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., 2010, 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;
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 × N) × 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 × 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...
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 \times N crosses are grown in microplots and then topcrossed with R-lines to produce the (A \times N) \times 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 \times 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 \times R hybrids thus resemble N \times 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 \times 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 \times R after the N-lines have been introgressed in the CMS cytoplasm and converted to A-lines. The single-cross (A \times R) and three-way ((A \times N) \times 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 \times 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 m² and sowing density was 280 viable seeds m²⁻¹, 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 plus sign. The single-stage model to calculate global variance components and best linear unbiased estimates (BLUEs) was:

\[ Y = G + L + G \cdot L + L \cdot R + L \cdot R \cdot B \]  \hspace{1cm} (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 (LSDs) 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:
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 $\text{GROUP}$, $\text{CHECK}$, $\text{FEMALE}$, and $\text{MALE}$ 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:

$$
\hat{y}_{\text{hybrid}(k)} = \bar{y} + \left( \frac{GCA_{Al(i)} + GCA_{Nl(j)}}{2} \right) + \frac{GCA_{Rk(i)}}{2} + \left( \frac{SCA_{Al(i)Rk(j)} + SCA_{Nl(j)Rk(i)}}{2} \right)
$$

with $\hat{y}_{\text{hybrid}(k)}$ 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 $GCA_{Al(i)} = GCA_{Nl(j)}$ reducing the model to:

$$
\hat{y}_{\text{hybrid}(i)} = \bar{y} + GCA_{Al(i)} + GCA_{Rk(i)} + SCA_{Al(i)Rk(i)}
$$

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:

$$
H^2 = 1 - \tilde{r}_{\text{BLUP}}^2 / 2 \sigma_G^2
$$

with $\tilde{r}_{\text{BLUP}}$ specifying the mean variance of a difference between two best linear unbiased predictors and $\sigma_G^2$ 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 (GCA2W) 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 (GCA3W: GCA–SCA3W) 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 GCA2W scenario (GCA_{lho}). 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 GCA3W and GCA–SCA3W 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 heritabilities, 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 the 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_{one}). 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 starchy 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 L⁻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 |
| \( \sigma^2_G \) 22.70 *** | 2.95 *** | 76.46 *** | 1.15 *** | 0.34 *** | 13.18 *** | 0.51 *** | 0.13 *** | 0.91 *** |
| \( \sigma^2_{GXL} \) 23.72 *** | 0.70 *** | 14.53 *** | 0.53 *** | 0.19 *** | 2.31 *** | 0.67 *** | 0.04 *** | 0.23 *** |
| \( \sigma^2_{S} \) 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 |

*Mean residual error variance.
***Significantly different from zero at the .001 probability level.
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 (GCA₂W) 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 (GCA₃W) or GCA and specific combining ability (SCA) effects (GCA-SCA₃W) estimated from three-way ((A × N) × R, N = 33) hybrids predicting single-cross hybrids (N × R, N = 8)

| Trait                          | MP   | GCA₂W | GCA_loho | GCA₃W | GCA-SCA₃W |
|-------------------------------|------|-------|----------|-------|-----------|
| Fusarium head blight          | 0.51 | 0.89  | 0.44     | 0.69  | 0.66      |
| Ear emergence                 | 0.75 | 0.98  | 0.83     | 0.99  | 0.91      |
| Plant height                  | 0.70 | 0.95  | 0.80     | 0.96  | 0.97      |
| Powdery mildew                | 0.85 | 1.00  | 0.88     | 0.78  | 0.64      |
| Grain yield                   | 0.23 | 0.78  | 0.45     | 0.96  | 0.74      |
| Thousand-kernel weight        | 0.74 | 0.45  | 0.83     | 0.93  | 0.77      |
| Test weight                   | 0.30 | 0.83  | 0.64     | 0.91  | 0.74      |
| Protein content               | 0.51 | 0.78  | 0.64     | 0.93  | 0.77      |
| Starch content                | 0.63 | 0.96  | 0.74     | 0.95  | 0.77      |

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
GCA₂W 0.89 0.98 0.95 1.00⁺ 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)

GCA₃W 0.69 0.99 0.96 1.00⁺ 0.59 0.97 0.91 0.53 0.25
GCA-SCA₃W 0.66 0.91 0.97 1.00⁺ 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

GCA₃W/MP 1.36 1.32 1.37 1.23 2.61 1.30 3.04 1.05 0.40
GCA-SCA₃W/MP 1.29 1.22 1.39 1.21 2.84 1.32 2.90 1.29 0.93

⁺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_{hoch}), 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 higher 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 number of N lines to be evaluated with the same effort. 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; Schultness 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 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 tested 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.

ACKNOWLEDGEMENTS

This research was funded by the German Federal Ministry of Food and Agriculture (BMEL) through its project management body Fachagentur für Nachwachsende Rohstoffe e.V. (FNR) within the project SENSELGO (Grant FKZ 22024515).

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.

*How to cite this article:* Trini J, Maurer HP, Weissmann EA, Würschum T. Fast-tracking the evaluation of novel female candidate lines in CMS-based hybrid breeding. *Plant Breeding*. 2021;140:432–441. https://doi.org/10.1111/pbr.12893