programs as it substantially influences lodging and disease susceptibility, is a main contributor to biomass yield, and is required to improve hybrid seed production by fine-tuning plant height in the female and male parental pools in hybrid breeding programs. In this study, we evaluated a panel of 846 diverse Central European triticale genotypes to dissect the genetic architecture underlying plant height by genome-wide association mapping. This revealed three medium- to large-effect QTL on chromosomes 5A, 4B, and 5R. Genetic and physical fine-mapping of the putative QTL revealed that the QTL on chromosome 5R most likely corresponds to *Ddw1* and that the QTL on chromosome 5A is likely to be *Rht12*. Furthermore, we observed a temporal trend in registered cultivars with a decreasing plant height during the past decades, accompanied by an increasing use of the height-reducing alleles at the identified QTL. In summary, our results shed new light on the genetic control of plant height in triticale and open new avenues for future improvement by breeding.

Keywords: triticale; plant height; genome-wide association mapping; fine-mapping; blast; *Ddw1*; *Rht12*

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1. Introduction

Hexaploid triticale (×*Triticosecale* Wittmack) is a man-made cereal that combines the genomes of tetraploid durum wheat (*Triticum durum* L.) and diploid rye (*Secale cereal* L.) [1]. The first triticale cultivars were released in the 1970s [1] and since then the triticale acreage has grown to approximately four million hectares worldwide as of 2019 [2]. Today's grain use is mainly restricted to animal fodder and bioethanol production [1,3,4]. Its biomass is used as fresh fodder [4] and more recently for biogas production due to a promotional policy in the European Union [5–8].

Knowledge about the genetic control of plant height is of interest in small-grain cereal breeding programs. Tall plants usually are more susceptible to lodging resulting in substantial grain yield losses, reduced quality, and high drying costs during the harvesting process [9–11]. They also have a less favorable harvest index and the tendency consequently was to breed for shorter types. However, taller genotypes are also associated with a reduced Fusarium head blight susceptibility [12–14] and generally show an increased biomass yield, for which plant height is an important component of biomass yield [15–17]. Biomass yield has gained increasing interest as a breeding goal in triticale breeding programs in recent years due to the high potential of triticale as a bioenergy crop [8,18]. In addition, hybrid breeding has shown potential in triticale, which requires the control of the plant height of the male and female parental components, as the male lines should be taller compared to their female mating partners in order to increase the efficiency of hybrid seed

production [19]. Thus, adjustment of plant height is required in triticale breeding programs, but the direction depends on the breeding goals.

Plant height in small-grain cereals is considered a quantitatively inherited trait controlled by many minor as well as by a few major quantitative trait loci (QTL) [17,20–22]. However, some height-reducing loci, such as the *Rht-1* homoeoloci, show adverse pleiotropic effects on other developmental processes, such as root elongation, early seedling vigor, or coleoptile length, which has been extensively studied in wheat [23–26]. In wheat and rye, the founding species of triticale, a large number of height-reducing loci are known [21,27–30]. Of the major QTL located on the A or B genome that are used in wheat breeding and are therefore potentially suitable to be used in triticale breeding, *Rht-B1* located on chromosome 4B has previously been reported in spring triticale [31]. QTL mapping in bi-parental triticale families has revealed several QTL on different chromosomes, but most of them with only small effects [14,17,32]. The only major QTL identified in triticale so far is *Ddw1*, located on chromosome 5 of the rye genome [14,17].

Consequently, the aims of this study were to investigate the phenotypic variation, the genetic architecture, and long-term genetic trends of plant height in Central European winter triticale. In particular, our objectives were to (i) perform genome-wide association mapping in a diversity panel of 846 Central European triticale genotypes, (ii) fine-map the genomic regions showing significant associations with plant height and determine their co-location with known height-reducing loci from wheat and rye, and (iii) evaluate long-term genetic trends and the allele frequencies of the detected QTL in registered cultivars.

2. Results

This study was based on 846 diverse triticale lines comprising 129 registered cultivars and 717 advanced breeding lines. All genotypes were evaluated in multi-location field trials for plant height and in addition for their developmental stage (BBCH scale) at a time point when the majority of the lines were heading, as this trait is often associated with plant height. We observed significant variation for both traits and high heritabilities of 0.81 for plant height and 0.80 for the developmental stage (Table 1). Plant height ranged from 87.3 cm to 126.6 cm and the BBCH stage ranged from 46.3 (opening of the flag leaf sheath) to 59.8 (completion of ear emergence). The correlation between the two traits was 0.24 ($p < 0.001$).

We used the 129 registered cultivars to investigate long-term trends resulting from breeding in the period from 1982 when the first cultivars of this panel were released until today. Plant height declined in the period from 1982 to 2010 from on average 110.4 cm to 102.4 cm and showed an increasing proportion of taller genotypes from 2011 on (Figure 1). By contrast, the developmental stage did not change over time, except that there may be a slight tendency towards earlier heading in the more recent cultivars, i.e., a higher and thus more advanced BBCH stage.

Table 1. Means and ranges of the best linear unbiased estimators as well as genotypic and genotype-by-environment interaction variance components of 846 diverse breeding lines and registered cultivars.

	Plant Height	Developmental Stage
Min	80.2	43.0
Mean	100.8	53.4
Max	126.6	59.8
$LSD_{0.05}$	4.0	1.3
σ^2_G	77.98 ***	6.09 ***
$\sigma^2_{G \times E}$	7.38 ***	1.21 ***
σ^2_e †	18.32	1.27
H^2	0.81	0.80

***   Significantly different from zero at the 0.001 probability level.
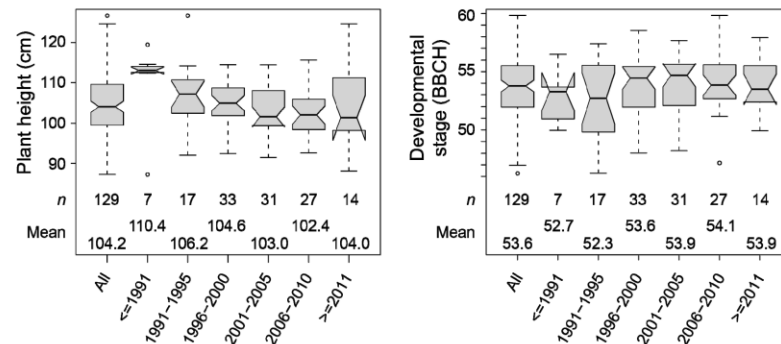†   Mean residual error variance across environment-trial combinations.

Figure 1. Development of plant height and developmental stage in the 129 registered cultivars dependent on their year of release.

We performed genome-wide association mapping using 31,823 markers to investigate the genetic architecture underlying the traits plant height and developmental stage (Figure 2). We identified 27 significantly associated markers for plant height and 17 for developmental stage (Supplementary Table S1). Jointly, these markers accounted for 42.16% of the total genotypic variance of plant height and 29.31% of that of developmental stage (Supplementary Table S1). The strongest association was found for the markers on chromosome 5R (Table 2). In a single fit, the QTL on 5A, 4B, and 5R explained more than 10% of the genotypic variance. In a joint fit, the QTL on chromosome 5R was fitted first and retained its high proportion of explained genotypic variance for plant height, whereas that of the 5A and 4B QTL was reduced to 0.40 and 2.34%, respectively. On chromosome 5R, three markers explained more than 1% of the genotypic variance even in a joint fit. This might indicate three separate QTL or that none of the markers alone is able to capture the full variance explained by a single QTL in this region. The fact that all three markers are located in close proximity, at 990.4, 997.0, and 1019.4 cm, suggests that they all identify the same QTL. Thus, the significantly associated markers might correspond to four QTL for plant height, located on chromosomes 5A, 4B, 4R, and 5R. The absolute values of the QTL allele substitution effects ranged between 1.03 and 5.30 cm (Table 2). Notably, the QTL effects varied substantially with the application rate of growth regulators and approximately doubled when only one instead of two rates were applied (Supplementary Figure S1). For the QTL on chromosome 5R, for example, the difference between the two allelic classes was 9.51 cm at the location Hohenheim when growth regulators were applied twice, which increased to 19.19 cm in the observation plots with only one application. For the developmental stage, three QTL were identified, the same QTL on chromosomes 5A, 4B, and 5R were also identified for plant height (Table 2). For these QTL, the allele that increased plant height also advanced the developmental stage, i.e., resulted in an earlier heading.
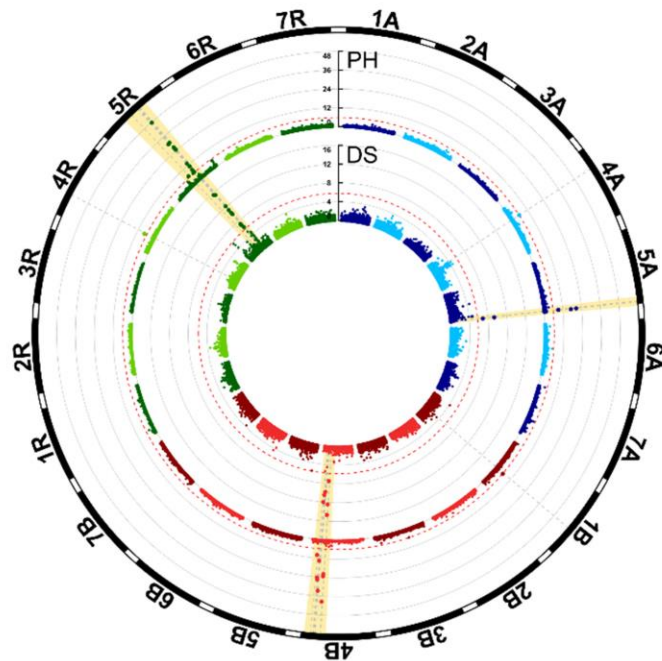
Figure 2. Circular Manhattan plot for plant height (PH) and developmental stage (DS). The red dashed lines indicate the Bonferroni-corrected significance threshold ($p < 0.05$) and the shaded area the QTL with pleiotropic effects on both traits.

Table 2. Results of the genome-wide association mapping for plant height and developmental stage.

QTL	Marker	Chr.	Pos. (cm)	p-Value	p_G Joint [a]	p_G Single [b]	α-Effect (Single Fit)	p [c]
			Plant height (42.16% p_G total)					
qPH.5A	D10506872	5A	150.6	2.3×10^{-18}	0.40	13.06	3.72	0.25
qPH.4B	D4371530 [d]	4B	70.6	1.2×10^{-23}	2.34	18.97	−4.02	0.36
qPH.4R	D10523748	4R	173.0	7.7×10^{-8}	1.99	1.35	1.03	0.48
qPH.5R.A	D10519777	5R	990.4	7.3×10^{-7}	1.79	4.56	2.26	0.23
qPH.5R.B	D4348428	5R	997.0	5.5×10^{-31}	1.91	19.12	−3.94	0.41
qPH.5R.C	S4341499 [d]	5R	1019.4	2.0×10^{-48}	29.38	29.38	5.30	0.63
			Developmental stage (29.31% p_G total)					
qEC.5A	D10506872	5A	150.6	3.3×10^{-10}	3.26	16.38	1.15	0.25
qEC.4B	D4372007	4B	70.6	2.7×10^{-15}	0.03	12.83	−0.88	0.51
qEC.5R.B	D4348428	5R	997.0	2.4×10^{-16}	16.74	16.74	−1.02	0.37
qEC.5R.C	S4341499 [d]	5R	1019.4	4.1×10^{-16}	5.35	19.43	1.19	0.63

[a] p_G values obtained by a joint fit of all significant markers for the respective trait in a linear model; markers were ordered according to their p-value (lowest first).
[b] p_G values obtained when each significant marker was fitted in a linear model for the respective trait.
[c] Frequency of trait-increasing allele, i.e., development stages (for earlier genotypes), plant height (for taller genotypes).
[d] Unmapped marker that was assigned to its most probable position based on its LD with mapped markers.

Next, we genetically and physically fine-mapped the plant height QTL on chromosomes 5A, 4B, and 5R (Figure 3a,b). The marker sequences were BLASTed against the respective reference genomes of wheat and rye to obtain their physical positions and to compare the QTL regions with those of known height-reducing loci. The significantly

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associated markers of the chromosome 5A QTL are located between 692 and 699 Mbp. For chromosome 4B, the significantly associated markers are located in the region between 657 and 666 Mbp, whereas *Rht-B1* is located at the front end of this chromosome. Physical map positions of all significant markers on chromosome 5R are located within the region from 842 to 875 Mbp. The dominant plant height locus *Ddw1* has recently been reported to be located at 862 Mbp [33]. Analysis of linkage disequilibrium (LD) among the significantly associated markers of these three QTL regions revealed a possible LD between some markers in the 4B and 5R regions (Figure 3c).
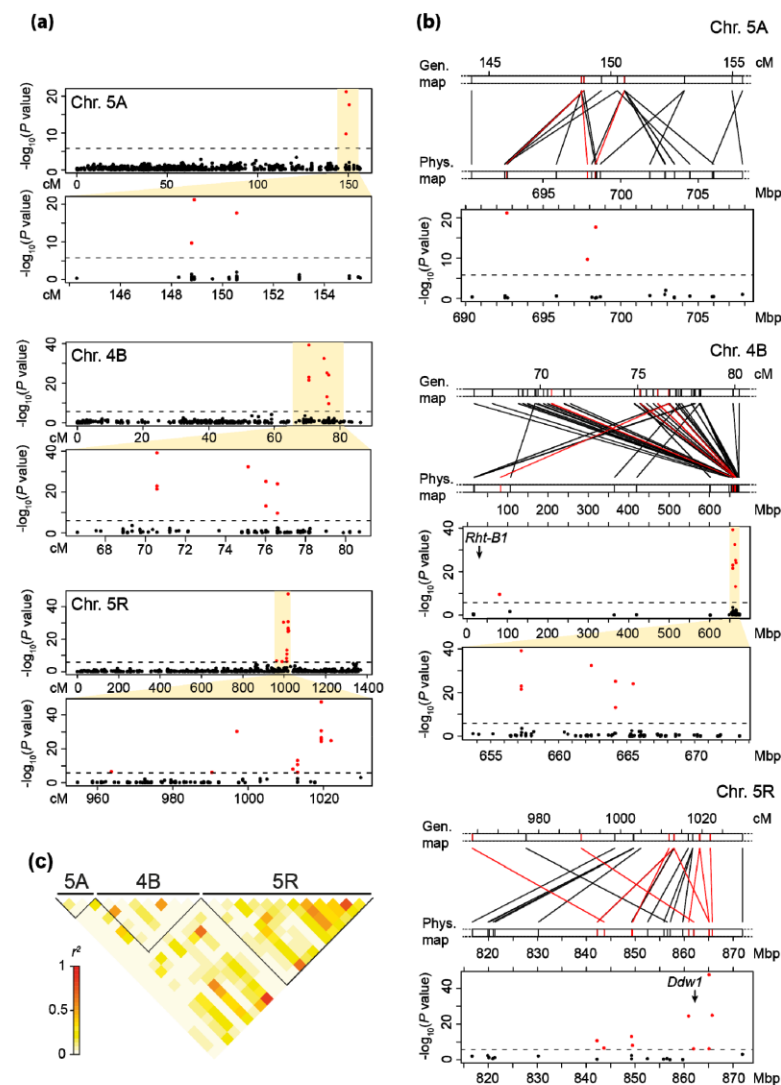

Figure 3. Fine-mapping of the identified QTL. (**a**) Genetic and (**b**) physical fine-mapping of the QTL on chromosomes 5A, 4B, and 5R. Red dots and lines indicate significantly associated markers after a Bonferroni multiple-test correction. (**c**) Linkage disequilibrium (r^2) among the markers from the three QTL regions.

To better understand the effect of the detected QTL on plant height and their utilization in triticale breeding, we used the marker with the strongest association to represent the QTL on chromosomes 5A, 4B, and 5R (Figure 4, significantly associated markers and their allele status for the 129 registered cultivars can be found in the Supplementary Tables S2–S5). We found that in the 129 registered cultivars all three QTL occurred with both alleles and the difference between the two homozygous classes ranged between 3.5 and 6.7 cm. Notably, however, our analysis revealed that the height-reducing alleles often occur in combination (Figure 5). Twenty-four of the cultivars carry none of the height-reducing alleles at these three QTL, 97 carry the height-reducing allele at the 5A QTL, 59 at the 4B QTL, and 20 at the 5R QTL. The 20 cultivars carrying the height-reducing allele at the 5R QTL all also carry the height-reducing alleles at the 5A and 4B QTL. Likewise, the 59 cultivars carrying the height-reducing 4B QTL allele also carry the height-reducing allele at the 5A QTL, except for two. Besides the latter two cultivars, this leaves 40 that only carry the height-reducing allele at the 5A QTL, so at only one of the three QTL. The analysis of the advanced breeding lines and the complete panel showed similar results (Supplementary Figures S2–S4). The only difference compared to the subset of registered cultivars was the higher frequency in the whole panel of genotypes carrying all three QTL and the lower frequency of genotypes carrying only the QTL on chromosome 5A (Figure 5, Supplementary Figure S4).
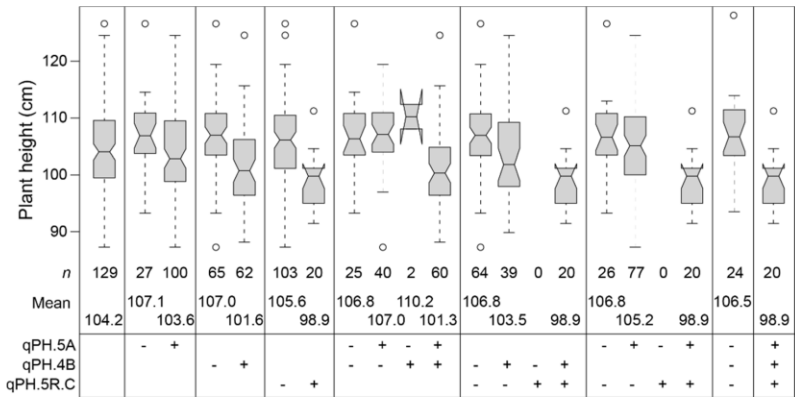

Figure 4. Effects of the QTL detected on chromosomes 5A, 4B, and 5R as well as their combinations on plant height, assessed in the 129 registered cultivars. '+' and '−' indicate presence or absence of the height-reducing QTL alleles. If no presence/absence labeling is shown, the allelic state of the respective QTL was not considered.

Last, we investigated the development of the frequencies of the height-reducing alleles at these three QTL in registered cultivars over time. This revealed an increase for all three QTL, as well as an increasing frequency of their combinations (Figure 6). For the 5A QTL, the frequency of the height-reducing allele was already around 0.5 in the cultivars registered before 1991, which then increased to above 0.9 in the cultivars registered in the past decade. For the 4B QTL, the same frequency started at around 0.2 and then increased to around 0.6, and for the QTL on chromosome 5R the frequency of the height-reducing allele was low in the cultivars registered until the year 2000 and only then increased to around 0.5 in the cultivars registered since 2011.
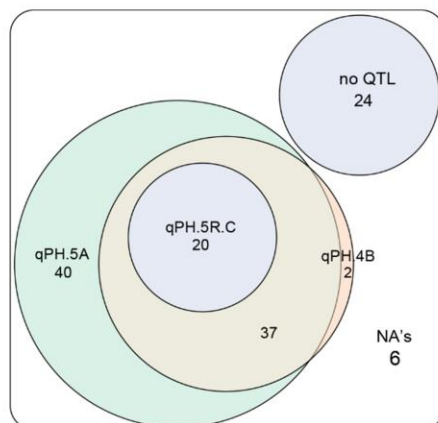
Figure 5. Number of cultivars with one or a combination of the plant height-reducing alleles at the detected QTL.
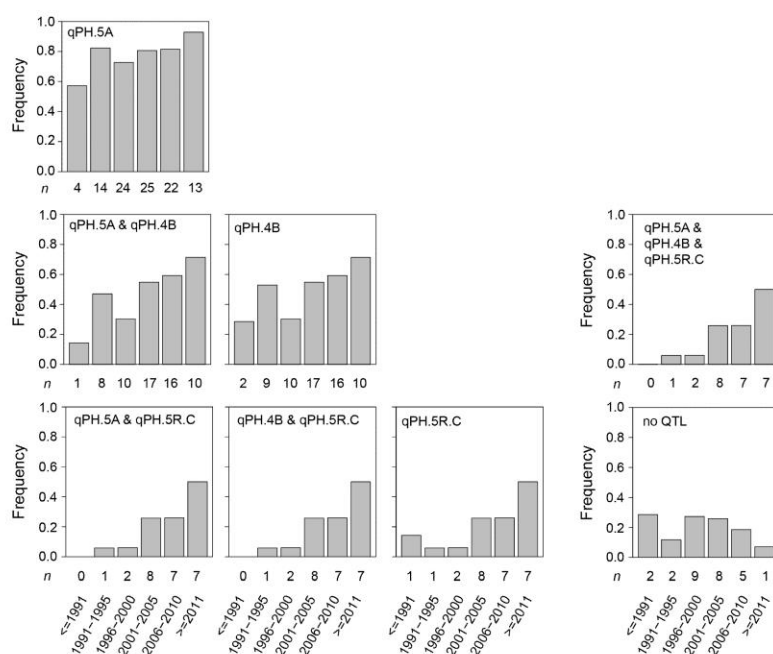

Figure 6. Temporal trends of the utilization of the identified QTL. Frequency of the height-reducing allele at the detected QTL qPH.5A, qPH.4B, and qPH.5R.C as well as their combinations in the registered cultivars dependent on their year of release.

3. Discussion

Plant height is of high importance in triticale breeding programs and in this study, we evaluated a diverse panel of 846 Central European triticale lines to investigate the genetic architecture underlying plant height, fine-map, and characterize identified QTL and draw conclusions for triticale breeding.

3.1. Genome-Wide Association Mapping and Characterization of QTL for Plant Height

Genome-wide association mapping revealed four putative QTL for plant height on chromosomes 5A, 4B, 4R, and 5R, of which the 5A, 4B, and 5R QTL can be considered as medium- to large-effect QTL. The QTL on chromosomes 5A, 4B, and 5R were also identified for developmental stage and thus have pleiotropic effects on both traits. The effects of these QTL on plant height may appear smaller than expected, but were shown to strongly depend on the application rate of growth regulators (Supplementary Figure S1). Notably, owing to the large variation present in the diversity panel, growth regulators were applied in these trials to prevent lodging and to mirror agronomic practice. Thus, the QTL effects can be expected to be larger in non-treated plants and reflect the substantial effect of the QTL even in combination with growth regulators.

In a single fit model with only one QTL, the 5A and 4B QTL explained 13.06 and 18.97% of the genotypic variance, respectively, which reduced to 0.40 and 2.34% in a joint fit of all QTL. Notably, the 5R QTL is fitted first in the joint fit model and thus retains its explained variance. The finding that the other two QTL do not, can have different reasons. It might be due to linkage disequilibrium among the QTL regions. However, at least for the 5A QTL, this LD with the 5R QTL was rather low and therefore LD among these QTL appears as a less likely explanation. A more likely reason is the joint occurrence of the height-reducing alleles of the three QTL in triticale lines observed in the registered cultivars but also in the entire panel (Figure 5). As the height-reducing allele of the 5R QTL occurs together with those of the 5A and 4B QTL, the genotypic classes are always confounded. Regarding the QTL on chromosomes 4B and 5R, the available genetic map positions as well as physical positions linked the significantly associated markers to regions on these two chromosomes. In an unrelated bi-parental population, however, we observed tight linkage among all these markers that segregated in that population (data not shown), indicating that they may actually identify only one QTL. Thus, further research using bi-parental families is required to disentangle the identities and effects of these QTL.

For the major QTL on chromosome 5R, three markers remained which in a joint fit still explained 1.79, 1.91, and 29.38% of the genotypic variance. While this could mean that there are three separate QTL, we concluded that it is more likely that they all identify the same QTL, as they are located in close proximity. Probably even the most strongly associated marker is not in perfect LD with the causal variant, such that also other markers can capture a small part of the variance explained by the QTL. Korzun et al. [34] mapped the dominant dwarfing gene *Ddw1* on the long arm of chromosome 5R. Braun et al. [33] recently identified a gibberellin 2-oxidase gene (*ScGA2ox12*) as a candidate for *Ddw1* in rye, which is located at 862 Mbp on chromosome 5R. As *Ddw1* has been reported in spring and winter triticale [14,17,31,35] and as the QTL region coincides with the position of *Ddw1*, we conclude that the 5R QTL corresponds to *Ddw1*. *Ddw1* is present in 20 of the 129 registered cultivars analyzed in this study and appears to have been increasingly used in recent years. However, previous work also illustrated adverse effects of *Ddw1*, for example on Fusarium head blight resistance [14]. The utilization of this height-reducing locus in triticale, therefore, requires further research on its advantages and limitations.

The QTL on chromosome 5A was located in the region between 692 and 699 Mbp (Figure 3b) and recently the dominant height-reducing *Rht12* locus has been reported to be located in the region between 700 and 710 Mbp of chromosome 5A in wheat [36]. It thus appears likely that the QTL on chromosome 5A corresponds to *Rht12*. To our knowledge, *Rht12* has not been reported in triticale germplasm so far, but its effect on plant height has been extensively investigated in wheat [23,37,38]. The height-reducing *Rht12* is reported to

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delay ear emergence in wheat [37,38] but this also depends on photoperiodic sensitivity loci [37]. We observed a pleiotropic effect of the QTL on chromosome 5A, with the allele that reduces plant height delaying development and resulting in later heading. Thus, further research is necessary to investigate the effects of the putative *Rht12* locus in triticale on other agronomic traits such as grain yield, ear emergence, disease resistance, early seedling vigor, or coleoptile length.

The height-reducing *Rht-B1* locus located on chromosome 4B has been investigated extensively in wheat [12,20,39–41], but much less so in triticale [31]. In this study, we identified a QTL on chromosome 4B with pleiotropic effects on plant height and developmental stage (Figure 2; Supplementary Table S1). However, *Rht-B1* is located on the short arm of chromosome 4B at approximately 30.8 Mbp, whereas the 4B QTL identified here is located at the opposite end of this chromosome (Figure 3b). Thus, *Rht-B1* is no candidate for this QTL, which is in line with previous findings in triticale, where *Rht-B1* did not contribute to the genetic control of plant height [32]. This QTL also showed a substantial plant height reduction and a high penetrance in the registered cultivars used in this study and is therefore of interest for future research.

3.2. Long-Term Trends of Plant Height in Triticale

The analysis of the development of plant height from the early 1980s—when the first cultivars of this panel were registered—until today, revealed a constant reduction in plant height (Figure 1) that is in line with previous findings [9]. In recent years, however, the demand for triticale genotypes with a greater biomass yield increased [15,42]. As plant height is one of the main contributors to biomass yield [15–17], taller genotypes were favored in breeding programs focusing on biomass yield.

In line with the phenotypic observation, we observed an increasing frequency of the height-reducing alleles at the three investigated QTL (Figure 6). The QTL on chromosome 5A, assumed to be *Rht12*, was constantly present at a medium to high frequency throughout the evaluated period. By contrast, especially the frequency of the QTL on chromosome 5R, likely *Ddw1*, increased only more recently since the turn of the millennium.

3.3. Conclusions

In this study, we identified three medium- to large-effect QTL and fine-mapping indicated that two of them correspond to *Rht12* and *Ddw1*. While *Ddw1* has been reported in triticale previously [14,17,31,35], *Rht12* has not yet been reported in triticale.

The widely used height-reducing locus *Rht-B1* has been one of the main contributors to improving grain yield and yield stability in wheat [20,39,43], but can negatively affect grain yield under drought conditions [43–45]. With the consequences of climate change [46], breeders will have to improve drought resistance also in triticale breeding material [47]. In wheat, the effect of height-reducing loci on seedling establishment and associated traits such as coleoptile length and early seedling vigor has been widely evaluated under dry conditions [25,26,48]. Genotypes carrying the dwarfing genes *Rht8* and *Rht12* have been reported to react less pronounced to heat and drought stress [23,37,49]. The effect of height-reducing loci on drought tolerance in triticale lacks behind what is known already in wheat and further research is required to evaluate the putative *Rht12* as well as other loci for their effects on drought tolerance and thus their potential for future breeding of climate-resilient triticale.

In conclusion, the diversity panel showed significant genotypic variation for plant height, which can be exploited to tailor plant height in breeding programs, either by phenotypic or marker-assisted selection. In addition, height-reducing loci from wheat, such as *Rht-B1* or *Rht24* [20,39], do not appear to be exploited in Central European triticale so far and could be introgressed from wheat.

4. Materials and Methods

4.1. Phenotypic Data

This study was based on a total of 846 triticale ($\times Triticosecale$ Wittmack) genotypes consisting of a diverse collection of officially registered Central European cultivars ($n = 129$) and lines in advanced breeding status ($n = 717$, for further details see Neuweiler et al. [50]). Phenotypic data of the diversity panel were taken from a large field trial ($n = 1280$), for which genotypes were grown in two separate sets comprising 800 (set A) and 500 (set B) individuals including 20 common checks. All genotypes were planted in yield plots (Y) with a plot size ranging from 5 to 10.5 m^2 and in addition, in double row observation plots (O) in the growing seasons 2014 and 2015. All field trials were designed as partially replicated α-lattice designs [51]. The yield plot trials had an average replication number of 1.3 and 1.2 for set A and B, respectively, observation plots were replicated twice. The field trials were grown in Eckartsweier (EWE, Y, 48°31′18′′ N, 7°52′18′′ E, 140 m above sea level, masl), Franconia (FRA, Y, 2014: 49°39′58′′ N, 9°47′30′′ E, 310 masl, 2015: 49°49′22′′ N, 10°6′19′′ E, 270 masl), Hohenheim (HOH, O, Y, 48°28′49′′ N, 9°11′16′′ E, 400 masl), Ihinger Hof (IHO, O, Y, 48°44′40′′ N, 8°55′25′′ E, 480 masl), Moosburg (MSB, Y, 48°26′36′′ N, 11°45′22′′ E, 420 masl), and Oberer Lindenhof (OLI, O, 48°28′49′′ N, 9°18′56′′ E, 700 masl) in 2014 and 2015 as follows: in 2014 only set A was evaluated as yield plots at the locations EWE, FRA, HOH, IHO, and MSB. In 2015, sets A and B were grown at HOH, IHO, and OLI as observation plots and in EWE, FRA, HOH, IHO, and MSB as yield plots. Genotypes grown in yield plots were treated with growth regulators twice and in observation plots once to prevent lodging. Plant height (cm) was assessed after flowering from the ground to the tip of the ears, excluding awns. In addition, the developmental stage was scored on a BBCH scale according to Zadoks et al. [52] when the ears of the majority of the genotypes were emerging.

4.2. Statistical Analysis

The linear mixed models used in this study followed the syntax as outlined by Piepho et al. [53], where dot operators specify crossed effects and fixed and random effects are separated by a colon, introducing fixed effects first. We used the following model to obtain best linear unbiased estimates (BLUEs) and least significant differences (LSD) as well as a full random model to determine variance components and heritability estimates:

$$G{:}E + E{\cdot}G + E{\cdot}T + E{\cdot}T{\cdot}R{\cdot}B + E{\cdot}T{\cdot}R{\cdot}B \tag{1}$$

where E, G, T, R, and B denotes environments, genotypes, trial effects as a combination between genotype sets A and B and plot types (yield plots, double row observation plots), replications within each environment-trial combination, and incomplete blocks within replications of each environment-trial combination. Trial main effects were nested within environments. Variance components for the diversity panel used in this study were calculated using dummy variables in the above model. Variance components and heritabilities were estimated with the full random model using the restricted maximum likelihood method implemented in the software package ASReml-R 3.0 [54]. We assumed heterogeneous error variances at an environment-trial level. Variance components were tested for significance ($p < 0.05, 0.01, 0.001$) using a likelihood ratio test [55]. LSDs ($p < 0.05$) were calculated as an approximation using the twofold of the average standard error or a difference. Broad-sense heritability (H^2) was estimated according to Cullis et al. [56] using the mean variance of a difference ($\overline{v}_{BLUP}$) between two best linear unbiased predictors and the genotypic variance component (σ_G^2) as:

$$H^2 = 1 - \frac{\overline{v}_{BLUP}}{2 * \sigma_G^2} \tag{2}$$

4.3. Molecular Data Analysis

Marker data were available for all of the 846 genotypes of the diversity panel and obtained by a genotyping-by-sequencing approach from Diversity Arrays Technology, Canberra, Australia (www.diversityarrays.com, accessed on 14 May 2021). Markers showing more than 20% missing values and a minor allele frequency of 5% or less were discarded. Unmapped significant markers were assigned to their most likely chromosomal position by their linkage disequilibrium with mapped markers. These measures yielded 31,823 markers in total that were used for the genome-wide association study. Positions for 10,192 markers on the A genome and 14,747 markers on the B genome were known [57]. Map positions for markers from the R genome were assigned as described in Neuweiler et al. [50] resulting in 6884 markers with a known map position. In total, 25,721 dominant silico-DArT and 6102 SNP markers were used. To differentiate between the silico-DArT and SNP markers, we assigned a "D" and an "S" prefix for silico-DArT and SNP markers, respectively. To determine the physical positions of markers in the QTL regions, the sequences of the markers were BLASTed against the reference genomes of wheat and rye (IWGSC RefSeq v1.0 and Secale cereale Lo7).

4.4. Genome-Wide Association Study

We performed genome-wide association mapping using a mixed linear model incorporating a kinship matrix [58] implemented in the GAPIT R package [59]. We used a Bonferroni-corrected significance threshold of $p < 0.05$. The genotypic data, filtered as stated above, were imputed using the k-nearest neighbor genotype imputation technique LD-kNNi implemented in LinkImpute [60]. Furthermore, we calculated the total proportion of the genotypic variance (p_G) explained by all detected QTL as $p_G = R^2_{adj}/H^2$, where R^2_{adj} was the adjusted R^2 from the linear model, and H^2 as the heritability of the trait [61]. To correct for collinearity, we ordered the QTL in the order of their strength of association and calculated their individual proportion of explained genotypic variance accordingly. The p_G values for individual QTL were derived by estimating their sums of squares in the linear model. The allele substitution (α) effect was derived as $\alpha = a(1 + k(p_1 - p_2))$ with a as genotypic value of the corresponding locus, k the degree of dominance, as well as p_1 and p_2 as the allele frequencies. The α-effect corresponds to one-half of the difference of the genotypic values of the corresponding genotypic classes of a QTL when inbred lines are considered. The α-effects were acquired by fitting a linear model for the marker of interest and the corresponding trait. Then the α-effect is represented by the determined regression coefficient [62].

Data Availability Statement: The study did not report any data.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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3 Hybrid breeding for biomass yield in winter triticale: II. Combining ability and hybrid prediction

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ORIGINAL ARTICLE

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Hybrid breeding for biomass yield in winter triticale: II. Combining ability and hybrid prediction

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Abstract

Accurate hybrid prediction and knowledge about the relative contribution of general (GCA) and specific combining ability (SCA) are of utmost importance for efficient hybrid breeding. We therefore evaluated 91 triticale single-cross hybrids in field trials at seven environments for plant height, heading time, fresh biomass, dry matter content and dry biomass. Fresh and dry biomass showed the highest proportion (23%) of variance due to SCA. Prediction accuracies based on GCA were slightly higher than based on mid-parent values. Utilizing parental kinship information yielded the highest prediction accuracies when both parental lines have been tested in other hybrid combinations, but still moderate-to-low prediction accuracies for two untested parents. Thus, hybrid prediction for biomass traits in triticale is currently promising based on mid-parent values as emphasized by our simulation study, but can be expected to shift to GCA-based prediction with an increasing importance of GCA due to selection in hybrid breeding. Moreover, the performance of potential hybrids between newly developed lines can be predicted with moderate accuracy using genomic relationship information.

KEYWORDS

biomass, genomic prediction, heterosis, hybrid prediction, prediction accuracy, simulation study, triticale

1 | INTRODUCTION

Owing to the increasing demand for renewable energy sources and a policy to promote them, the increase of plant biomass yield has become a breeding goal in crops suited for biomass production (Monforti, Bódis, Scarlat, & Dallemand, 2013; Wit & Faaij, 2010). A promising bioenergy crop is triticale (× *Triticosecale* Wittmack), as it possesses a high biomass and biogas yield potential compared to other small grain cereals (LTZ, 2013; Mergoum et al., 2009; Weiland, 2010). It could therefore become an attractive alternative or complement to the predominant, highly productive silage maize production

in Central Europe (Weiland, 2010). To date, triticale breeding programmes mainly focus on increasing grain yield, but also have a high potential to increase biomass yield due to the large genetic variation observed in European breeding material (Gowda et al., 2011).

Triticale is primarily improved by line breeding, but hybrid breeding has recently received increased attention to improve the trait performance of autogamous crops (Fischer et al., 2010; Gowda et al., 2013; Longin et al., 2012). Interestingly, Losert, Maurer, Weissmann, and Würschum (2016) reported a considerable commercial heterosis for biomass yield in triticale, indicating the potential economic value of hybrid breeding in this crop. Consequently, switching the variety type in triticale from line to hybrid cultivars appears worthwhile to investigate

further. A major bottleneck for hybrid breeding, however, is the fact that the number of possible hybrid combinations increases quadratically with the number of parental lines, thus resulting in huge numbers of possible hybrid combinations (Bernardo, 2010). Producing and testing all these combinations in the field is not feasible, and hence, approaches to preselect the most promising hybrid combinations are required.

Predicting the performance of single-cross hybrids based on mid-parent values is supposed to provide good results for qualitatively inherited and highly heritable traits as shown in previous studies (Boeven, Würschum, Weissmann, Miedaner, & Maurer, 2016; Gowda et al., 2013; Oettler, Tams, Utz, Bauer, & Melchinger, 2005). However, biomass yield is a highly quantitative trait (Alheit et al., 2014), and therefore, predictions based on mid-parent values are expected to be less powerful due to masking non-additive effects (Bernardo, 2010; Smith, 1986). Predictions of single-cross performance based on general combining ability (GCA) effects have shown promising results in triticale for complex traits such as grain yield or Fusarium head blight (Boeven et al., 2016; Fischer et al., 2010; Gowda et al., 2013). The ability to predict hybrid performance precisely by GCA effects strongly depends on the variance ratio between specific combining ability (SCA) effects and the total genotypic variance (Hallauer, Carena, & Miranda Filho, 2010; Melchinger, 1999). Previous results in triticale showed that this ratio strongly depended on the investigated trait (Boeven et al., 2016; Gowda et al., 2013; Oettler et al., 2005).

A limitation of the GCA-based hybrid prediction is that it is only possible when GCA estimates are available, which requires the production of hybrids and subsequent time- and resource-intensive field trials. Predicting hybrid performance from parental lines without their prior evaluation is possible, when pedigree or genomic relationship information is exploited. This can be used to preselect single-crosses to be tested in the field (Bernardo, 2010) and has previously been shown to result in promising prediction accuracies in different crops, including maize (Bernardo, 1994, 1995, 1996a, 1996b; Charcosset et al., 1998), sunflower (Reif et al., 2013) and triticale (Boeven et al., 2016; Gowda et al., 2013).

The aim of this study was to assess the potential of hybrid prediction for biomass yield in triticale. To this end, a set of 91 single-cross hybrids, derived from 33 parental inbred lines crossed in an incomplete factorial mating design, were evaluated in multi-location field trials. In particular, the goals of our study were to (a) evaluate the variances due to GCA and SCA effects, (b) compare different approaches to predict single-cross hybrid performance and (c) draw conclusions for triticale hybrid breeding.

2 | MATERIALS AND METHODS

2.1 | Phenotypic data

This study was based on a total of 178 winter triticale genotypes, comprising 23 female parental lines, 10 male parental lines, 91 single-cross hybrids and 54 check genotypes as described previously (Losert et al., 2016). The single-cross hybrids were derived by crosses between female and male parental lines in an unbalanced and incomplete factorial mating design (Table S1) using a cytoplasmic male sterility (CMS) inducing cytoplasm based on *Triticum timopheevii* Zhuk. The check genotypes comprised 8 triticale cultivars officially registered in Central Europe ('Agostino', 'Amarillo 105', 'Balu PZO', 'Cosinus', 'Grenado', 'SW Talentro', 'Tarzan' and 'Tulus') as well as 46 advanced breeding lines.

The field experiment was carried out in the growing seasons 2011/12 and 2012/13 at five locations: Bohlingen (BOH, 47°43′12″N, 8°53′46″E, 420 metres above sea level, masl), Eckartsweier (EWE, 48°31′18″N, 7°52′18″E, 140 masl), Hohebuch (HET, 49°12′29″N, 9°39′29″E, 360 masl), Hohenheim (HOH, 48°28′49″N, 9°11′16″E, 400 masl) and Oberer Lindenhof (OLI, 48°28′49″N, 9°18′56″E, 700 masl). The locations Hohebuch and Eckartsweier were only used for field trials during the growing season 2011/12.

Due to limited seed availability, not all single-cross hybrids were cultivated in both growing seasons (Table S1), and in addition, a partially replicated field design was chosen (Williams, Piepho, & Whitaker, 2011) with an average replication number across locations of 1.5 for females, hybrids and checks, and 1.6 for males. Plot sizes ranged from 6.0 to 9.0 m^2, and sowing density was 280 viable seeds/m^2. The following traits were evaluated in these trials: heading time (HT, EC stage according to Zadoks, Chang, & Konzak, 1974), plant height (PH, cm), fresh biomass (FBM, Mg/ha), dry matter content (DMC, %) and dry biomass (DBM, Mg/ha).

2.2 | Molecular data analysis

DNA of all parental lines was extracted from the leaves of young plants using a modified CTAB protocol (Doyle & Doyle, 1990) and genotyped by genotyping-by-sequencing by Diversity Arrays Technology, Canberra, Australia (www.diversityarrays.com). Markers showing a minor allele frequency of 5% or less, or more than 20% missing values were removed, resulting in a set of 56,722 dominant DArT markers used for further analyses.

A principal coordinate analysis (PCoA) based on Rogers' distances between pairs of inbred lines (Rogers, 1972) was performed according to Gower (1966). Elements of the additive relationship matrices of the female (A_F) and male (A_M) parental lines as well as the dominance relationship matrix D were calculated based on (a) the suggestions of Bernardo (1993) and (b) VanRaden (2008) method two. However, estimates exploiting relationship information according to VanRaden (2008) showed only minor differences compared to Bernardo (1993) and are therefore not reported further. Bernardo (1993) calculates the coefficients of coancestry θ_{ij} used for A_F, A_M and D between inbred lines i and j based on molecular marker data as:

$$\theta_{ij} = 1 + \frac{(S_{ij} - 1)}{1 - T}$$

where S_{ij} is the proportion of marker loci with shared variants between inbred lines i and j. The measure of T denotes the average probability that a variant from a parent of inbred line i and a variant

from a different inbred line *j* are alike in state, given that they are not identical by descent and was set to min (1–S_{ij}).

2.3 | Statistical analysis

The models used in this study follow the syntax outlined by Piepho, Büchse, and Emrich (2003). Briefly, crossed effects are denoted with a dot operator and fixed effects are separated from random effects by a colon, with fixed effects first. The model to obtain global variance components and best linear unbiased estimates (BLUEs) was:

$$G + E + E \cdot R : E \cdot G + E \cdot R \cdot B$$

with *G*, *E*, *R* and *B* denoting genotypes, environments, replications and incomplete blocks, respectively. Outliers were determined following method four (Bonferroni–Holm with rescaled MAD standardized residuals) presented by Bernal-Vasquez, Utz, and Piepho (2016). Best linear unbiased predictors (BLUPs) and variance components were calculated with a fully random model for each trait and BLUEs according to the model described above.

To estimate group-specific BLUPs and variance components, dummy variables were introduced for the genotypic groups females, males, hybrids and checks. This allowed to calculate GCA and SCA effects of the hybrids independently from the other genotypic groups. For simplification, dummy variables were suppressed in the following model used to estimate group-specific variance components:

$$E + GR7OUP + E \cdot R : FEMALE + MALE + CHECK +$$
$$FEMALE \cdot E + MALE \cdot E + CHECK \cdot E +$$
$$GCA_F + GCA_M + SCA +$$
$$GCA_F \cdot E + GCA_M \cdot E + SCA \cdot E +$$
$$GROUP \cdot E + E \cdot R \cdot B$$

with *E*, *R* and *B* as environments, replications and incomplete blocks, respectively, as well as *GROUP*, *FEMALE*, *MALE*, *CHECK*, *GCA* and *SCA* as the genotypic groups, females, males, checks, GCA effects of the female (GCA_F) or male (GCA_M) parental lines and the SCA effects.

Exploiting the relatedness between the parental genotypes, we specified a variance–covariance structure for the random effects of GCA_F, GCA_M and SCA (Piepho, Möhring, Melchinger, & Büchse, 2008). We assumed $\mathrm{var}(GCA_F) = \boldsymbol{A}_F \sigma^2_{GCA-F}$, $\mathrm{var}(GCA_M) = \boldsymbol{A}_M \sigma^2_{GCA-M}$ and $\mathrm{var}(SCA) = \boldsymbol{D} \sigma^2_{SCA}$, where σ^2_{GCA-F}, σ^2_{GCA-M} and σ^2_{SCA} refer to the variance of GCA_F, GCA_M and SCA effects estimated by REML for the female or male as well as the hybrid combinations derived by crosses between the female and male parental lines, respectively. $\boldsymbol{A}_F$ was a 23 × 23 matrix and $\boldsymbol{A}_M$ a 10 × 10 matrix containing genomic relationship coefficient estimates defining the degree of genetic covariance between all pairs of female and male parental components, respectively. $\boldsymbol{D}$ was a 91 × 91 matrix containing the dominance relationships for the 91 single-cross hybrids derived from the probability that both alleles at a locus are identical by descent. Elements of $\boldsymbol{D}$ correspond to the

dominance relationship between two hybrids *a × b* and *c × d* with their parents *a* and *b* or *c* and *d*, respectively, and were calculated according to the suggestions of Reif et al. (2013) as $\delta_{ab,cd} = \left\{ \left(\theta_{ac} \theta_{bd} + \theta_{ad} \theta_{bd} \right) / 2 \right\}$, where θ_{ac}, θ_{bd}, θ_{ad} and θ_{bd} refer to the coefficient of coancestry between corresponding parents estimated based on marker data. The matrices $\boldsymbol{A}_F$, $\boldsymbol{A}_M$ and $\boldsymbol{D}$ were calculated as outlined above.

Global and group-specific variance components were calculated with a fully random model using the restricted maximum likelihood method (REML) implemented by the software package ASReml-R 3.0 (Butler, Cullis, Gilmour, & Gogel, 2009). Residual error variances were assumed to be heterogeneous among environments (Kelly, Smith, Eccleston, & Cullis, 2007) for both, the global and the group-specific variance component estimation. Test of significance ($p < .05$, .01, and .001) of the variance components was performed with a likelihood ratio test according to Stram and Lee (1994). Global and group-specific heritabilities (H^2) were calculated as $H^2 = 1 - \bar{v}_{BLUP} / \left(2 * \sigma^2_G \right)$ (Cullis, Smith, & Coombes, 2006), with $\bar{v}_{BLUP}$ being the mean variance of a difference of two BLUPs and σ^2_G the genotypic variance estimate across all groups or the nested genotypic variance estimate of the corresponding genotypic group.

Hybrid performance was estimated based on GCA and SCA effects of the parental inbred lines *i* and *j* as:

$$\hat{y}_{hybrid(ij)} = \mu + \widehat{GCA}_{F(i)} + \widehat{GCA}_{M(j)} + \widehat{SCA}_{F(i) \times M(j)}$$

with $\hat{y}_{hybrid(ij)}$, μ, $\widehat{GCA}_{F(i)}$, $\widehat{GCA}_{M(j)}$ and $\widehat{SCA}_{F(i) \times M(j)}$ being the estimated phenotypic performance of the hybrid derived from the parental lines *i* and *j*, the overall mean, the GCA effect of the *i*-th female line, the GCA effect of the *j*-th male line and the SCA effect of the cross between the *i*-th female parental line with the *j*-th male parental line, respectively.

Pearson's product–moment correlations (*r*) were estimated between (a) Rogers' distance and the mid-parent heterosis of the corresponding hybrid, as well as (b) per se performance of the parental lines and the corresponding GCA effects of the female and male parental inbred lines. Mid-parent heterosis of each single-cross hybrid was estimated as the difference between the mean per se performance of the two parental inbred lines *i* and *j* of the corresponding single-cross and the hybrid's per se performance.

2.4 | Hybrid prediction

Prediction accuracies were calculated by dividing Pearson's product–moment correlation between predicted and observed values by the square root of the global heritability estimate (Table S2) of the corresponding trait (Legarra, Robert-Granié, Manfredi, & Elsen, 2008). Hybrids were predicted based on (a) mid-parent (MP) performance, (b) the sum of parental GCA effects, as well as the sum of parental (c) GCA (GCA$_{kin}$), or (d) GCA and SCA effects (GCA-SCA$_{kin}$) exploiting relationships between parental inbred lines as outlined above.

We evaluated the prediction accuracies by cross-validation approaches to correct for autocorrelation (Schrag, Melchinger,

Sørensen, & Frisch, 2006). First, we used a leave-one-hybrid-out cross-validation to predict each untested single-cross based on parental GCA effects, not exploiting genetic relationship (GCA$_{loho}$). For this scenario, all parental lines were included and only the hybrid to be predicted was not considered for the estimation of the parental GCA effects. Second, we applied four scenarios, where (a) both (T2), (b) only the male (T1$_{female}$), (c) only the female (T1$_{male}$), or (d) no (T0) parental component(s) contributed to the prediction of the untested single-cross, using relationship information based on (a) parental GCA effects only (GCA$_{kin}$) or (b) GCA and SCA effects (GCA-SCA$_{kin}$). For these three scenarios, one (T1$_{female}$ and T1$_{male}$) or two (T0) of the parental lines were removed from the training set, which was achieved with the dummy variables mentioned for the model above.

In addition, we expanded the dominance relationship matrix **D** to all 230 possible hybrid combinations, predicting the 139 untested hybrids by using the sum of GCA or GCA and SCA effects. For a better comparison between the five traits, we normalized the predicted phenotypic values for each trait, including tested and untested hybrids, as:

$$z_i = \frac{x_i - \bar{x}}{\sigma_x}$$

where z_i is the normalized phenotypic value, x_i is the predicted phenotypic value of the i-th hybrid, and $\bar{x}$ and σ_x are the mean and the standard deviation across the predicted values of tested and untested hybrids, respectively.

2.5 | Simulation study

We performed a simulation study to demonstrate what different prediction accuracies actually mean in a breeding programme, regarding the identification and thus the production of superior hybrids based on predicted hybrid values. We assessed the rate of success of having produced and tested (a) the best or (b) one of the five best hybrids from a 10 × 10 or a 100 × 100 factorial, when the hybrids are predicted with varying prediction accuracies, and only a subset of all possible hybrids from the factorial is actually produced. The simulation was based on 1,000,000 runs.

3 | RESULTS

Rogers' distance estimates based on genome-wide molecular marker data revealed a high variation in relatedness within as well as among the female and male parental lines (Figure 1a). Rogers' distances of the produced hybrids provided a good coverage of all possible hybrid combinations. Principal coordinate axes one and two explained 14.5% and 11.7% of total genotypic variation, respectively. A distinct clustering of the female and male parental lines was not observable (Figure 1b).
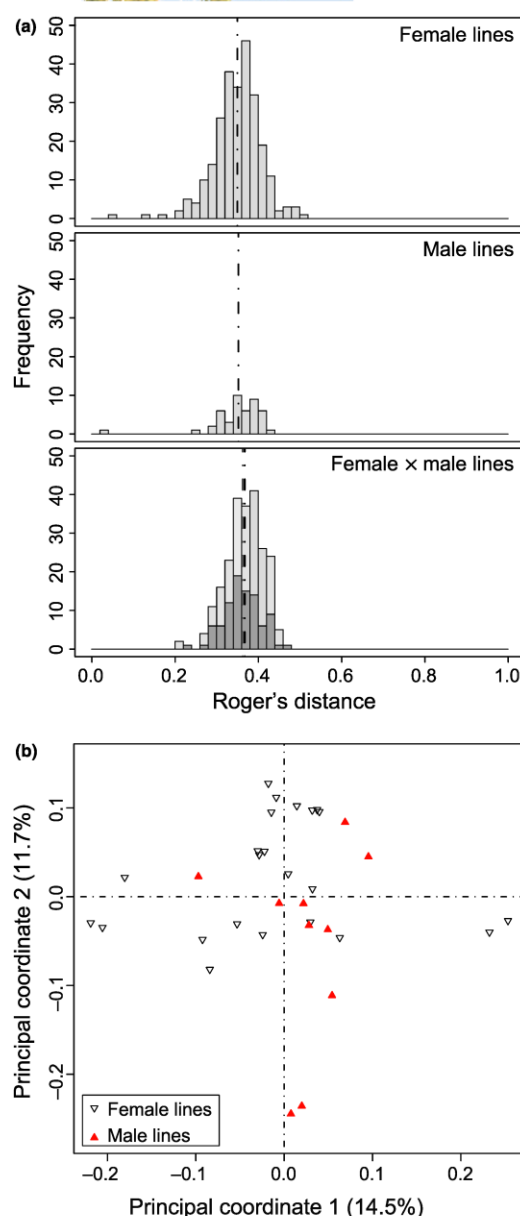
FIGURE 1 (a) Histogram of Rogers' distances based on genome-wide marker data among the 23 female and the 10 male parental lines, as well as between these parental lines. The dark grey bars indicate the distribution of Rogers' distances between the female and male parental lines of the triticale hybrids evaluated in this study. (b) Principal coordinate analysis of the 23 female and 10 male parental lines based on Rogers' distances. Values in parentheses refer to the proportion of variance explained by the principal coordinates [Colour figure can be viewed at wileyonlinelibrary.com]

Phenotypic assessment of the single-cross hybrids in multi-location trials yielded high heritability estimates for all five evaluated traits, ranging from 0.83 for heading time to 0.95 for plant height (Table 1). Also for the highly quantitative traits fresh biomass and dry biomass, high heritabilities of 0.91 and 0.92, respectively, could be obtained. Most variance components of the single-cross hybrids were significantly different from zero. The ratio of the SCA variance to the total genetic variance was low for heading time, plant height and dry matter content, ranging between 0.01 and 0.04. By contrast, for fresh biomass and for dry biomass this ratio was higher, amounting to 0.23 for both traits.

The correlation between the per se performance of the parental lines and their GCA effects was high for heading time, plant height and dry matter content, and moderate for fresh and dry biomass (Table 2). The correlation between Roger's distance between the two parental lines of a hybrid and the hybrid's mid-parent heterosis, by contrast, was low and non-significant. Hybrid prediction based on the mean of the parental lines yielded a high prediction accuracy between 0.83 and 0.89 for heading time, plant height and dry matter

content, and a moderate prediction accuracy of 0.67 and 0.63 for fresh and dry biomass, respectively (Table 3). Using GCA estimates to predict single-cross hybrids increased the prediction accuracy for all traits, but particularly for fresh and dry biomass with a relative increase of around 30% compared to prediction based on mid-parent values. Exploiting genetic relationships among the parental lines did not increase prediction accuracy based on GCA effects (GCA_{kin}), but for the prediction based on GCA and SCA effects (GCA-SCA$_{kin}$) the accuracy increased by around 0.15 for fresh and dry biomass (Table 3). The use of relationship information of all 230 potential hybrid combinations resulted in higher median estimates for the predicted phenotypic values of the 91 tested hybrids compared to the 139 untested hybrids for all traits (Figure S1). The inclusion of SCA effects in the prediction showed only minor changes.

To account for autocorrelation in the GCA-based prediction approaches, we applied different cross-validation schemes. In brief, we used a leave-one-hybrid-out cross-validation not using kinship information (GCA_{loho}) and two approaches exploiting genetic relationship information based on either GCA (GCA_{kin}) or GCA and SCA effects (GCA-SCA$_{kin}$). For the latter two, three scenarios were evaluated that represent the situations where both parental components (T2), only the female (T1$_{male}$) or the male (T1$_{female}$) parent, or none of the two parental components (T0) have been tested in other hybrid combinations before. Cross-validated prediction accuracies of GCA_{loho} were considerably lower compared to the GCA-based predictions (Table 4). For dry biomass yield, this cross-validated prediction accuracy dropped to nearly the level of the mid-parent-based prediction (Table 3). Estimates of the GCA_{kin} T2 scenario yielded similar prediction accuracies compared to GCA_{loho} for all traits, except fresh biomass yield, for which it dropped even below the corresponding value of mid-parent prediction (Table 3). As a general trend, we observed a decrease of the prediction accuracy from the T2 to the T1 and the T0 scenario. Including dominance relationships between parental inbred lines (GCA-SCA$_{kin}$) did not yield substantially higher estimates compared to using only GCA effect estimates (GCA_{kin}) (Table 4).

We performed a simulation study to demonstrate what the different prediction accuracies mean for hybrid prediction applied in a breeding programme (Figure 2). We assumed a 10 × 10 or a 100 × 100 factorial, for which the hybrids were predicted with a prediction accuracy ranging from 0 to 1. Out of these possible hybrids, only a certain fraction, that is selected based on the predicted hybrid values, is produced and tested in field trials. The question is then, what is the probability of having included the best or one of the five best hybrids in this selected fraction. Obviously, if the selected

TABLE 1 Means and ranges of best linear unbiased estimators, heritability estimates across environments and variance components for 91 single-cross hybrids for the traits heading time (HT), plant height (PH), fresh biomass (FBM), dry matter content (DMC) and dry biomass (DBM)

	HT	PH	FBM	DMC	DBM
Min	52.7	92.5	30.7	34.7	12.1
Max	60.5	128.7	44.5	39.9	16.7
Mean	56.8	113.7	39.5	37.2	14.7
σ^2_{GCA-F}	0.44***	44.39***	1.26**	0.21***	0.15*
$\sigma^2_{GCA-F \times E}$	0.11**	1.64***	0.00	0.14***	0.00
σ^2_{GCA-M}	1.43***	92.26***	5.36***	0.78***	0.65***
$\sigma^2_{GCA-M \times E}$	0.46***	6.24***	1.18***	0.29***	0.15***
σ^2_{SCA}	0.08**	4.12***	2.02***	0.01	0.24***
$\sigma^2_{SCA \times E}$	0.06*	1.48*	0.86+	0.00	0.10+
$\sigma^2_{SCA}/\sigma^2_{sum}$ [a]	0.04	0.03	0.23	0.01	0.23
H^2	0.83	0.95	0.91	0.89	0.92
σ^2_e [b]	0.97	9.76	6.89	1.27	0.89

[a]Sum of $\sigma^2_{GCA-F} + \sigma^2_{GCA-M} + \sigma^2_{SCA}$.
[b]Pooled residual error variance across all genotypic groups.
+, *, **, ***Significantly different from zero at the 0.1, 0.05, 0.01 and 0.001 probability level, respectively.

TABLE 2 Pearson's product–moment correlations (r) between parental per se performance (per se) and general combining ability (GCA) effects as well as between Rogers' distance (RD) and mid-parent heterosis (MPH), shown for heading time (HT), plant height (PH), fresh biomass (FBM), dry matter content (DMC) and dry biomass (DBM)

	HT	PH	FBM	DMC	DBM
r(per se, GCA)	0.81***	0.89***	0.57***	0.73***	0.54***
r(RD, MPH)	0.08	−0.07	−0.02	−0.00	−0.01

***Significantly different from zero at the 0.001 probability level.

fraction is 1.0, that is if all possible hybrids from the factorial are produced, these will include the best hybrid and the rate of success is 1. Likewise, if the prediction accuracy is 1.0, the prediction is perfect, and irrespective of the number of produced hybrids, these will include the best one, even if just one hybrid is produced. Now if, for example, we assume a moderate prediction accuracy of 0.6 and we expect a success rate of at least 0.95 of having included the best hybrid in the selected fraction, then we need to produce approximately 46% of all possible hybrids in the smaller factorial and 17% in the larger factorial. If, instead, we aim to identify at least one of the five best hybrids, which can be expected to not be significantly different anyhow, then the fraction of hybrids that needs to be produced decreases substantially to around 10% in the smaller and 2% in the larger factorial. As a general trend, the fraction of hybrids that has to be produced to include the best or at least one of the five best hybrids, increased with decreasing prediction accuracy.

TABLE 3 Prediction accuracy for heading time (HT), plant height (PH), fresh biomass (FBM), dry matter content (DMC) and dry biomass (DBM), for prediction based on mid-parent value (MP), the sum of general combining ability (GCA) effects, the sum of GCA effects (GCA$_{kin}$) and the sum of GCA and specific combining ability (SCA) effects (GCA-SCA$_{kin}$) using kinship estimates

	HT	PH	FBM	DMC	DBM
MP	0.89	0.86	0.67	0.83	0.63
GCA	0.97	0.99	0.87	0.99	0.85
GCA$_{kin}$	0.97	0.99	0.85	0.99	0.84
GCA-SCA$_{kin}$	1.00[a]	1.00[a]	1.00[a]	0.99	1.00[a]

[a]Prediction accuracy values ≥ 1 were set to 1.

TABLE 4 Cross-validated prediction accuracy for heading time (HT), plant height (PH), fresh biomass (FBM), dry matter content (DMC) and dry biomass (DBM), shown for GCA-based leave-one-hybrid-out cross-validation (GCA$_{loho}$), the sum of GCA effects (GCA$_{kin}$) and the sum of GCA and specific combining ability (SCA) effects (GCA-SCA$_{kin}$) using kinship estimates. For the cross-validation of the GCA$_{kin}$ and GCA-SCA$_{kin}$ approaches, three scenarios were simulated, where both (T2), only the male (T1$_{female}$) or only the female (T1$_{male}$), or no (T0) parental component(s) of the predicted hybrid has yet been tested in other hybrid combinations

	HT	PH	FBM	DMC	DBM
GCA$_{loho}$	0.89	0.95	0.74	0.93	0.67
GCA$_{kin}$					
T2	0.88	0.95	0.60	0.92	0.66
T1$_{female}$	0.72	0.72	0.64	0.74	0.60
T1$_{male}$	0.63	0.52	0.44	0.78	0.22
T0	0.08	0.31	0.39	0.52	0.27
GCA-SCA$_{kin}$					
T2	0.88	0.95	0.70	0.92	0.66
T1$_{female}$	0.72	0.77	0.64	0.74	0.60
T1$_{male}$	0.63	0.52	0.46	0.78	0.22
T0	0.09	0.31	0.41	0.52	0.28

4 | DISCUSSION

The first commercial triticale hybrids have already been registered (Longin et al., 2012), and the study of Losert et al. (2016) demonstrated that hybrids possess competitive biomass yield characteristics compared to line cultivars. However, the number of single-cross hybrids that could potentially be generated increases quadratically with the number of parental lines (Bernardo, 2010). Identifying the most promising single-cross hybrids out of the huge number of potential hybrid combinations is one of the biggest challenges for a successful hybrid breeding programme. The aim of this study was, therefore, to compare different approaches to predict single-cross hybrid performance based on mid-parent value, GCA and SCA effects, as well as by exploiting relationship information between the parental lines.

4.1 | Relationship between parental line per se performance and GCA effects

No significant correlation between mid-parent heterosis and Rogers' distance estimates was found for any of the traits evaluated in this study (Table 2), which is consistent with previous results (Gowda et al., 2013; Tams, Bauer, Oettler, Melchinger, & Schön, 2006). The correlation between parental line per se performance and their GCA effects, by contrast, was moderate to high, which corroborates earlier results from triticale and wheat (Gowda et al., 2013; Longin et al., 2013; Miedaner, Schulthess, Gowda, Reif, & Longin, 2017). This can be explained by the fact that the GCA reflects the additive and the additive × additive effects of a line, which are also selected during the improvement of the line per se performance in line breeding. As GCA estimates require the time- and resource-intensive establishment of hybrids and their evaluation in field trials, the parental line per se performance can be used as an efficient first predictor to select lines to be tested in hybrid combinations in triticale hybrid breeding programmes.

4.2 | Hybrid prediction based on mid-parent performance and general combining ability estimates

Estimates of the prediction accuracy based on mid-parent value were moderate for the genetically more complex traits fresh and dry biomass and high for the presumably less complex traits heading time, plant height and dry matter content. The use of GCA estimates instead of mid-parent values to predict hybrid performance resulted in an increased prediction accuracy for all traits, especially for fresh and dry biomass (Table 3). For the latter two traits, the prediction accuracy was still lower compared to the other three traits, which can be explained by a higher contribution of SCA as illustrated by the substantially higher ratio of the variance component of SCA to the total genetic variance. In contrast to prediction based on mid-parent value, GCA-based prediction
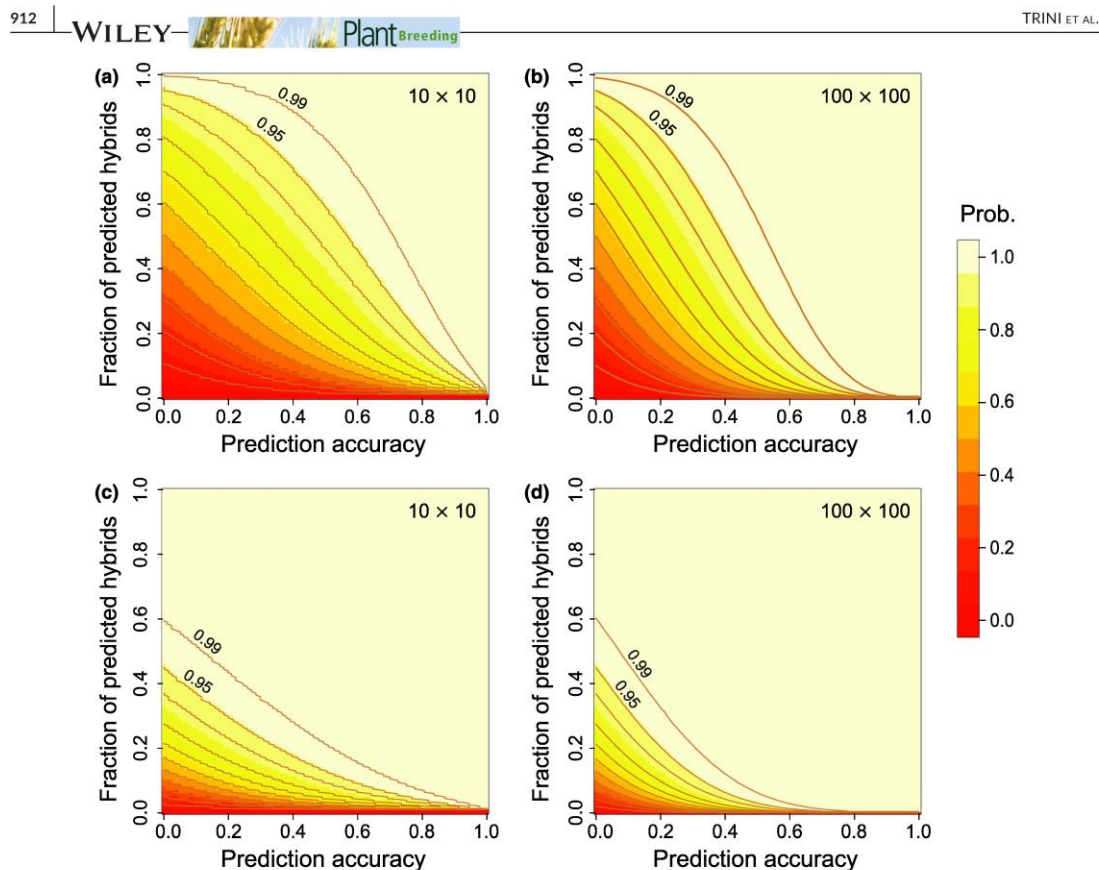
FIGURE 2 Probability of having selected and produced the best (a, b) or at least one of the five best hybrids (c, d), depending on the fraction of predicted hybrids that are actually produced and the prediction accuracy, shown for a 10 × 10 (a, c) or a 100 × 100 (b, d) factorial. Dark colour indicates low, bright colour a high rate of success, with the lines indicating different levels of success in steps of 0.1 and additionally of 0.95 and 0.99 [Colour figure can be viewed at wileyonlinelibrary.com]

also exploits non-additive effects, which was particularly evident for fresh and dry biomass (Bernardo, 2010; Hallauer et al., 2010; Reif et al., 2013; Smith, 1986; Wricke & Weber, 1986). However, dominance effects can only be exploited using SCA effects and seem to have a considerable influence on fresh and dry biomass (Reif, Gumpert, Fischer, & Melchinger, 2007). The influence of dominance effects on single-cross prediction was also reported in previous studies (Technow, Riedelsheimer, Schrag, & Melchinger, 2012; Zhao, Mette, Gowda, Longin, & Reif, 2014).

As a main goal of a hybrid breeding programme is to predict untested hybrids, we applied different cross-validation schemes to mimic this situation. The leave-one-hybrid-out approach (GCA$_{loho}$) predicts the hybrid of interest without including it in the estimation of the parental GCA effects. As expected, cross-validation reduced the GCA-based prediction accuracy (GCA$_{loho}$), but it was still higher than the prediction accuracy obtained by mid-parent values.

Mid-parent heterosis for fresh and dry biomass averaged around 5% in this panel, illustrating that hybrid performance for the

biomass yield traits in triticale is to a large extent based on effects that are captured by the GCA (Losert et al., 2016). In line with the high correlation between the line per se performance and the GCA estimates, hybrid prediction based on mid-parent value provided somewhat lower, but still promising prediction accuracies compared to GCA-based prediction. This, along with the results from our simulation, again underscores the potential to use the per se performance as a first criterion to select not only promising lines, but also hybrid combinations in current triticale hybrid breeding programmes as proposed for maize (Longin, Mi, Melchinger, Reif, & Würschum, 2014) and wheat (Longin, Utz, Melchinger, & Reif, 2007).

4.3 | Hybrid prediction exploiting genetic relationship

Genome-wide markers nowadays offer the possibility to estimate genetic kinship, which can be exploited for hybrid prediction.

The use of relationship information did not improve the prediction accuracy based on GCA effects alone (GCA$_{kin}$), but did so when also SCA effects were included for prediction (GCA-SCA$_{kin}$), particularly for the traits fresh and dry biomass with their higher contribution of SCA (Table 3). However, this difference was not discernible any more when cross-validation was applied, which is in line with previous studies in triticale (Boeven et al., 2016; Gowda et al., 2013), rapeseed (Werner et al., 2018) and sunflower (Reif et al., 2013). Furthermore, no differences between the GCA$_{kin}$ or GCA-SCA$_{kin}$ and the GCA$_{loho}$ estimates were observed, which is probably due to the already high prediction accuracies of the latter (Table 4).

Establishing new hybrid combinations is especially time- and labour-consuming in triticale, as the female component needs to be introgressed into a CMS cytoplasm and tested for sterility, whereas the male component requires a sufficient restoration ability. Predicting hybrids of yet untested parental lines is therefore of high interest in triticale hybrid breeding. We, therefore, considered three scenarios to predict untested single-cross combinations, where either the female (T1$_{female}$), the male (T1$_{male}$), or both (T0) parental lines have not been tested in hybrid combinations before. The reference is the T2 scenario, where both parents have already been used as hybrid components. In general, we observed a decrease in prediction accuracy from T2 to T0 and only slight differences between models considering GCA alone or GCA and SCA estimates (Table 4). This decrease in prediction accuracy following the decreasing number of highly related genotypes in the training set is well known in hybrid prediction and has, for example, been reported in triticale (Boeven et al., 2016; Gowda et al., 2013), wheat (Gowda et al., 2014; Liu et al., 2016; Mirdita et al., 2015; Zhao, Zeng, Fernando, & Reif, 2013), maize (Technow et al., 2012, 2014) and sunflower (Reif et al., 2013). The in part considerably higher prediction accuracies for the T1$_{female}$ compared to the T1$_{male}$ scenario are probably caused by a higher σ^2_{GCA} of the male parental lines (Table 1) (Boeven et al., 2016; Gowda et al., 2013; Reif et al., 2013). Interestingly, except for heading time, the prediction accuracies for the T0 scenario were still promising, ranging between ~0.3 and 0.5. As demonstrated by our simulation study, even such seemingly moderate prediction accuracies allow to substantially reduce the number of hybrids to be established while maintaining a high probability of including one of the best hybrids. Notably, this prediction accuracy can be expected to be improved further if larger training data sets for effect estimation become available.

The medians of predicted phenotypic values of tested compared to untested hybrids differed substantially, and only a small fraction of untested hybrids appear to have promising performance for future testing in the field (Figure S1). This is likely due to the high correlation between the per se performance of the parental lines and their GCA, so that a focus on better performing lines as parental components resulted in the establishment of better performing hybrids. Nevertheless, prediction of hybrids between as yet untested lines is promising and has the potential to become a valuable tool for triticale hybrid breeding.

5 | CONCLUSIONS

We did not observe any population structure separating the female and male lines used in this study (Figure 1), which corroborates previous results in triticale (Fischer et al., 2010; Gowda et al., 2013; Losert, Maurer, Marulanda, & Würschum, 2017; Tams, Bauer, Oettler, & Melchinger, 2004; Tams et al., 2006). This was suspected, as all parental lines were taken from the same triticale breeding germplasm and grouped into the female or male parents based on their maintainer and restorer characteristics, respectively. The establishment of heterotic pools is always mentioned as one of the major goals in hybrid breeding, as they allow to increase the contribution of GCA relative to SCA (Fischer et al., 2010; Reif et al., 2007). This in turn would increase the accuracy of GCA-based predictions. Thus, the establishment of heterotic groups remains a long-term goal in triticale hybrid breeding, but given the difficulties in identifying female and male lines with the required characteristics, the goal for the near future is to identify additional lines for each parental group as well as to improve their performance through intra-group recurrent as well as inter-group reciprocal recurrent selection.

Regarding the considerably high prediction accuracies obtained by mid-parent value raises the question whether the laborious and costly estimation of GCA effects and marker data for genetic relationship information are worthwhile. Indeed, at present the high prediction accuracies make mid-parent value a suitable instrument to predict single-cross hybrids, under the prerequisite of available field data for per se performance of both parental lines. This is in accordance with the results from our simulation, as well as findings from triticale (Boeven et al., 2016; Gowda et al., 2013), durum wheat (Gowda et al., 2010) and wheat (Liu et al., 2016; Longin et al., 2013; Miedaner et al., 2017). However, if selection in hybrid triticale breeding programmes is shifted to GCA instead of per se performance and potentially accompanied by the establishment of heterotic groups, GCA-based prediction can be expected to increasingly outperform prediction based on mid-parent value. Then, the further improvement of the promising prediction accuracies for the T1 and T0 scenarios through targeted compilation of larger training sets with higher predictive power will become a task for hybrid triticale breeding, as this circumvents the necessity to develop parental lines to a level, where testing of hybrid performance in multi-environment trials is possible.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

4   Fast-tracking the evaluation of novel female candidate lines in CMS-based hybrid breeding

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ORIGINAL ARTICLE

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Fast-tracking the evaluation of novel female candidate lines in CMS-based hybrid breeding

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Abstract

Time- and resource-efficient identification of promising lines is of utmost importance in hybrid breeding. Here, we present a novel approach to evaluate female candidate lines for single-cross hybrids, which saves four to five generations by obtaining general (GCA) and specific combining ability (SCA) effect estimates prior to the introgression of the female lines into a male sterility-inducing cytoplasm. The approach is based on three-way crosses and we exemplarily demonstrate its power by predicting 57 single-cross hybrids of triticale. Prediction accuracies based on GCA or GCA and SCA effects estimated in three-way hybrids were generally superior to those based on mid-parent values. Notably, a high proportion of SCA variance had only little influence on the prediction accuracies based on three-way hybrids. Simulation studies support the empirical findings and illustrate approaches for further optimization. Thus, the presented approach appears highly valuable and has the potential to increase selection gain in hybrid breeding.

KEYWORDS
combining ability, cytoplasmic male sterility, hybrid prediction, prediction accuracy, single-cross hybrid, three-way cross, triticale

1 | INTRODUCTION

The systematic use of heterosis through hybrid breeding is widespread in many commercially important outcrossing crops like maize, rye, sugar beet, or sunflower (Carena, 2009; Cooke & Scott, 1993; Coors & Pandey, 1999). In recent years, it has also gained renewed interest in self-pollinating small-grain cereals like wheat, barley, or triticale (Longin et al., 2012). However, the evaluation of new hybrid combinations is challenging as their number increases quadratically with the number of parental lines to be included in the hybrid breeding process (Bernardo, 2010). Consequently, not all possible hybrid combinations can be generated and tested and thus, efficient methods for an accurate

prediction of the performance of hybrids is essential in order to focus on the most promising ones.

Hybrid prediction based on mid-parent performance has the advantage that producing and testing of experimental hybrids is not required. Predictions based on mid-parent performance have been evaluated previously and have shown high predictive values in autogamous small-grain cereals regarding less complex inherited traits such as heading time or plant height, meaning that a preselection for such traits is feasible (Boeven et al., 2016; Gowda et al., 2013; Oettler et al., 2005). However, for more complex inherited traits, such as grain yield or thousand-kernel weight, predictions based on mid-parent performance are usually less accurate and can vary substantially making them less reliable (Gowda et al., 2010, 2013;

Mühleisen et al., 2015; Oettler et al., 2005). This is due to the fact that hybrid predictions based on mid-parent performance disregard non-additive effects (Bernardo, 2010), which leads to less powerful predictions compared to predictions based on general combining ability (GCA) (Smith, 1986). Therefore, hybrid prediction based on GCA effects is the standard procedure in many hybrid breeding programmes to select superior hybrid combinations (Guimarães, 2009; Hallauer et al., 2010; Henzell & Jordan, 2009).

The use of genomic prediction approaches has been evaluated for many crops and promising results in terms of prediction accuracy have been reported (Bernardo, 1994, 1995, 1996a, 1996b; Gowda et al., 2013; Li et al., 2017; Liu et al., 2016; Mirdita et al., 2015; Philipp et al., 2016; Reif et al., 2013; Technow et al., 2012, 2014; Wang et al., 2017; Werner et al., 2018; Zhao et al., 2013). However, the genomic approach is still associated with significant costs, requires large training populations to achieve even moderate prediction accuracies, and realizing sufficiently high prediction accuracies is still not possible for all crops, especially when heterotic groups are missing and variance due to specific combining ability (SCA) plays a significant role (Gupta et al., 2019; Liu et al., 2016; Mirdita et al., 2015; Werner et al., 2018; Xu et al., 2014; Zhao et al., 2013).

For the prediction of single-cross hybrids based on GCA effects, the GCA of parental candidates needs to be estimated, which requires efficient methods for the routine production of experimental hybrids. Several techniques have been proposed for hybrid seed production (Gupta et al., 2019), but for small-grain cereals, systems using cytoplasmic male sterility (CMS) or chemical hybridization agents (CHA) inducing male sterility are suitable (Longin et al., 2012). Producing hybrid seeds via CHAs has several limitations such as their narrow and developmental stage-specific window of application, environmental toxicity, phytotoxicity resulting in reduced hybrid vigor and hybrid seed set, and high costs due to chemical application (Adugna et al., 2004; Cisar & Cooper, 2002; Gupta et al., 2019). These factors make hybrid seed production based on CHAs economically inferior compared to using a CMS system (Hede, 2001). A disadvantage of the CMS-based approach is, however, that it requires the introgression of promising inbred lines into a CMS cytoplasm, which is expensive and time-consuming as several backcrossing steps and an additional step for seed multiplication are required before topcrosses can be made to generate the experimental hybrid seeds (Figure 1). This means that GCA estimates of candidate parental lines are often only obtained in the tenth or eleventh generation after the initial cross, depending on the organization of the hybrid breeding programme (Figure 1b). A significant reduction in time can be achieved by using greenhouses to reduce the cycle time and/or marker-assisted backcrossing, but both are associated with high additional costs. Moreover even if markers are utilized, at least three marker-assisted backcrossing steps are necessary (Herzog & Frisch, 2013).

For these reasons, the widening of the female parental pool in CMS-based hybrid breeding is very time-consuming. To overcome the limitations of hybrid prediction based on mid-parent values, we here propose a rapid and efficient approach to evaluate new potential lines in the female pool without their prior introgression into a male sterility-inducing cytoplasm by using GCA and SCA estimates obtained from three-way hybrids. These three-way hybrids are coded as $(A \times N) \times R$, where A are existing parental female lines in the male sterile cytoplasm, N are novel female parental candidates in normal cytoplasm to be evaluated for their hybrid potential before introgression into the CMS cytoplasm, and R characterizes male inbred lines with restorer properties, which recover the male fertility of the produced hybrids. In a first step, the male sterile $A \times N$ crosses are produced in single-row isolation plots with female parental candidate lines of interest used as N-lines (Figure 1c). The N-lines are

FIGURE 1 Comparison between (a, b) a classical breeding scheme to obtain general combining ability (GCA) effects by the production of cytoplasmic male sterile (CMS) single-cross hybrids with doubled-haploid technology and marker-assisted backcrossing versus (c) single-cross hybrid prediction based on GCA or GCA and specific combining ability (SCA) effects derived from three-way hybrids [Colour figure can be viewed at wileyonlinelibrary.com]
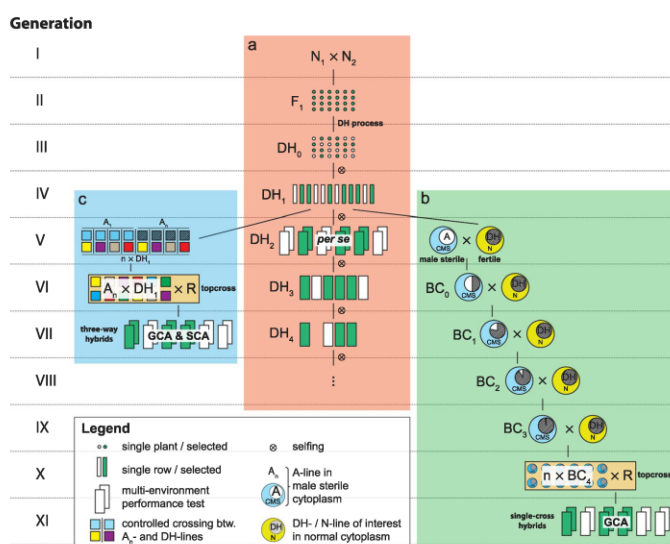
produced by an initial cross between two promising lines from the female pool, followed by either a doubled haploid (DH) or single-seed descent procedure to obtain homozygous inbred lines (Figure 1a). In a second step, the male sterile A × N crosses are grown in microplots and then topcrossed with R-lines to produce the (A × N) × R three-way hybrids. In a final step, the three-way hybrids are evaluated in field trials to obtain GCA and SCA effects for the prediction of the untested single-cross hybrids N × R. Only the N-lines with a high GCA and/or superior performance in a specific hybrid combination are then introgressed into the CMS cytoplasm and converted into A-lines. This novel approach saves four to five generations compared to an ordinary hybrid breeding scheme. The objectives of this study were to (a) introduce this concept to bypass the time- and resource-consuming introgression of female parental candidates into a CMS cytoplasm and to predict single-cross hybrids based on GCA and SCA effects from three-way hybrids, (b) perform a proof-of-concept study with triticale, (c) use a simulation study to complement the experimental data and evaluate additional strategies, and (d) draw conclusions for hybrid breeding.

2 | MATERIALS AND METHODS

2.1 | Phenotypic data

This study was based on a total of 122 winter triticale genotypes, comprising 12 female maintainer lines in normal cytoplasm (denoted here as N-lines, as for the present study they represent female candidates not yet introgressed into the CMS cytoplasm), 11 A-lines, 13 male lines with dominant nuclear restorer genes to recover the male fertility of the produced hybrids (R-lines), 7 check genotypes, and 90 hybrids consisting of 57 single-cross as well as 33 three-way hybrids. The 11 A-lines were produced from the 12 N-lines via crossing with a CMS donor and were repeatedly backcrossed to introgress the nuclear genome into the CMS cytoplasm. The A × R hybrids thus resemble N × R hybrids after the N-lines have been converted to A-lines by an introgression into the CMS cytoplasm. In a breeding programme, this conversion would only be done for the most promising N-lines. However, this experimental setup allowed to predict the performance of untested N × R hybrids by the novel approach, but then also to assess the accuracy of the prediction when comparing it to the observed performance of this hybrid, which is A × R after the N-lines have been introgressed in the CMS cytoplasm and converted to A-lines. The single-cross (A × R) and three-way ((A × N) × R) hybrids were derived from crosses between female and male parental lines in an unbalanced and incomplete factorial mating design (Table S1) using the *Triticum timopheevii* Zhuk. CMS inducing cytoplasm. The female parental components of the three-way hybrids (A × N) were produced by crosses between a male sterile A-line and a fertile N-line of interest.

The field experiments were carried out in the growing season 2016/2017 at six locations in two trials with observation plots (O) or yield plots (Y): Ammeloe (AMM, Y, 52°4′37″N, 6°49′2″E,

37 meters above sea level, masl), Bohlingen (BOH, O, 47°43′12″N, 8°53′46″E, 420 masl), Eckartsweier (EWE, Y, 48°31′18″N, 7°52′18″E, 140 masl), Hohenheim (HOH, O, Y, 48°28′49″N, 9°11′16″E, 400 masl), Oberer Lindenhof (OLI, O, Y, 48°28′49″N, 9°18′56″E, 700 masl), and Weimar (WEM, Y, 51°1′5″N, 11°21′8″E, 330 masl). A partially replicated field design was chosen (Williams et al., 2011) with an average replication number of 1.8. Plot sizes for the yield trials ranged from 5 to 12 m^2 and sowing density was 280 viable seeds m^{-2}, whereas the observation plots were grown in double rows with a length of 1 m and a total of 100 grains per plot. We evaluated the following traits at all locations: heading time (EC-stage according to Zadoks et al., 1974) and plant height (cm). The traits thousand-kernel weight (g), test weight (kg hL^{-1}), protein content (%), and starch content (%) were evaluated at all locations, except BOH. Grain yield (Mg ha^{-1}) was assessed at the locations AMM, EWE, HOH, and WEM, powdery mildew (1–9) at AMM, BOH, HOH, and OLI, and Fusarium head blight (FHB, %) at BOH, HOH, and OLI. For FHB resistance testing in the observation plots, a well-established standard protocol with multiple artificial inoculations of a single-spore isolate during flowering was used. For details see Boeven et al. (2016).

2.2 | Statistical analysis

The linear mixed models used in this study followed the syntax outlined by Piepho et al. (2003), where crossed effects are denoted with a dot operator and fixed and random effects are separated by a colon, with fixed effects in the first place. The single-stage model to calculate global variance components and best linear unbiased estimates (BLUEs) was:

$$Y = G:L + G \cdot L + L \cdot R + L \cdot R \cdot B \quad (1)$$

with Y, G, L, R, and B denoting the response variable, genotypes, locations, replications, and incomplete blocks within replications, respectively. The response variable Y has n observations and G, L, R, and B have g, l, r, and b levels, respectively, with g, l, r, and b representing the total number of genotypes ($g = 122$), locations ($l = 6$), replications within locations ($r = 2$), and incomplete blocks within locations and replications ($b = 11$), respectively. BLUEs and least significance differences (LSD) were calculated with the model described above. Global variance components and heritabilities were calculated with a fully random model using the restricted maximum likelihood method implemented in the software package ASReml-R 3.0 (Butler et al., 2009). LSDs ($p < .05$) were calculated as an approximation using the twofold of the average standard error of a difference.

To estimate group-specific variances, best linear unbiased predictors (BLUPs) and the variance components of GCA and SCA effects, we introduced dummy variables for the genotypic groups females, males, single-cross and three-way hybrids, as well as check genotypes. To simplify the following model notation, dummy variables were suppressed. Variance components and BLUPs were calculated with a full random model, BLUEs with the model:

$$Y = G + GROUP{:}L + GROUP \cdot L + L \cdot R + L \cdot R \cdot B +$$
$$CHECK + CHECK \cdot L +$$
$$FEMALE + FEMALE \cdot L +$$
$$MALE + MALE \cdot L + \qquad (2)$$
$$GCA_A + and\,(GCA_N) + GCA_R +$$
$$GCA_A \cdot L + and\,(GCA_N \cdot L) + GCA_R \cdot L +$$
$$SCA_{A \times R} + and\,(SCA_{N \times R}) +$$
$$SCA_{A \times R} \cdot L + and\,(SCA_{N \times R} \cdot L)$$

where Y, L, R, and B denote the response variable, locations, replications and incomplete blocks within replications with the factor levels as defined in model (1). The notations GROUP, CHECK, FEMALE, and MALE were used to distinguish the genotypic groups in the model with the following number of levels: $gr = 5$, $c = 7$, $f = 12$, $m = 13$, where gr, c, f, and m correspond to groups, checks, females, and males, respectively. The terms GCA and SCA represent the GCA and SCA effects of the A-, N-, and R-lines and of their related crosses. The levels for the factor GCA were $gca_A = gca_N = 12$ and $gca_R = 13$, and for the factor SCA $sca_{A \times R} = sca_{N \times R} = 57$. We assumed a pooled variance structure of the A- and N-lines for the GCA and SCA effects as well as for their location interactions, which is indicated by the notation $and()$ in the model described above. For a joint analysis of single-cross and three-way hybrids, we specified the A- and N-lines of the single-cross hybrids to be identical, as they were near isogenic lines in distinct cytoplasm. The suggestions of the model above were based on the findings of Melchinger et al. (1987), who predicted three-way hybrids based on GCA and SCA effects of the female parental lines i, j and the male parental line k as follows:

$$\hat{y}_{hybrid(ijk)} = \hat{\mu} + \left(\frac{\widehat{GCA}_{A(i)} + \widehat{GCA}_{N(j)}}{2} \right) + \widehat{GCA}_{R(k)} + \left(\frac{\widehat{SCA}_{A(i) \times R(k)} + \widehat{SCA}_{N(j) \times R(k)}}{2} \right)$$
$$(3)$$

with $\hat{y}_{hybrid(ijk)}$, $\hat{\mu}$, $\widehat{GCA}_{A(i)}$, $\widehat{GCA}_{N(j)}$, and $\widehat{GCA}_{R(k)}$, $\widehat{SCA}_{A(i) \times R(k)}$, $\widehat{SCA}_{N(j) \times R(k)}$, being the estimated phenotypic performance of the hybrid derived from the parental lines i, j, and k, the overall mean, the i-th and j-th female, as well as the k-th male parental GCA, and the estimated SCA effects of the crosses between the i-th or j-th female and the k-th male parental line, respectively. For single-cross hybrids, we assumed $\widehat{GCA}_{A(i)} = \widehat{GCA}_{N(j)}$ reducing the model to:

$$\hat{y}_{hybrid(ij)} = \hat{\mu} + \widehat{GCA}_{A(i)} + \widehat{GCA}_{R(k)} + \widehat{SCA}_{A(i) \times R(k)} \qquad (4)$$

For the trait FHB, we realized a covariate adjustment following the suggestions of Emrich et al. (2008) to correct for correlations with heading time and plant height at a single location level. This is supposed to reduce the impact of increased infection levels with *Fusarium* spp. due to early flowering or short genotypes. Residual error variances were assumed to be heterogeneous and independent among locations in models (1) and (2) (Kelly et al., 2007). Variance components were tested for significance ($p < .05$, .01, .001) with a likelihood ratio test according to Stram and Lee (1994). Broad-sense heritability (H^2) was estimated according to Cullis et al. (2006) as:

$$H^2 = 1 - \bar{v}_{BLUP} / 2 * \sigma_G^2 \qquad (5)$$

with $\bar{v}_{BLUP}$ specifying the mean variance of a difference between two best linear unbiased predictors and σ_G^2 the genotypic variance component across all groups in the global or the respective group-specific variance component derived by the group-specific model.

2.3 | Hybrid prediction

Prediction accuracies were estimated by dividing the Pearson's product-moment correlation between predicted and observed values by the square root of the global heritability estimate of the corresponding trait (Legarra et al., 2008). We predicted the hybrid performance of the A × R single-cross hybrids based on the mid-parent (MP) performance of the parental A- and R-lines and the sum of parental GCA effects of the A- and R-lines (GCA_{2W}) estimated based on single-crosses. In addition, the N × R single-cross hybrids were predicted based on the sum of parental GCA or GCA and SCA effects of the parental N-lines and the R-lines from the three-way hybrids (GCA_{3W}, GCA-SCA_{3W}) and compared to the performance of the A × R hybrids with the A-line corresponding to the respective N-line. To account for autocorrelation we applied a leave-one-hybrid-out cross-validation approach (Schrag et al., 2006) for the GCA_{2W} scenario (GCA_{loho}). Furthermore, we excluded those A × R single-cross hybrids identical to the N × R combinations represented in the three-way hybrids to obtain GCA and SCA effects for the GCA_{3W} and GCA-SCA_{3W} scenarios.

2.4 | Simulation study

To further evaluate the presented approach, we performed a simulation study using a model similar to that shown in Equation (2). The simulation assumes a phenotypic evaluation at one location with two replications to estimate GCA and SCA effects in three-way hybrids in order to predict the performance of single-cross hybrids by GCA and SCA effects. We evaluated two scenarios with a ratio of SCA to the total genotypic variance of 0.5 and 0.1. The residual error variance was assumed twice the genotypic variance. For each scenario 15,000 simulation runs were performed. For the evaluation of novel female candidate lines (N) in three-way hybrids, we assumed one or two female A-lines and one, two, or five male restorer lines (R) to be used in a full factorial mating design, that is, each N-line is tested in three-way hybrids with all available A- and R-lines. In addition, we evaluated a single and a double round robin design, where each A × N-cross is only crossed to one or two of the male parental lines, respectively (Figure S1). This leads to a decreased number of experimental hybrids tested or alternatively increases the number of N-lines that can be evaluated with the same amount of resources. Due to model restrictions, a reduced model incorporating only female GCA effects was used for the full factorial when only one R-line was assumed. Likewise, no results were reported for the single round robin design with one A-line, as no effects can be

estimated for the generated and tested N-lines. R code is available upon request.

3 | RESULTS

All evaluated traits showed high heritability, ranging from 0.82 for powdery mildew to 0.97 for plant height (Table 1). In addition, the quantitative traits, grain yield, Fusarium head blight, and protein content showed high heritabilities of 0.83, 0.83, and 0.87, respectively. Genotypic and genotype-times-location interaction variances were highly significant ($p < .001$) for all evaluated traits, with the genotypic variances being larger than the genotype-times-location interaction variances (Table 1). Almost all group-specific variance components were significantly different from zero (Table 2). Females showed higher variance component estimates compared to males for almost all traits, except for powdery mildew, thousand-kernel weight, and protein content (Table 2). All female GCA variance components, except that for protein content, were higher than the corresponding male GCA variance component (Table 2). The SCA variance accounted for a low proportion relative to the total genotypic variance of hybrids for the traits ear emergence, plant height, powdery mildew, and thousand-kernel weight, and ranged from 4% to 17% (Table 2). In contrast, the traits Fusarium head blight and grain yield showed very high proportions for SCA variance with regard to the total genotypic variance of the hybrids.

We observed low prediction accuracy estimates for the prediction of single-cross hybrids (A × R) based on mid-parent values for the traits grain yield and test weight, moderate estimates for the traits Fusarium head blight, protein content, and starch content, but high estimates for the traits ear emergence, plant height, powdery mildew, and thousand-kernel weight (Table 3). Using GCA effects to predict single-cross hybrid performance increased the prediction accuracies of all traits, ranging from 0.78 for grain yield to 1.00 for powdery mildew. To account for autocorrelation when predicting

single-cross hybrids based on their respective GCA effects, we applied a leave-one-hybrid-out cross-validation approach (GCA_{loho}). Briefly, we recalculated GCA effects for all 57 single-cross hybrids not taking the hybrid of interest into consideration when it was predicted (Table 3). These cross-validated prediction accuracies of single-cross hybrids dropped between 12% and 51% for powdery mildew or Fusarium head blight, respectively. However, estimates still exceeded the prediction accuracies based on mid-parent values, except for Fusarium head blight.

Last, we evaluated the prediction accuracies of potential female lines not yet introgressed into the CMS cytoplasm, employing the novel three-way cross approach introduced here. Prediction accuracies for these N × R hybrids based on GCA or GCA and SCA effects estimated from the three-way hybrids ((A × N) × R, GCA_{3W}, GCA-SCA_{3W}) already account for autocorrelation, because we excluded the single-cross hybrid of interest when predicting it. In summary, with the exception of starch content, the prediction accuracy estimates based on GCA or GCA and SCA together, were much higher compared to the prediction accuracies based on mid-parent performance (Table 3). For GCA_{3W} the prediction accuracy was 0.25 for starch content but otherwise ranged between 0.53 and 1.00, and for GCA-SCA_{3W} ranged between 0.59 and 1.00. Consequently, the ratio between the prediction accuracy achieved with GCA_{3W} and mid-parent values was 0.40 for starch content and for the other traits ranged between 1.05 for protein content and 3.04 for test weight, with an average of 1.52. For GCA-SCA_{3W} this ratio with mid-parent prediction ranged between 0.93 for starch content and 2.90 for test weight, averaging 1.60. For grain yield, the ratios were 2.61 and 2.84 for GCA_{3W} and GCA-SCA_{3W}, respectively.

To complement the experimental results, we performed a simulation study. We tested two scenarios with either a high or a low amount of SCA variance compared to the total genotypic variance. The novel female candidate lines were evaluated in three-way hybrids with one or two A-lines and one, two or five R-lines. The simulated genotypic values of the N × R hybrids were then compared

TABLE 1 Means and ranges of best linear unbiased estimators, least significant differences at the $p < .05$ level ($LSD_{0.05}$), heritability estimates across environments and variance components for the traits Fusarium head blight (1–9), ear emergence (EC code), plant height (cm), powdery mildew (1–9), grain yield (Mg ha^{-1}), thousand-kernel weight (g), test weight (kg hL^{-1}), protein content (%), and starch content (%) for all genotypes ($N = 122$)

	Fusarium head blight	Ear emergence	Plant height	Powdery mildew	Grain yield	Thousand-kernel weight	Test weight	Protein content	Starch content
Min	36.2	50.2	83.3	0.5	7.5	37.9	63.3	10.5	66.5
Mean	48.0	55.2	106.7	2.4	9.2	49.3	66.7	11.8	69.0
Max	72.8	59.4	127.0	5.5	10.5	56.9	71.4	12.8	71.5
$LSD_{0.05}$	5.9	1.2	4.5	1.5	0.8	2.3	1.4	0.4	0.8
σ_G^2	22.70***	2.95***	76.46***	1.15***	0.34***	13.18***	1.51***	0.13***	0.91***
$\sigma_{G \times L}^2$	23.72***	0.70***	14.53***	0.53***	0.19***	2.31***	0.67***	0.04***	0.23***
σ_e^{2a}	17.05	0.58	6.33	0.93	0.14	1.27	0.73	0.09	0.20
H^2	0.83	0.94	0.97	0.82	0.83	0.95	0.87	0.87	0.91

[a]Mean residual error variance.

***Significantly different from zero at the .001 probability level.

TABLE 2 Group-specific variance components across locations for the traits Fusarium head blight (1–9), ear emergence (EC code), plant height (cm), powdery mildew (1–9), grain yield (Mg ha^{-1}), thousand-kernel weight (g), test weight (kg hL^{-1}), protein content (%), and starch content (%) for four different genotypic groups: N-lines ($N = 12$), R-lines ($N = 13$), pooled single-cross and three-way hybrids ($N = 90$), as well as checks ($N = 7$)

	Fusarium head blight	Ear emergence	Plant height	Powdery mildew	Grain yield	Thousand-kernel weight	Test weight	Protein content	Starch content
N-lines									
σ^2_G	47.64**	3.46***	78.08***	0.92***	46.97***	9.46***	3.92***	0.09***	1.33***
$\sigma^2_{G \times L}$	36.34***	0.53**	8.96***	0.39$^+$	0.00	2.16***	0.37*	0.04*	0.04
R-lines									
σ^2_G	21.86*	3.40***	44.04***	1.08***	10.88$^+$	19.90***	1.74***	0.10***	0.38***
$\sigma^2_{G \times L}$	24.78**	0.74***	14.60***	0.00	13.97*	1.36***	0.73***	0.06**	0.22**
Hybrids									
σ^2_{GCA-F}	10.72**	1.25***	27.93***	0.79***	21.76***	5.15***	0.58***	0.02*	0.30***
$\sigma^2_{GCA-F \times L}$	3.94*	0.22***	7.54***	0.16***	5.35***	1.06***	0.24***	0.02***	0.11***
σ^2_{GCA-M}	3.13	1.00***	19.59***	0.70***	0.56	2.26***	0.19**	0.05***	0.23***
$\sigma^2_{GCA-M \times L}$	4.07**	0.13***	1.89***	0.34***	5.73***	0.49***	0.20***	0.01**	0.03**
σ^2_{SCA}	8.70**	0.32***	9.72***	0.06*	14.59***	1.54***	0.26***	0.02***	0.21***
$\sigma^2_{SCA \times L}$	14.34***	0.35***	0.00	0.10*	1.25	0.37**	0.16**	0.00	0.04*
$\sigma^2_{SCA}/\sigma^2_{sum}{}^{a}$	0.39	0.12	0.17	0.04	0.40	0.17	0.25	0.26	0.28
Checks									
σ^2_G	24.19*	2.65***	173.06***	0.22$^+$	11.28*	18.96***	1.08**	0.30***	2.64***
$\sigma^2_{G \times L}$	23.55***	0.63***	6.51**	0.31*	10.45*	2.26***	1.37***	0.01	0.21**
σ^{2b}_e	20.67	0.74	15.48	1.03	28.99	1.72	1.00	0.11	0.33

Note: $^+$, *, **, ***Significantly different from zero at the 0.1, 0.05, 0.01, and 0.001 probability level, respectively.

^{a}Sum of $\sigma^2_{GCA-F} + \sigma^2_{GCA-M} + \sigma^2_{SCA}$.

^{b}Mean residual error variance across all genotypic groups.

to their predicted values based on the estimated GCA and SCA effects. The prediction was better when two instead of only one A-line were used (Figure 2a,c). Likewise, the prediction improved with increasing number of R-lines. Notably, if the N-lines are tested with several A- and R-lines, the number of experimental three-way hybrids to be generated and assessed in the field increases rapidly which soon limits the number of novel candidate lines that can be evaluated (Figure 2e). We therefore also evaluated single and double round robin as alternative crossing designs. For the same numbers of N-, A-, and R-lines, these substantially reduce the number of experimental hybrids to be generated and tested (Figure 2e,f, Figure S1). The hybrid predictions of these crossing designs were only slightly lower compared to those obtained with the full factorial (Figure 2b,d).

4 | DISCUSSION

Hybrid breeding is widely established in many crops (Carena, 2009; Cisar & Cooper, 2002; Longin et al., 2012). Primarily, systems using CHA or CMS are used to produce experimental hybrids (Carena, 2009; Longin et al., 2012), but utilizing a CMS system

has several advantages compared to the use of CHAs (Adugna et al., 2004; Hede, 2001). However, introgressing female inbred lines into a CMS cytoplasm via backcrossing is time- and cost-intensive and is thus the major bottleneck for hybrid breeding, considering the high number of experimental hybrids produced and discarded afterwards due to low performance. The aim of our study was therefore to present a novel concept using three-way hybrids that allows to evaluate new female inbred lines of interest for their GCA and SCA effects prior to their embedding in a CMS cytoplasm.

4.1 | Hybrid prediction based on single-cross hybrids (A × R)

Prediction accuracies based on mid-parent performance have recently shown promising results for simply inherited and highly heritable traits (Boeven et al., 2016; Gowda et al., 2013; Miedaner et al., 2017; Oettler et al., 2005), but are expected to be lower for more complex traits (Bernardo, 2010; Oettler et al., 2005; Smith, 1986). Our results corroborate this, as the prediction accuracies based on mid-parent values varied, but were low for the

TABLE 3   Prediction accuracies of single-cross (A × R, $N = 57$) hybrids for the traits Fusarium head blight (1–9), ear emergence (EC code), plant height (cm), powdery mildew (1–9), grain yield (Mg ha^{-1}), thousand-kernel weight (g), test weight (kg hL^{-1}), protein content (%), and starch content (%) based on mid-parent value (MP), the sum of general combining ability (GCA$_{2W}$) effects, and the sum of GCA effects derived by leave-one-hybrid-out cross-validation (GCA$_{loho}$) using single-cross hybrids as well as the sum of GCA (GCA$_{3W}$) or GCA and specific combining ability (SCA) effects (GCA-SCA$_{3W}$) estimated from three-way ((A × N) × R, $N = 33$) hybrids predicting single-cross hybrids ($N × R$, $N = 8$)

|	Fusarium head blight	Ear emergence	Plant height	Powdery mildew	Grain yield	Thousand-kernel weight	Test weight	Protein content	Starch content
Prediction accuracies of single-cross hybrids (A × R) with estimates based on single-cross hybrids (A × R) and their parental components									
MP	0.51	0.75	0.70	0.85	0.23	0.74	0.30	0.51	0.63
GCA$_{2W}$	0.89	0.98	0.95	1.00[a]	0.78	0.96	0.91	0.93	0.95
GCA$_{loho}$	0.44	0.83	0.80	0.88	0.45	0.83	0.64	0.74	0.77
Prediction accuracies of single-cross hybrids (N × R) with estimates based on three-way hybrids ((A × N) × R)									
GCA$_{3W}$	0.69	0.99	0.96	1.00[a]	0.59	0.97	0.91	0.53	0.25
GCA-SCA$_{3W}$	0.66	0.91	0.97	1.00[a]	0.64	0.98	0.87	0.66	0.59
Ratio between hybrid prediction based on the novel three-way cross approach and mid-parent prediction									
GCA$_{3W}$/MP	1.36	1.32	1.37	1.23	2.61	1.30	3.04	1.05	0.40
GCA-SCA$_{3W}$/MP	1.29	1.22	1.39	1.21	2.84	1.32	2.90	1.29	0.93

[a]Prediction accuracy values ≥1 were set to 1.
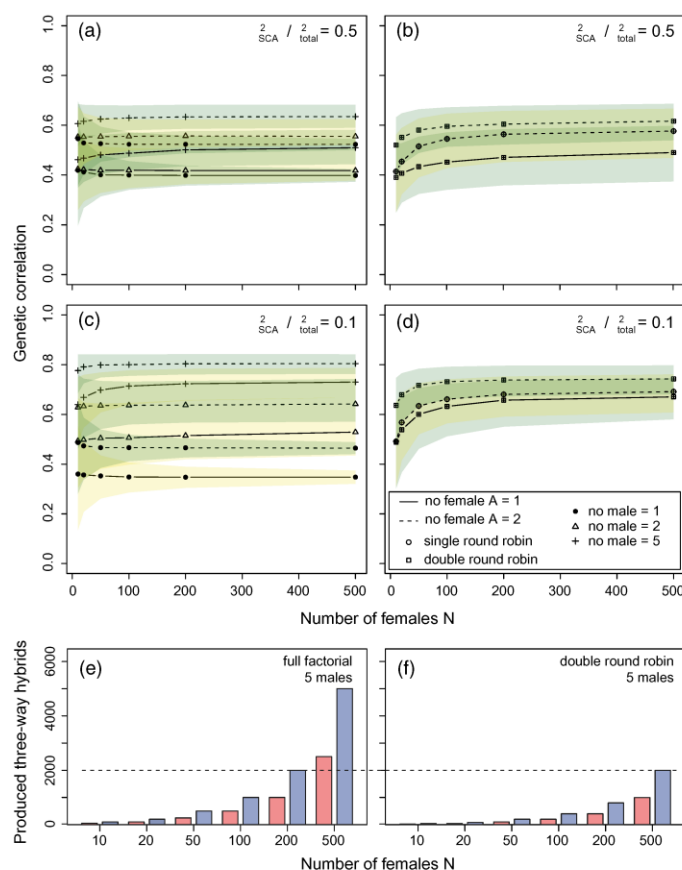
FIGURE 2   Results of the simulation study. Correlation of the simulated genotypic value of single-cross (N × R) hybrids and their hybrid performance predicted based on general and specific combining ability effects estimated in three-way hybrids. Results are based on a full factorial (a, c) or applying a single and double round robin design (b, d). Medians and 50% quantiles from 15,000 simulation runs are displayed. Two scenarios with a SCA to the total genotypic variance ratio of 0.5 (a, b) and 0.1 (c, d) are shown. Due to model restrictions, a reduced model incorporating only female GCA effects was used for the full factorial with only one R-line. Likewise, no results are shown for the single round robin design with one A-line, as no effects can be estimated for the generated and tested N-lines. The total number of produced hybrids is shown for the scenario with five R-lines and one (red) or two (blue) A-lines for the full factorial (e) and a double round robin design (f) [Colour figure can be viewed at wileyonlinelibrary.com]

complex inherited traits, including grain yield, which is an important breeding target in all breeding programmes (Table 3). Predictions based on GCA effects are expected to show higher prediction accuracies due to their additional exploitation of non-additive effects, but are hampered by increasing importance of SCA variance (Lynch & Walsh, 1998). Indeed, utilizing GCA effects to predict hybrid performance yielded substantially higher prediction accuracies for all traits, but especially for the more complex inherited traits Fusarium head blight, grain yield, test weight, protein content, and starch content. To mimic a scenario predicting untested hybrids, we used a leave-one-hybrid-out cross-validation approach (GCA$_{loho}$), which resulted in reduced but more realistic prediction accuracies for all traits. In this study, we observed high amounts of SCA variance for the traits Fusarium head blight (39%) and grain yield (40%) resulting in considerably lower prediction accuracies compared to the other traits (Table 3). In conclusion, hybrid prediction based on GCA estimates is possible with an acceptable prediction accuracy, but requires the prior introgression of candidate female parental lines into the CMS cytoplasm in order to obtain these GCA estimates.

4.2 | Potential of single-cross hybrid prediction (N × R) based on estimates from three-way hybrids ((A × N) × R)

The approach predicting single-cross hybrids by GCA and SCA effects obtained from three-way hybrids yielded generally high prediction accuracies using only GCA effects which for some traits increased further when taking also SCA effects into consideration (Table 3). While the novel N lines share the additive genetic variance on the female side in the three-way hybrids, this still allowed robust and accurate effect estimates. The results of our simulation study further substantiated the power of the presented approach. Moreover, alternative crossing designs, as for example the single or double round robin design, enable comparably high predictions of promising hybrid combinations with a strongly reduced number of experimental hybrids (Figure 2). For example, the number of experimental hybrids evaluated in a full factorial with one or two male restorer lines is equal to the number of hybrids tested with five male restorer lines in a single or double round robin design, respectively (Figure 2e,f). Alternatively, these crossing designs allow a higher number of N lines to be evaluated with the same effort. Jointly, the experimental data and the simulation study show that the novel approach is by far superior to prediction based on mid-parent values.

Philipp et al. (2016) predicted single-crosses by means of three-way hybrids based on a genomic approach, which resulted in only low prediction accuracies. Furthermore, encouraging prediction accuracies were reported for small-grain cereals using genomic prediction based on single-crosses, but estimates were often less accurate when compared to our results (Akel et al., 2019; Boeven et al., 2016; Gowda et al., 2013; Liu et al., 2016; Philipp et al., 2016; Schulthess et al., 2018; Trini et al., 2020; Zhao et al., 2015). Importantly, large training populations are required to achieve acceptable genomic

prediction accuracies, which in many crops still hampers the efficiency of this genomic approach. In summary, the novel approach demonstrated here shows great potential due to the observed prediction accuracies and the fact that the time- and resource-intensive introgression of candidate lines into a CMS cytoplasm becomes limited to only the most promising candidates.

5 | CONCLUSIONS

Generally, the prediction accuracies using GCA effects based on single-cross and three-way hybrids was lower when the proportion of SCA compared to the total genotypic variance of the hybrids was high. This is expected for the absence of heterotic groups in breeding populations (Reif et al., 2007), as is the case for triticale (Fischer et al., 2010; Gowda et al., 2013; Losert et al., 2017; Tams et al., 2004, 2006). The establishment of heterotic pools would therefore further increase the prediction accuracies utilizing GCA effects regardless of whether they are derived from single-cross or three-way hybrids. Thus, the maintenance or establishment of heterotic pools remains a long-term goal in hybrid breeding programmes as it increases the predictive power of GCA-based predictions.

Compared to predictions based on mid-parent values, the prediction accuracies based on combining ability estimates obtained by the novel approach with three-way crosses showed a high potential for the prediction of single-cross hybrids of yet untested lines, which was strongly supported by the results of our simulation study. Furthermore, hybrids can be predicted four to five generations earlier compared to an ordinary hybrid breeding programme and in addition, a higher number of potential hybrid combinations can be tested, when an appropriate mating design is applied. Moreover if superior three-way hybrids are identified these can also be registered directly. Another interesting side aspect is that with the presented approach it is easy to select against unwanted fertility restoration genes in the female parental pool, if the supposedly sterile single-cross hybrids (A × N) show pollen fertility. Consequently, the production of three-way hybrids for the evaluation of lines of interest in normal cytoplasm is an attractive alternative for the selection of lines to be introgressed into a male sterility-inducing cytoplasm.

When utilizing this approach in a breeding programme, one can either use a single line from the male parental pool (R-line) to be test-crossed with the two-way crosses (A × N), or several R-lines. If only one R-line is used, GCA and SCA are confounded, but nevertheless, promising hybrid combinations (N × R) can be identified. If several R-lines are used, more three-way crosses need to be generated and tested, but this allows to separately estimate GCA and SCA. Notably, the abovementioned single or double round robin mating designs can be applied to reduce the number of experimental hybrids even when more R-lines are used. Prediction of untested hybrid combinations is then based on GCA estimates alone and SCA of promising hybrids needs to be determined by generating and evaluating them.

In combination with molecular markers, however, SCA information for untested experimental hybrids and additional information regarding newly developed male inbred lines can be gained (Trini et al., 2020). Apart from that, female candidate lines with a high GCA can be identified and used to improve the GCA of the female parental pool. In conclusion, the novel approach presented here can be a valuable tool to substantially improve the efficiency of hybrid breeding programmes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

HPM designed the experiments. HPM, EAW, and JT collected data. JT analysed the data. JT, HPM, and TW wrote the manuscript. All authors read and approved the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

5 General discussion

The history of triticale is a success story of its own as it has started only 130 years ago (Rimpau 1891) and triticale is grown worldwide on about 4 million ha today (FAOSTAT 2021) predominantly as line but also as hybrid cultivar (Longin et al. 2012). However, triticale faces more and more challenges, such as an increasing disease pressure (Losert et al. 2017a; Oettler 2005) or challenges converting triticale to a hybrid crop (Longin et al. 2012). On the other hand, new technologies, such as molecular markers (Badea et al. 2011) have been introduced in triticale breeding programs recently and can be used today to assist breeders by marker-assisted or genomic selection. Therefore, this dissertation was conducted to evaluate potentials improving triticale line and hybrid breeding programs using phenotypic and genomic concepts.

Genetic control of plant height in triticale

Many breeding programs are evaluating plant height routinely as it is a very important trait in many small-grain cereals. This study identified several putative QTL for plant height and therefore provided new insights into its genetic control in Central European winter triticale cultivars. Two of these QTL most likely correspond to the height-reducing genes *Rht12* and *Ddw1* originating from wheat (Sun et al. 2019) and rye (Braun et al. 2019). So far, no study reported the height-reducing gene *Rht12* in triticale, but its use and application has been investigated in wheat recently (Chen et al. 2018; Chen et al. 2013; Sun et al. 2019; Worland et al. 1994). On the contrary, the height-reducing gene *Ddw1* has been detected in many studies working on triticale (Chernook et al. 2019; Kroupin

et al. 2019; Kalih et al. 2014; Alheit et al. 2014) and the fine-mapping results in this study likely confirmed its broad occurrence in Central European winter triticale.

However, the use of such height-reducing genes often are accompanied with adverse effects on other traits, such as developmental stage, Fusarium head blight, or grain yield and has, to our best knowledge, only been described in detail for *Ddw1* in Central European winter triticale before (Kalih et al. 2014). Our results confirmed these findings as most markers detected to significantly reduce plant height also delayed flowering time and registered cultivars showed the slight tendency of flowering at a later point of time (Trini et al. 2021a). Until now the effects of height-reducing genes under drought conditions, as shown in wheat (Worland et al. 1998; Mathews et al. 2006; Butler et al. 2005), were not evaluated in triticale but should be considered as a future possibility facing the challenges of climate change (Fahad et al. 2017). The height-reducing gene *Rht12*, for example, showed favorable effects on seedling establishment under drought conditions in wheat (Singh and Khanna-Chopra 2010; Bai et al. 2013) and might therefore be a candidate for future research in triticale.

Triticale breeding theoretically could also use other height-reducing genes from its parental species wheat such as *Rht-A1, Rht*-B1, *Rht7*, *Rht9*, *Rht12*, *Rht22*, *Rht24* (McIntosh et al. 2017; McIntosh et al. 2013) or the dominant (*Ddw1*, *Ddw2*, *Ddw3*, *Ddw4*) and the recessive (*ct1*, *ct2*, *np*, *dw9*) height-reducing genes from rye (Kantarek et al. 2018; Stojałowski et al. 2015; Braun et al. 2019; Grądzielewska et al. 2020). However, to date only the use of *Rht-B1* and *Ddw1* have been reported in triticale (Chernook et al. 2019; Kroupin et al. 2019) leaving a lot of room for improvement. The most promising height-reducing genes of wheat, which could be used in triticale are probably *Rht-B1* and *Rht24* as they are widely used in wheat breeding programs today and have a large height-reducing effect on plant height (Würschum et al. 2015; Würschum et al. 2017a). For the height-reducing gene *Rht-B1*, however, adverse effects under heat conditions were reported (Würschum et al. 2017a), which have to be kept in mind if introgressing it into triticale germplasm. Promising candidates in the context of adverse drought effects through climate change, though, seem to be *Rht12* located on chromosome 5A and the height-reducing loci *Rht14*, *Rht16*,

Rht18, *Rht24*, and *Rht25* located on chromosome 6A (Mo et al. 2018; Sun et al. 2019). The genetic positions of the latter, however, were not evaluated in detail and therefore it is not clear until now whether these loci represent alleles belonging to the same gene or closely linked genes (Mo et al. 2018). Besides *Ddw1*, the probably most promising height-reducing genes, which could be introduced into triticale are *Ddw3* lying on chromosome 1R, and *Ddw4* on chromosome 3R. Both are gibberellin-sensitive indicating that they might show positive properties under drought conditions similar as reported for *Rht12* in wheat (Singh and Khanna-Chopra 2010; Bai et al. 2013) and showed substantial height-reduction of around 40% to 50% in rye (Stojałowski et al. 2015; Kantarek et al. 2018). Effects on other traits, such as number of spikelets per ear, spike length, or grain yield have partly been evaluated in rye, but did not allow a final evaluation in terms of negative influences, especially in regards on grain yield (Stojałowski et al. 2015; Kantarek et al. 2018). However, before applying the mentioned height-reducing genes from wheat and rye in triticale their genetic effects on plant height and other traits need to be evaluated in the genetic background of triticale and therefore remain a future research question.

Nevertheless, the general trend of decreasing plant height observed during the last four decades in this study is a success owed to the efforts of triticale breeders and resulted in an increasing frequency of genotypes carrying height-reducing QTL. On the contrary, a growing number of taller genotypes is owed the fact, that biomass yield gained in importance as a breeding goal in recent years (EU 2001, 2018, 2009; Ayalew et al. 2018) and the fact that plant height is one of the major contributors to biomass yield in triticale (Losert et al. 2017a; Gowda et al. 2011). The future trend of plant height in triticale is thus less clear, as it is very likely that taller genotypes continue to be of interest for biomass production, for an increased straw production, and dual use cultivars improving biomass and grain yield simultaneously may increase further. However, opposing relationships between agronomically important traits, such as susceptibility to lodging and plant height or biomass yield (Losert et al. 2017b; Losert et al. 2016; Gowda et al. 2011; Alheit et al. 2014), facilitate the necessity to evaluate efficient measures for a simultaneous improvement of such traits in triticale by, *e.g.*, using index selection as recently proposed by Neuweiler et al. (2021).
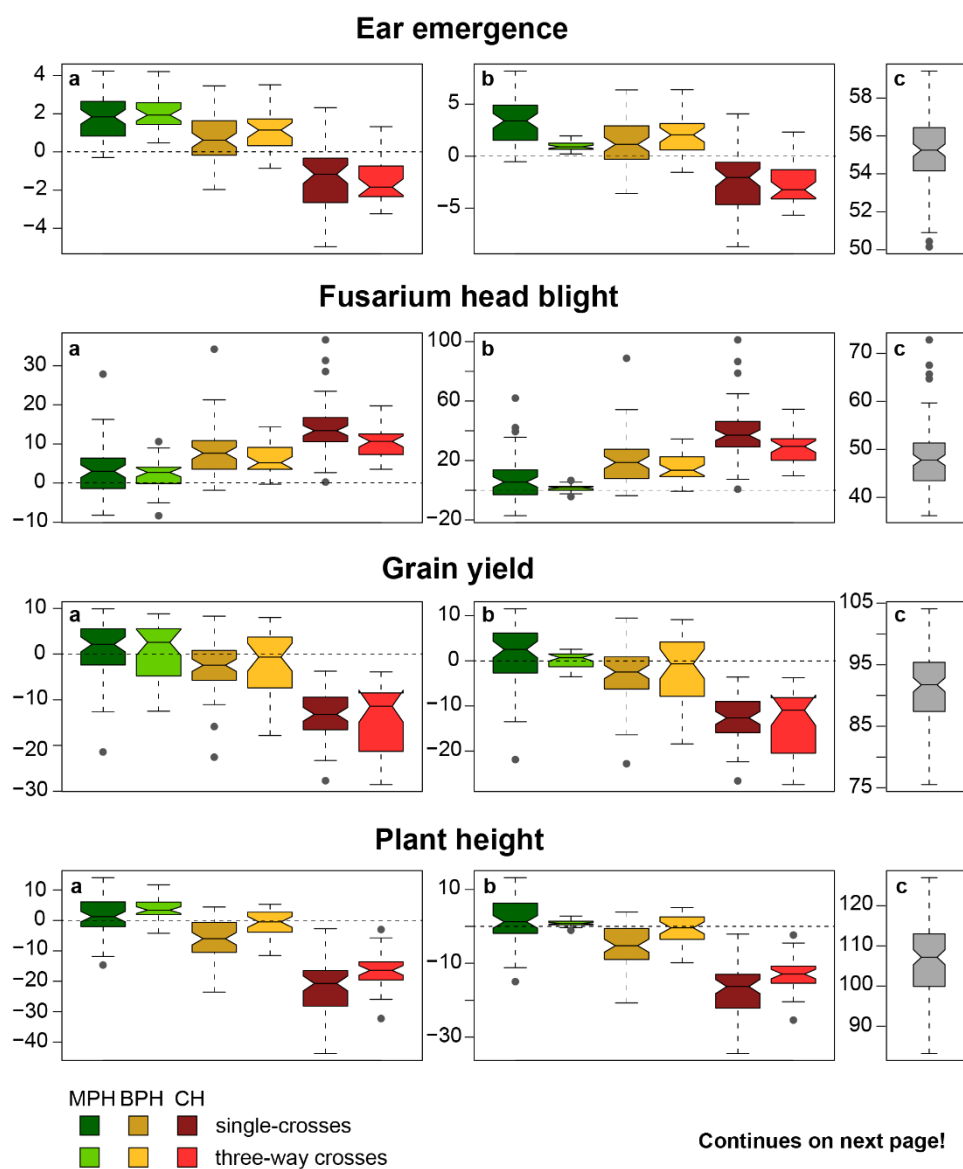
Hybrid breeding in triticale

Hybrid breeding in autogamous small-grain cereals did not prevail entirely until today, but many efforts have been attempted and resulted in first CMS-based triticale hybrid cultivars released in 2012 (Longin et al. 2012).The major goal and advantages of hybrid breeding are to systematically exploit heterosis (Shull 1908) and taking advantage of stacking favorable genes, *e.g.*, for disease resistance, to improve stress tolerance of hybrids over lines especially in marginal environments (Oettler et al. 2005; Hallauer et al. 2010).

As positive heterosis for grain and biomass yield has been observed, its exploitation moved into the focus of triticale breeders (Tams et al. 2006; Oettler et al. 2001; Oettler et al. 2005; Losert et al. 2016). Nonetheless, heterotic pools are not established in triticale breeding so far (Fischer et al. 2010; Gowda et al. 2013; Losert et al. 2017b; Tams et al. 2004; Tams et al. 2006) what hampers the exploitation of heterosis (Reif et al. 2007). This was confirmed by molecularly evaluating a panel of triticale hybrids and their parents in this study, showing that female and male lines did not cluster into heterotic groups (Trini et al. 2020). The two main reasons for the absence of heterotic pools though are that in triticale the heterotic pool establishment is still at a very beginning (Fischer et al. 2010) and the predominance of line breeding accompanied by a strong exchange of breeding material among breeders (Góral et al. 2015).

With the absence of heterotic pools, however, the exploitation of useful heterosis is hampered and hybrid breeding cannot utilize its full potential to create hybrids with an improved performance compared to line breeding. Nevertheless, a great amount of heterosis can be observed in triticale for numerous traits and for some traits even commercial heterosis – meaning that a hybrid is outperforming the best check cultivar in the dataset or more generally in the market – was observed (Figure 1; Losert et al. 2016; Boeven et al. 2016; Oettler et al. 2005; Oettler et al. 2001). If considering grain yield, for example, we observed experimental hybrids showing negative mid-parent, better-parent, and commercial heterosis reflected in both, single- and three-way crosses (Figure 1). However, the positive mid-parent and better-parent heterosis values of up to 11.5% and 9.5%, respectively, indicate that substantial heterosis can be

achieved. The commercial heterosis values, though were still negative reflecting the timely backlog in the development process of hybrids compared to line breeding at the moment. This, however, is likely to change with an increasing *per se* performance of the parents. To develop heterotic pools in triticale and exploit favorable heterosis, efficient methods as proposed by Cowling et al. (2020), Fischer et al. (2010), and Melchinger and Gumber (1998) are required and need to be implemented to further improve triticale hybrid breeding.
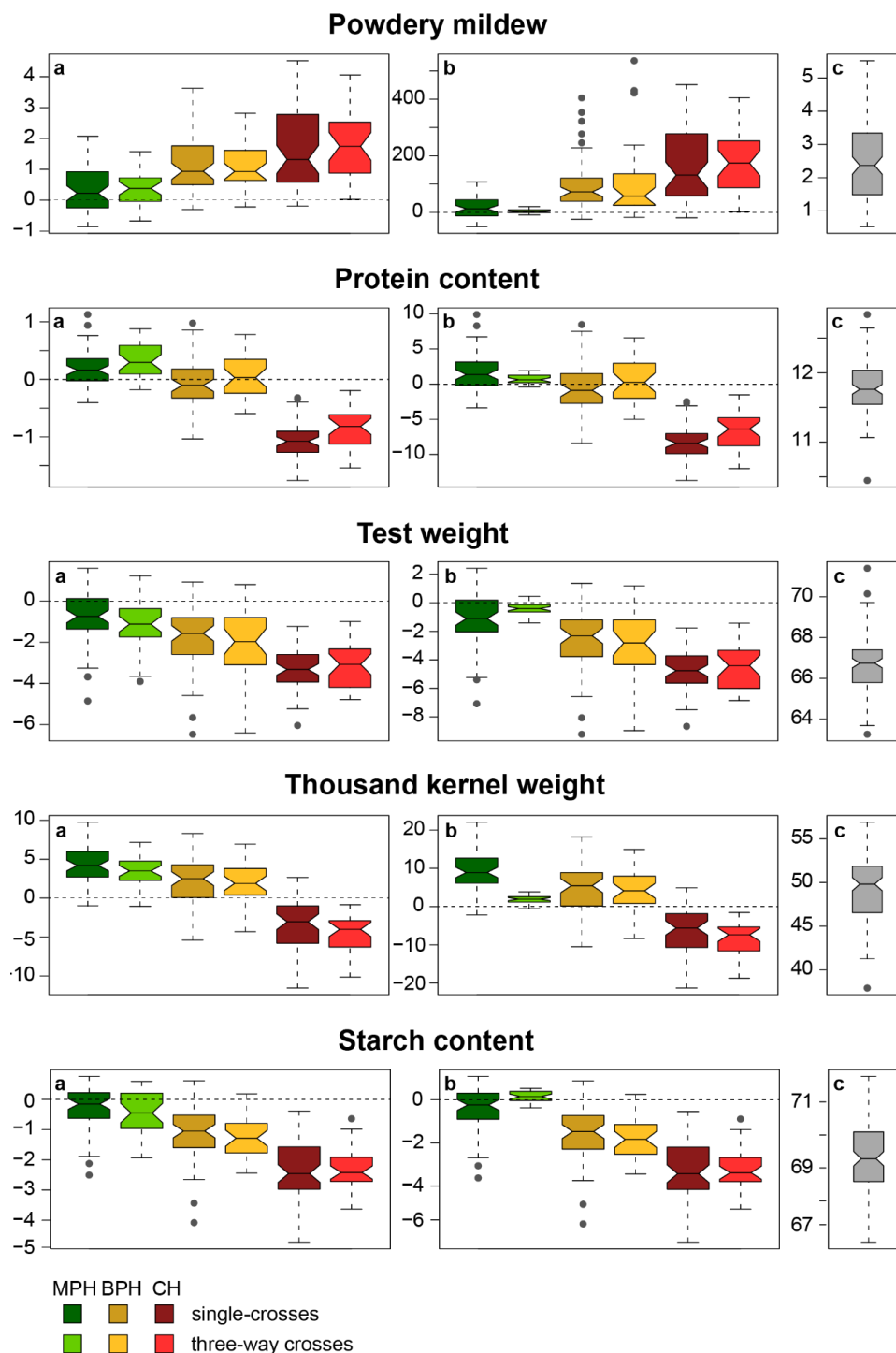
Ear emergence

Fusarium head blight

Grain yield

Plant height

MPH BPH CH

single-crosses
three-way crosses

Continues on next page!

Powdery mildew

Figure 1: Absolute (**a**) and relative (%, **b**) mid-parent (MPH), better-parent (BPH), and commercial heterosis (CH) derived with the best linear unbiased estimates (BLUEs, **c**) from Trini et al. 2021b for the traits ear emergence (BBCH), Fusarium head blight (0–100%), grain yield (Mg ha^{-1}), plant height (cm), powdery mildew (0-9), protein content (%), test weight (kg hL^{-1}), thousand kernel weight (g), and starch content (%). For all traits, except Fusarium head blight and powdery mildew, more positive heterosis estimates are better.

Breeding for biomass yield in hybrid triticale

Biomass yield became an important breeding goal in triticale just recently due to a policy promoting energy utilizing crop biomass (EU 2001, 2018, 2009; Monforti et al. 2013) and its potential as high-quality fodder for ruminants (Ayalew et al. 2018). This led to a shift from breeding primarily shorter cultivars to also breed for taller genotypes with an increased biomass yield as plant height is significantly associated with biomass yield (Losert et al. 2016; Alheit et al. 2014; Gowda et al. 2011). This development is displayed by an increasing number of taller genotypes released since 2011 in comparison to previous years (Trini et al. 2021a). Furthermore, triticale showed a great variation regarding biomass yield (Losert et al. 2016) indicating the great potential to breed for cultivars with improved biomass yield. On the contrary, taller genotypes are associated with a higher susceptibility to lodging (Losert et al. 2017b) meaning that sufficient selection methods, *e.g.*, selection indices or marker-assisted selection, need to be implemented into a breeding program to simultaneously improve lodging tolerance and biomass yield.

The potential of hybrid breeding to improve biomass yield traits has also been evaluated and has shown promising results (Losert et al. 2016). The observed commercial heterosis estimates for biomass yield showed values up to 11% using parental lines which did not represent the latest developed lines and whose performance was originally optimized for grain yield (Losert et al. 2016). This means, that the full potential using hybrid methodology could not be exploited but still demonstrated great potential increasing biomass yield using hybrid methodology. To evaluate the most promising hybrid combinations in terms of biomass yield, though, we evaluated different prediction approaches showing great potential to further increase biomass yield (Trini et al. 2020). As grain yield is still the most important breeding goal in triticale, efficient measures are needed to focus on a simultaneous improvement of grain and biomass yield in joint breeding programs improving both traits simultaneously as proposed by Gowda et al. (2011) and Liu et al. (2017). However, such an improvement has not been evaluated for triticale hybrid breeding programs so far and should thus be an objective for future studies.

Hybrid prediction in triticale

Hybrid breeding is facing multiple challenges. One of the biggest is the quadratically increasing number of experimental hybrids when the number of parental lines increases (Bernardo 2010). Therefore, efficient methods to preselect the most promising parental lines and hybrid combinations before testing them in the field are required and were evaluated for the purpose of triticale hybrid breeding in this study.

Hybrid prediction based on phenotypic estimates

According to quantitative genetic theory, phenotypic hybrid prediction estimates based on the parental *per se* performance is expected to be inferior compared to predictions based on GCA effects, as GCA effects additionally exploit dominance effects partially (Lynch and Walsh 1998; Smith 1986). For some traits in this study, however, prediction accuracies based on the parental *per se* performance and GCA effects were similar or equally high (Trini et al. 2020). In particular, this was observed for traits that generally show a less complex inheritance and high heritabilities, such as ear emergence and plant height. This was in concordance with previous findings in triticale and also observed for other traits, *e.g.*, Fusarium head blight and thousand-kernel weight (Gowda et al. 2013; Boeven et al. 2016). Accordingly, a bigger difference between the two prediction approaches was observed for traits showing a more complex inheritance, such as biomass related traits, grain yield, protein content, starch content, and test weight (Trini et al. 2021b; Trini et al. 2020; Gowda et al. 2013). In triticale breeding programs, however, the relatively high prediction accuracies based on the parental mid-parent performance make it relatively easy for breeders to preselect the most promising parental lines, *i.e.*, before their introgression into a male sterile cytoplasm. This is supported by recent findings, as Boeven et al. (2016) showed that the resistance to Fusarium head blight severity is highly correlated between lines in a male sterile and in a normal cytoplasm.

Genomic hybrid prediction in triticale

The incorporation of molecular markers into breeding programs (Mammadov et

al. 2012; Collard et al. 2005) during the last years opened new possibilities for the prediction of experimental hybrids. Various methods were proposed with diverse advantages and disadvantages, but these mostly yielded the same prediction accuracy (Thorwarth 2019). Therefore, we constrained the used approach to genomic best linear unbiased prediction (gBLUP) to predict hybrid performance. Prediction accuracies based on the parental GCA effects including genomic data showed great potential for the evaluated biomass traits in this study and for other traits, such as ear emergence, flowering time, Fusarium head blight, grain yield, and plant height in previous studies (Gowda et al. 2013; Boeven et al. 2016). However, the genomic data did only increase the prediction accuracies slightly compared to predictions solely based on phenotypic data in this study making it necessary for breeders to carefully consider how to apply molecular markers in a breeding program. Great improvement, however, has been observed previously in triticale hybrids using such an approach to predict grain yield (Gowda et al. 2013).

Another field for the application of molecular markers in a hybrid breeding program is the evaluation of newly introduced parental lines into a breeding program and its evolving experimental hybrids. This is probably one of the most challenging fields as no phenotypic estimates of the possible hybrid combinations are available and prediction accuracies are usually decreasing with a lower degree of relatedness among the genetic material (Thorwarth 2019; Crossa et al. 2014). In this study, prediction accuracies incorporating molecular marker data decreased with an increasing number of untested parental lines (Trini et al. 2020). However, some prediction accuracies incorporating only one or no parental line in the estimation set still showed promising properties and therefore can be used for a preselection of newly introduced lines before their complex production and evaluation of experimental hybrids in the field.

Hybrid prediction and its use for biomass yield and related traits

The use of hybrid prediction approaches to improve the performance of biomass yield and related traits was not evaluated before. In this study, we proved that besides phenotypic also genotypic hybrid prediction approaches can be used to effectively predict the performances of experimental hybrids for biomass related

traits (Trini et al. 2020). The fact that prediction accuracies based on the parental *per se* performance were already very high give breeders the possibility to preselect the most promising parental lines already before the production of experimental hybrids and evaluate the most promising pre-selected lines using experimental hybrids in the up-following steps of a breeding program. The prediction accuracies based on GCA effects – using phenotypic and/or molecular data – showed promising properties to efficiently predict untested hybrids but were substantially hampered for biomass yield by a high ratio of variance due to SCA effects compared to the total genetic variance (Trini et al. 2020). Nevertheless, hybrid breeding including hybrid prediction has shown to be a very promising tool to further increase the performance of biomass yield in triticale.

Challenges of triticale hybrid prediction

Although the prediction accuracies showed promising results, triticale hybrid prediction still faces several challenges. Genomic prediction in triticale just recently became cheaper but is still not broadly used by triticale breeders (H. P. Maurer, personal communication) as relatively large training population sizes are required for sufficiently high prediction accuracies which are mostly not available as hybrid methodology is still not widely spread in triticale breeding programs (Crossa et al. 2014; H. P. Maurer, personal communication; Jannink et al. 2010). Furthermore, the efficiency of predicting experimental hybrids is strongly dependent on the genetic relationship among the training and the prediction set (Thorwarth 2019; Crossa et al. 2014; Würschum et al. 2017b) and therefore the selection of a sample of genotypes representing the whole population which should be predicted is of utmost importance. Prediction accuracies based on the parental *per se* performance showed encouraging results for a few traits, *e.g.*, dry matter content, heading date, or plant height, what was verified by simulation studies but are neglecting dominance effects and therefore their use cannot exploit its full potential achieving the highest possible prediction accuracies (Trini et al. 2021b; Trini et al. 2020; Lynch and Walsh 1998; Smith 1986). The lack of heterotic pools in triticale hampers both, GCA-based prediction accuracies based on solely phenotypic or incorporating genomic data (Gowda et al. 2013; Boeven et al. 2016). This is displayed in high proportions of variance due to SCA

compared to the total genetic variance. As dominance effects are partly neglected in predictions solely based on GCA effects (Lynch and Walsh 1998) prediction accuracies are reduced for traits with a high observed SCA variance ratio compared to the total genetic variance (Reif et al. 2012; Melchinger 1999; Hallauer et al. 2010). We observed this in our study for traits such as fresh and dry biomass yield, Fusarium head blight severity, protein and starch content, as well as grain yield (Trini et al. 2021b; Trini et al. 2020). Finally, the production of experimental hybrids using CMS is very time- and resource-consuming due to the required introgression into a male sterile cytoplasm illustrating the necessity for new approaches evaluating novel candidate lines in hybrid breeding.

Novel approach evaluating candidate lines for hybrid breeding

To overcome the disadvantages of the introgression of female candidate lines into a male sterile cytoplasm for the purpose of testing them, we introduced a novel approach (Trini et al. 2021b). The prediction of single-crosses using GCA or GCA and SCA estimates derived from experimental three-way hybrids was promising for all evaluated traits and its efficiency was further substantiated by computer simulations. The newly presented approach could be used routinely in triticale hybrid breeding programs to (i) increase the number of female candidate lines tested as a potential hybrid component, (ii) decrease the quantity of produced experimental hybrids especially with the use of suitable mating designs like single and double round robin design, (iii) identify female lines with high GCA effects recurrently improving the female pool, and (iv) gaining additional information about the candidate line under investigation regarding its level of sterility and response to restorer genes increasing the annual selection gain. A further step would be the inclusion of molecular markers to estimate SCA effects of untested single-cross hybrids, particularly when single or double round robin designs are used, to further increase prediction accuracies and increase the efficiency of triticale hybrid breeding programs.

Conclusions and prospects

Unraveling the genetic architecture of complex traits and using this knowledge to improve germplasm and breeding programs is very important. In triticale, though, molecular markers in great numbers are available only recently, since the introduction of genotyping-by-sequencing (Edwards et al. 2013). Therefore, this study was a breakthrough in triticale, as only a very limited number of fine-mapping studies are available so far and none of them evaluated the genetic architecture of plant height in Central European winter triticale. We observed only a small number of putative height-reducing QTL, which likely are associated with known height-reducing genes from wheat and rye (Trini et al. 2021a). The great majority of height-reducing genes, which could possibly be utilized from wheat and rye, were not used in triticale germplasm to date, *e.g.*, *Rht-B1*, *Rht24*, and *Ddw3*, leaving a lot of space for the improvement of triticale plant height by introducing such height-reducing genes. Moreover, side effects of these height-reducing genes, such as lodging tolerance or drought resistance, were not evaluated in triticale and should therefore be subject of future research, especially facing difficulties of changing weather conditions due to climate change (Fahad et al. 2017). However, over the time we observed an increasing frequency of registered cultivars carrying one or several putative height-reducing QTL indicating the potential of marker-assisted breeding in Central European winter triticale.

The evaluated hybrid prediction approaches showed great potential for the future improvement of triticale regarding biomass traits and was supported by our simulation studies (Trini et al. 2020). However, the missing heterotic pools observed in this and previous studies (Fischer et al. 2010; Gowda et al. 2013; Losert et al. 2017b; Tams et al. 2004; Tams et al. 2006) resulted in high ratios of SCA variance compared to the total genetic variance for some traits (Trini et al. 2021b; Trini et al. 2020). This reduced prediction accuracies solely based on phenotypic GCA effects or additionally incorporating molecular marker data and in some cases, these were then only as high as the prediction accuracies based on the hybrids' parental *per se* performances. Therefore, heterotic pools in triticale have to be established and efficient strategies have to be tailored to the specific requirements of triticale. Until then, though, promising hybrid

combinations can be pre-selected using the parental *per se* performances before producing and evaluating them based on their phenotypic GCA estimates.

In triticale breeding programs grain yield will most probably stay the most important trait in the future. Despite this, biomass yield has gained sufficient interest in recent years due to a promoting policy (EU 2018) and its high digestion quality in ruminant feed (Ayalew et al. 2018). This indicates that biomass yield will also play an important role in triticale breeding in the future and therefore its improvement has to be considered in breeding programs. However, it is important to examine if breeding programs only designed to improve biomass yield in triticale are able to prevail as the demand for cultivars used for biomass production is to some extend limited. It is more probable, that breeding programs will work on the simultaneous improvement of grain and biomass yield in the near future. This, however, still leaves obstacles as farmers prefer shorter cultivars, whereas biomass yield is highly dependent on tall genotypes in triticale (Gowda et al. 2011). Thus, sufficient methods and their use in triticale breeding programs need to be evaluated in the future, *e.g.*, by index and marker-assisted selection, to ensure efficient breeding programs.

The novel approach introduced in this study phenotypically evaluating novel female candidate lines with respect to hybrid production showed promising results for all evaluated traits using field data and simulation studies (Trini et al. 2021b). This approach, however, was not evaluated for biomass traits leaving space for future evaluations. Nevertheless, it can be expected to increase the efficiency of CMS-based hybrid breeding programs as it showed promising results and could also be applied in other hybrid crops using a CMS system as hybrid mechanism.

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Summary

Triticale (×*Triticosecale* Wittmack) breeding is a success story as it evolved to a serious alternative in farmer's crop rotations since the 1970s and is grown globally on around 4 million hectares today. New developments, however, pointed out additional possibilities to improve triticale line and hybrid breeding programs increasing its future competitiveness and were evaluated in this study. In more detail, these were to (i) examine the genetic control and evaluate long-term genetic trends of plant height in Central European winter triticale, (ii) evaluate the potential of triticale hybrid breeding and hybrid prediction approaches in triticale with a focus on biomass yield, (iii) introduce and examine a concept bypassing the time- and resource-consuming evaluation of female candidate lines in cytoplasmatic male sterility (CMS) based hybrid breeding, and (iv) to draw conclusions for the future improvement of triticale line and hybrid breeding programs.

The genome-wide association study detected markers significantly associated with plant height and developmental stage, respectively. These explained 42,16% and 29,31% of the total genotypic variance of plant height and development stage and are probably related to four and three quantitative trait loci (QTL), respectively. The two major QTL detected for plant height were located on chromosomes 5A and 5R which most likely could be assigned to the known height-reducing genes *Rht12* from wheat and *Ddw1* from rye. The third major QTL detected located on chromosome 4B could not be assigned to a known height-reducing gene and it cannot be precluded, that these significantly associated markers are identifying one and the same QTL as the markers located on chromosome 5R, as these showed a high linkage disequilibrium amongst each

other. Evaluating the 129 registered cultivars showed that plant height decreased since the 1980's. Evaluating their genetic constitution revealed that most cultivars carried at least one height-reducing QTL and that plant height could be reduced even further in cultivars combining more than one height-reducing QTL. It was further observed that the frequency of cultivars carrying one or a combination of height-reducing QTL increased since the 1980's.

A considerable amount of heterosis has been observed for biomass related traits in triticale hybrids before. However, the use of hybrid prediction approaches for these traits has not been evaluated. Hybrid prediction based on mid-parent values already showed very good results illustrating their potential to preselect the most promising parents as prediction accuracies based on parental general combining ability (GCA) effects were only slightly better. When incorporating molecular markers into GCA-based prediction accuracies, prediction accuracies decreased slightly compared to prediction accuracies solely based on phenotypic GCA effects. Predicting hybrids incorporating one or two untested parental lines, imitating a scenario where novel female and/or male candidate lines are introduced into a hybrid breeding program, reduced genomic prediction accuracies even further due to the decreasing amount of information which could be exploited from the parents. Additionally including specific combining ability (SCA) effects in the genomic prediction models did not yield additional use. A high proportion of SCA variance compared to the total genetic variance decreased prediction accuracies for the traits fresh and dry biomass yield. In this study simulation studies were used to demonstrate what a prediction accuracy of a specific value actually means for a hybrid breeding programs.

Further, an approach was introduced and evaluated showing great potential to evaluate novel female candidate lines for their use in a CMS-based hybrid breeding program by bypassing their time- and resource-demanding introgression into a male sterile cytoplasm using three-way hybrids. Prediction accuracies obtained by this novel approach showed highly promising results for most evaluated traits compared to prediction accuracies based on GCA effects or mid-parent performance. Additionally incorporating SCA effects into the prediction models showed only a little increase of the prediction accuracies. Further, the results were supported by simulation studies adjusting different

parameters, such as the number of parents or the proportion of SCA variance compared to the total genetic variance.

Zusammenfassung

Die Züchtung von Triticale (×*Triticosecale* Wittmack) ist eine Erfolgsgeschichte, da sie sich seit den 1970er Jahren zu einer ernstzunehmenden Alternative in der Fruchtfolge von Landwirten entwickelt hat und heute weltweit auf rund 4 Millionen Hektar angebaut wird. Jüngere Entwicklungen jedoch identifizierten zusätzliches Potential zur Verbesserung von Triticale Linien- und Hybridzüchtungsprogrammen, die die Konkurrenzfähigkeit von Triticale weiter erhöhen können und wurden deshalb in dieser Studie näher beleuchtet. Genauer betrachtet waren die Ziele dieser Studie (i) die genetische Struktur und genetischen Langzeittrends des Merkmals Wuchshöhe in Mitteleuropäischer Wintertriticale zu untersuchen, (ii) die Potenziale der Hybridzüchtung und Konzepte zur Hybridvorhersage in Triticale, mit dem Fokus auf Biomasseertrag, zu evaluieren, (iii) ein Konzept, welches die zeit- und ressourcenintensive Beurteilung weiblicher Mutterkomponenten in der zytoplasmatisch männlich sterilen (CMS) Hybridzüchtung vereinfacht, vorzustellen sowie bezüglich seiner Zweckdienlichkeit zu bewerten, und (iv) um Rückschlüsse für die zukünftige Verbesserung von Triticale Linien- und Hybridzuchtprogrammen zu ziehen.

In einer genomweiten Assoziationsstudie wurden Marker entdeckt, die signifikant mit den Merkmalen Wuchshöhe und Entwicklungsstadium assoziiert waren. Diese erklärten 42,16% beziehungsweise 29,31% der Gesamtvarianz der genannten Merkmale und repräsentieren wahrscheinlich vier beziehungsweise drei merkmalsbeeinflussende Genorte (QTL). Es wurden zwei bedeutende QTL für das Merkmal Wuchshöhe auf den Chromosomen 5A und 5R entdeckt, welche höchstwahrscheinlich den Wuchshöhe reduzierenden Genen *Rht12* von Weizen

und *Ddw1* von Roggen zuzuschreiben sind. Das dritte bedeutende QTL, welches auf Chromosom 4B gefunden wurde, konnte keinem bekannten Wuchshöhe reduzierenden Gen zugeordnet werden. Ferner kann nicht ausgeschlossen werden, dass diese als signifikant identifizierten Marker ein und dasselbe QTL identifizieren, wie die auf Chromosom 5R liegenden Marker, da diese untereinander ein hohes Kopplungsungleichgewicht aufweisen. Die Wuchshöhe der 129 untersuchten zugelassenen Sorten nahm seit den 1980er Jahren kontinuierlich ab. Die Untersuchung der zugrunde liegenden genetischen Ursachen hat ergeben, dass die meisten Sorten mindestens ein Wuchshöhereduzierendes QTL trugen und dass sich die Wuchshöhe noch weiter verringerte, wenn eine Sorte mehrere Wuchshöhe reduzierende Gene vereinte. Außerdem konnte beobachtet werden, dass sich seit den 1980er Jahren die Häufigkeit von zugelassenen Sorten die ein oder eine Kombination aus mehreren Wuchshöhe reduzierenden QTL trugen erhöht hat.

Ein beträchtlicher Umfang an Heterosis wurde in früheren Studien für Biomassemerkmale beobachtet. Nichtsdestotrotz wurden die Nützlichkeit von Hybridvorhersageansätzen bisher nicht evaluiert. Hybridvorhersagen, welche auf dem Mittelwert ihrer Elternlinien basieren zeigten vielversprechende Vorhersagegenauigkeiten und spiegelte somit ihr hohes Potential für die Vorselektion der vielversprechendsten Elternkomponenten wider, da Vorhersagegenauigkeiten basierend auf den elterlichen allgemeinen Kombinationsfähigkeiten (GCA) nur geringfügig besser waren. Mit der Aufnahme von molekularen Markerdaten in die GCA-basierten Vorhersagen verringerten sich die Vorhersagegenauigkeiten im Vergleich zu den Vorhersagen, welche lediglich auf phänotypischen GCA Effekten basierten. Bei Vorhersagen, welche ein oder zwei ungetestete Elternlinien beinhalteten, d.h. in einem Szenario welches neue weibliche und/oder männliche Kandidatenlinien in ein Hybridzuchtprogramm integriert, verringerten sich die genomischen Vorhersagegenauigkeiten noch weiter, da nur von einem beziehungsweise von keinem Elter Information genutzt werden konnte. Die zusätzliche Einbeziehung von spezifischen Kombinationseffekten (SCA) in die genomischen Vorhersagemodelle ergab keinen zusätzlichen Nutzen. Ein hoher Anteil an SCA Varianz an der genetischen Gesamtvarianz verringerte die

Vorhersagegenauigkeiten für die Merkmale Frisch- und Trockenbiomasseertrag. Ferner wurde anhand von Simulationsstudien abgeleitet, was eine bestimmte Vorhersagegenauigkeit eigentlich für ein Hybridzüchtungsprogramm bedeutet.

Es wurde ein neuer Ansatz auf seinen Nutzen in einem CMS-basierten Hybridzuchtprogram hin evaluiert der die zeit- und ressourcenintensive Rückkreuzung weiblicher Kandidatenlinien für deren Evaluierung in ein männlich steriles Zytoplasma umgeht. Die durch diesen neuen Ansatz erhaltenen Vorhersagegenauigkeiten zeigten großes Potential für die meisten untersuchten Merkmale im Vergleich zu Vorhersagegenauigkeiten basierend auf GCA Werten oder ihrem elterlichen Mittelwert. Das zusätzliche Miteinbeziehen von SCA Effekten in die Vorhersagemodelle zeigte nur eine geringfügige Verbesserung der Vorhersagegenauigkeiten. Außerdem konnten die Ergebnisse durch Simulationsstudien unter Anpassung verschiedener Parameter, wie die Anzahl der Eltern oder der Anteil der SCA Varianz an der genetischen Gesamtvarianz, untermauert werden.

Acknowledgements

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Eitensheim, 15/09/2021

_____   _____
Place, Date   Signature

Declaration in lieu of an oath on independent work

According to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics, and Social Sciences

1. The dissertation submitted on the topic

"Genomic and phenotypic improvement of triticale (×*Triticosecale* Wittmack) line and hybrid breeding programs"

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken work-for-word or paraphrased from other works.

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