Received: 7 September 2023 | Revised: 21 December 2023 | Accepted: 27 December 2023
DOI: 10.1111/pbr.13160

ORIGINAL ARTICLE

Plant Breeding WILEY

First insights into the quantitative genetic composition of the female seed yield for an efficient hybrid seed production in wheat (*Triticum aestivum* L.)

Johannes Schneider[1] | Valentin Hinterberger[1] | Monika Spiller[2] | Thierry Moittié[3] | Mario Gils[4] | Markus Wolf[5] | Jochen C. Reif[1] | Albert W. Schulthess[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany
[2]KWS LOCHOW GMBH, Northeim, Germany
[3]ASUR Plant Breeding SAS, Estrées Saint-Denis, France
[4]Nordsaat Saatzucht GmbH, Langenstein, Germany
[5]Saaten-Union Biotec GmbH, Gatersleben, Seeland, Germany

Correspondence
Albert W. Schulthess, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany.
Email: schulthess@ipk-gatersleben.de

Funding information
Federal Ministry of Food and Agriculture, Grant/Award Number: FKZ2818401A18; Federal Ministry of Education and Research, Grant/Award Number: FKZ031B0184B; Deutsche Forschungsgemeinschaft, Grant/Award Number: 491250510

Abstract

Hybrid breeding of wheat (*Triticum aestivum* L.) is limited by its self-pollinating nature. Past cross-pollination improvements mostly focused on optimizing male traits. We tested the hybrid seed yield of 100 diverse elite lines treated with a chemical hybridization agent (CHA) and pollinated by non-sterilized male plants in multi-environmental field trials. Plant height and phenological traits of female plants were also assessed. In parallel, control experiments without CHA sterilization were conducted to measure per se yield of the tested material. Hybrid seed yield variation is of quantitative genetic nature, and, despite the large environmental influence, this trait has a strong genotypic component and is highly heritable ($h^2 = .77$). The lack of correlation between hybrid seed yield and per se yield suggests a non-shared genetic control. Phenological traits and their interactions are important factors explaining together $\sim$1/3 of hybrid seed yield variation. In contrast to plant height and flowering traits, which are influenced by major genetic factors, no significant marker–trait associations were found for the hybrid seed yield, thus suggesting a highly polygenic genetic architecture and the need of larger populations to investigate female hybrid seed yield.

KEYWORDS
confounding traits, direct selection, female hybrid seed yield, hybrid seed production, marker–trait associations, polygenic genetic architecture, wheat

1 | INTRODUCTION

Breeding hybrid varieties in bread wheat (*Triticum aestivum* L.) promises benefits in terms of higher grain yield due to heterosis as well as yield stability (Liu et al., 2017; Longin et al., 2012; Mühleisen et al., 2014; Pickett, 1993). These advantages could cope with major challenges of future agricultural production, like an increasing world population or the negative effects of climate change (Fróna et al., 2019; Tester & Langridge, 2010).

Despite several efforts in recent decades, hybrid breeding programmes in wheat showed only limited success, and the interest of growers in these varieties has increased only marginally through time (Gupta et al., 2019; Ter Steeg et al., 2022). This is mainly due to the fact that the market success of hybrid wheat is influenced by the level of seed production costs and the profit from an increased grain yield compared to inbred lines for the farmers (Longin et al., 2014). In this context, various requirements still need to be overcome. For example, the level of commercial heterosis of varieties should be increased at least 20% above the best locally adapted line varieties so that it counterbalances the higher costs of hybrid seeds for farmers (Singh et al., 2010). In addition, since wheat is an autogamous self-pollinating species, an efficient and affordable hybrid seed production is another major bottleneck for every breeding programme (Longin et al., 2014; Whitford et al., 2013).

For profitable hybrid seed production, the female parent component has to be male sterile. Despite the existence of several cytoplasmatic/nuclear genetic male sterility systems, treating plants with chemical-hybridizing agents (CHA) is still one of the most widely applied methods for plant emasculation in commercial hybrid seed production of wheat (Farooq et al., 2023; Whitford et al., 2013). This is probably because the use of a CHA treatment is in principle much simpler than implementing alternative fertility control systems dependent on restorer/maintainer genetics and has therefore a relatively wide application scope for the stable production of hybrids with a large number of different female lines (Easterly et al., 2019; Parodi & de los Angeles Gaju, 2009). Regardless of the sterility system, hybrid seed yield on the female parents is limited by the structure and the development of the wheat flower and modulated by various environmental factors (Fábián et al., 2019; Selva et al., 2020). Both female and male parent components should be improved with respect to favourable traits for cross-pollination (Selva et al., 2020). While the properties and genetic architecture of suitable male components, such as anther extrusion, pollen amount and viability, have been described in various studies (Boeven et al., 2016, 2018; El Hanafi et al., 2021), information on the female component are rare. Genotypic variation underlying the hybrid seed yield of female lines has been reported before (Schneider et al., 2021). Knowledge on the trait components that improve pollen receptivity and increase the female hybrid seed yield could support the design and selection of plant ideotypes (Selva et al., 2020). In the literature, several traits like the stigma length, the openness of floret or the ovary swelling have been well described (Okada et al., 2018; Singh & Joshi, 2003), but until now, their causal relation to the female hybrid seed yield has not been reported. Moreover, phenotyping these characteristics is very time consuming and labour intensive, which makes it difficult to study in commercial breeding programmes that handle large numbers of plants (Millan-Blanquez et al., 2022). As an alternative, the selection of favourable female ideotypes could be supported by using molecular markers closely linked with major quantitative trait loci (QTL) for the female hybrid seed yield (Boeven et al., 2018). In this regard and to the best of our knowledge, the genetic architecture of the suitability of female lines for hybrid seed production has not been directly assessed so far.

In this study, the objectives were to (1) study the genotypic variation for the female hybrid seed yield of an elite wheat panel in the context of hybrid seed production, (2) explore the relationship between the hybrid seed yield and confounding traits like flowering time, heading date, plant height or the per se yield of female lines and (3) give first insights for the genetic architecture of the female hybrid seed yield through genome-wide association mapping.

2 | MATERIALS AND METHODS

2.1 | Plant material and field experiments

A population of 100 Central European winter wheat (*T. aestivum* L.) lines were used as female parents in hybrid seed production. This panel contained released modern varieties as well as current breeding material from the private breeding industry. Female parents were preselected based on visual performance favouring traits that are in general beneficial for crop production under continental environment and rainfed agricultural conditions of Central Europe such as late flowering time (FT), reduced plant height (PH) and good leaf health. To ensure sufficient pollen availability during the whole flowering period, a seed mix of male lines composed of the wheat German elite varieties Piko (released in 1994) and KWS Ferrum (released in 2012) as well as two advanced breeding lines from the German company Nordsaat Saatzucht GmbH was used. These wheat genotypes are characterized by their excellent pollination properties and cover together a wide range of flowering times. In the male mix, seeds of wheat genotypes were combined in equal proportion.

Multi-environmental field trials were carried out in Böhnshausen (Germany, latitude 51°51′14.0″N, longitude 10°56′41.8″E, growing seasons 2020 and 2021), Wohlde (Germany, latitude 52°50′05.1″N, longitude 9°57′31.9″E, growing season 2020) and Estrées-Saint-Denis (France, 49°25′59.2″N, longitude 2°38′11.5″E, growing season 2022). The overview of weather conditions in these experiments are presented in Figure S1. The experiments were conducted following an alpha lattice design with two replications and 10 female lines per incomplete block. The wheat lines were grown in 10.95 m^2 plots in Wohlde, while 9 m^2 plots were considered in both Böhnshausen and Estrées-Saint-Denis. The sowing density amounted to 450 grains per m^2 for the female and 250 grains per m^2 for the male lines. The higher plant density in female plots was meant to inhibit the production of late tillers, which often carry male fertile ears. In contrast, a low plant density for male lines would benefit the late tillering, thus prolonging the pollen shedding period of plants. To avoid uncontrolled outcrossing and ensure the targeted cross-pollination, male and female plots were sown side by side in a crossing block surrounded by isolating male sterile rye plants. Female lines were emasculated by using the commercial CHA Croisor® 100 (ASUR Plant Breeding SAS), which contains the active ingredient sintofen (1-(4-chlorophenyl)-5-(2-methoxyethoxy)-4-oxo-1,4-dihydrocinnoline-3-carboxylic acid). For each female plot, CHA application took place only once, when wheat ears—still inside of the main shoot—reached a length of 2.5 cm during stem elongation (Zadoks scale 31 [Zadoks et al., 1974], BBCH scale 31). The sterility level of each female plot was controlled by counting the number of kernels of individual plants, which were completely bagged, directly after the CHA treatment.

The hybrid seed yield was quantified as the grain weight harvested from each cross-pollinated female plot and expressed in g m^{-2} at a 14% moisture content. In this work, we also refer to this trait as female hybrid seed yield or simply as female seed yield. Moreover, during the growth period, five traits potentially influencing female hybrid seed yield variation were evaluated on the CHA-treated female population: (1) Heading date (HD) was quantified in days after the 1st of January when 75% of the spike per plot have emerged (Zadoks scale 59; BBCH scale 59). FT was also assessed in days after the 1st of January as (2) the start of flowering (FT begin: Zadoks scale 60; BBCH scale 61), (3) the end of flowering (FT end: Zadoks scale 69; BBCH scale 69) and (4) the flowering duration (FT dur) as difference between both traits. (5) PH was measured in cm from the ground to the erected ear after the end of flowering time (Zadoks scale 71; BBCH 71). While FT begin, FT end and FT dur were recorded in Wohlde and Böhnshausen during the 2020 growing season, HD and PH were measured in all four environments considered in this study.

In parallel, control experiments were conducted within each environment to test an additional trait potentially influencing the female hybrid seed yield: (6) the per se yield of each female genotype without applying the CHA treatment. For this, each female line was sown directly nearby its corresponding crossing block following the exact same experimental design and using the same plot sizes as well as sowing rate as described above for the CHA-treated crossing blocks. Grain yield was then recorded as the harvested grain weight in g m^{-2} at a 14% moisture content. With the exception of the CHA treatment, all experiments (CHA-treated and control) were conducted under standard cultural practice of plant protection and nutrition.

2.2 | Phenotypic data analyses

Before parameter estimation, we subjected the raw data to a quality assessment. In the case of the CHA-treated experiment, all datapoints across traits from non-sterile plots were completely removed. In the case of the control experiments, we extracted the residuals from a mixed model that included design effects as random factors plus genotypes and a common mean as fixed effects. After normalizing residuals, outliers were identified experiment-wise by following Anscombe and Tukey (1963) and assuming a nominal alpha level of .01. These significant outliers were removed from the datasets. Experiment-specific heritabilities—a.k.a. repeatabilities—were computed from outlier-corrected data according to Schulthess et al. (2022) and served as an additional measure of quality control before analysing data across environments.

For each trait, the best linear unbiased estimations (BLUEs) for genotypes across environments, replications and blocks were calculated with the following mixed model:

$$y_{ijkm} = \mu + g_i + e_j + g_i \times e_j + r_{k(j)} + b_{m(kj)} + \varepsilon_{ijkm} \tag{1}$$

where y_{ijkm} is the observed phenotypic value of the *i*th genotype tested in the *m*th incomplete block of the *k*th replication within the *j*th environment, μ is the common population mean effect, g_i refers to the effect of the *i*th genotype, e_j– to the effect of the *j*th environment, $g_i \times e_j$ to the interaction effect of the *i*th genotype with the *j*th environment, $r_{k(j)}$ stands for the *k*th replication effect, $b_{m(kj)}$ for the effect of the *m*th incomplete block and ε_{ijkm} is the residual. For BLUE calculation, all effects in Equation (1), except μ and genotype effects, were modelled as random. In addition, variance components were estimated by assuming all factors in Equation (1) except μ as random, while their significance was tested using a log-likelihood ratio test. Mixed models were fitted by using the ASReml V4 package (Butler et al., 2018) in R V4.1.3. (R Core Team, 2020).

The heritability for every evaluated trait was calculated across environments as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g\times e}^2}{E} + \frac{\sigma_\varepsilon^2}{E\times R}}, \tag{2}$$

where σ_g^2 represents the genotypic variance, $\sigma_{g\times e}^2$ is the variance of the interaction between the genotype and the environment, σ_ϵ^2 stands for the error variance, while E and R refer to the effective number of testing environments and replicates, respectively.

We estimated the genetic correlation among two traits as (Burdon, 1977)

$$r_{g_{no}} = \frac{r_{p_{no}}}{\sqrt{h_n^2 \times h_o^2}}, \tag{3}$$

where $r_{g_{no}}$ stands for the genetic correlation between the trait n and o, $r_{p_{no}}$ is the phenotypic correlation between BLUEs, while h_n^2 and h_o^2 are their corresponding trait heritabilites.

In addition, we investigated the contribution of female plant traits and their interactions potentially underlying female seed yield in the context of CHA-dependent hybrid seed production. We implemented therefore a parsimonious model selection method to maximize the percentage of explained variation of female hybrid seed yield while avoiding overfitting. In a first step, a multiple regression model including the main effects of all six potential confounding traits and their two-way interactions was fitted to the BLUEs of female hybrid seed yield using the lm() R function. Here, main effects of female plant traits with the highest correlations with the female hybrid seed yield entered first the model, while the sequence order of interactions was determined by the absolute value of the correlation cross-product with hybrid seed yield. From this model, main and interaction effects with significant partial sum of squares (F test, *P*-value <.05) as well as non-significant main effects with their significant interactions were retained for further analyses. In the second step, the selected linear model was progressively extended with higher-order components for the significant main effects by using a sequential polynomial regression method. For each main effect, model extension was stopped after observing two consecutive non-significant sequential sums of squares. The final selected model included therefore the last significant high-order term for the main effects of female plant traits in addition to their lower order terms as well as the significant interactions from the first selection step. This model was used to compute the partial R^2 values for each of the significant components.

2.3 | Genotypic characterization and marker imputation

The DNA of all female lines was extracted from 10-day-old seedling in compliance with known standard procedures (Stein et al., 2001). The genotyping was carried out by TraitGenetics GmbH (https://www.traitgenetics.com/) using a 70,000 single-nucleotide-polymorphism (SNP) array, which derived from the original 90,000 Illumina Infinium Chip array (Wang et al., 2014). Soleimani et al. (2020) placed the SNP array marker sequences onto the pseudomolecule reference sequence of Chinese Spring (The International Wheat Genome Sequencing Consortium [IWGSC], 2018), which allowed the obtention of a physical map for the Illumina Infinium Chip array. Particularly, the physical map of our study is based on RefSeq V1.0 and was downloaded from the BioStudies (Sarkans et al., 2018) database (https://www.ebi.ac.uk/biostudies/arrayexpress/arrays/A-MTAB-675). SNP markers with more than 25% missing values or a minor allele frequency of <0.05 were eliminated from the dataset. After this quality check, 10,194 markers remained for downstream analysis. Missing genotypic information were imputed using the random forest method as implemented in the missForest R package V1.5 (Stekhoven & Bühlmann, 2012).

2.4 | Population structure and association mapping

To investigate population structure, a principal coordinate analysis (PCoA) was conducted based on the modified Rogers' distance (RD) matrix (Wright, 1978). Here, the first two PCos were retained to portray the molecular diversity using a biplot. In addition, we also investigated the relationship between population structure and female hybrid seed yield variation through a Mantel correlation test (Mantel, 1967) between RDs and the Euclidean distances among phenotypic values as implemented in the vegan R package V2.6-4 (Oksanen et al., 2022). To determine which loci are related to the female hybrid seed yield, a genome-wide association study (GWAS) was conducted based on BLUEs by using the R package rrBLUP V4.6.2 (Endelman, 2011). Under simplified assumptions, kinship coefficients between two homozygous genotypes can be approximated by the expression $2*(1-\mathrm{RD})$ (Melchinger et al., 1991). Computed kinship coefficients were compiled into a matrix and used to correct for genetic relatedness in GWAS. For the detection of significant markers, a threshold after Gao et al. (2008) was applied using the nominal alpha level of .05. For the sake of data integrity, GWAS scans were also run for HD, FT begin, FT end, FT dur and PH. For a given trait, the amount of phenotypic variation explained by significant markers (R^2) was estimated by fitting a linear regression model to the BLUEs including all corresponding significant markers. Here, significant markers with the lowest *P*-value entered first the model. All computational methods were implemented in R V4.1.3 (R Core Team, 2020).

3 | RESULTS

3.1 | Despite environment effects, the female hybrid seed yield has a strong genetic component

Female hybrid seed yield was evaluated for 100 diverse lines in multiple environments. In these experiments, the environment component was 4.6 times larger than the genotypic component for total variation on female hybrid seed yield (Table 1). This large influence of the environment on the female hybrid seed yield was also reflected in the significant mean environmental difference ($\Delta = 384.53$ g m^{-2}, *P*-value <.001) between Böhnshausen 2021 and Böhnshausen 2020 (Figure S2). The

TABLE 1 Summary statistics for the traits female hybrid seed yield (g m^{-2}), yield per se (g m^{-2}), heading date (days from 1st of January, HD), beginning of flowering (days from 1st of January, FT begin), duration of flowering (days, FT dur), end of flowering (days from 1st of January, FT end) and plant height (cm, PH) assessed for a population of 100 Central European female winter wheat lines in up to four environments. For each trait, summary statistics include minimum, median, mean and maximum value among the best linear unbiased estimations of genotypes, variance components of environments (σ^2_E), genotypes (σ^2_G), their interactions ($\sigma^2_{G\times E}$) and residual error (σ^2_e) as well as the heritability (h^2) estimated across environments. Significance of variance components was tested using a log-likelihood ratio test.

Parameter	Hybrid seed yield	Yield per se	HD	FT begin	FT dur	FT end	PH
Min	300.57	737.21	142.62	148.01	3.55	156.07	66.21
Median	533.80	844.95	147.88	155.60	5.93	161.17	81.08
Mean	528.79	844.71	147.60	154.98	6.09	161.08	80.76
Max	746.17	930.56	151.20	161.01	11.06	166.04	93.76
σ^2_G	6153.08***	591.45***	3.04***	5.99***	0.43	1.68***	28.37***
σ^2_E	28560.90**	39443.8***	56.34*	8.47	2.45	2.02**	45.67***
$\sigma^2_{G\times E}$	3480.44***	1189.16**	0.11*	1.01**	0.42	0.67	14.61***
σ^2_e	5276.94	1564.57	1.67	1.73	1.75	1.43	6.45
h^2	0.77	0.47	0.90	0.83	0.32	0.65	0.85

Note: *, ** and *** indicate significantly different from zero with *P*-value <.05, .01 and .001, respectively.

genotypic component was 1.8 times larger than its interaction with the environment (Table 1). The strong genotypic effect was also evidenced by the high heritability of $h^2 = .77$. The significant correlations with values above .4 between the BLUEs of female hybrid seed yield from individual environments also underline the importance of the genetic effect (Table S1). According to these correlations, the environments Wohlde 2020 and Böhnshausen 2020 presented the most contrasting genotypic rankings. Moreover, the quantitative nature of the female hybrid seed yield was reflected in the Gaussian-like distribution of genotypic values (BLUEs) estimated across environments, ranging between 300.57 and 746.1 g m^{-2} (Figure 1).

3.2 | Phenological traits had the greatest confounding effect on female hybrid seed yield

Phenological traits (HD, FT begin, FT end, FT dur), PH and per se yield performance were assessed in parallel to the hybrid seed yield for female lines. The heritability of each trait ranged between moderate to high, with the least heritable trait being FT dur ($h^2 = .32$) and the most heritable one being HD ($h^2 = .9$) (Table 1). Genotypic values for phenological traits and PH corresponded to those expected from preselection for limited PH and late FT (see Section 2.1), thus favouring adaptation to Central European modern wheat production environments. Particularly for per se yield (CHA-untreated females), the experiment conducted at Böhnshausen during the 2020 season presented a much lower repeatability than the other three environments, that is, 0.19 versus 0.60–0.80. By discarding per se yield data of this outlier experiment from the dataset, the final heritability for this trait amounted to 0.47. Genotypic values of per se yield were in general 1.3 times higher (average: 844.71 g m^{-2}) and less variable (range: 737.21–930.56 g m^{-2}) than those of female hybrid seed yield (Figure 1a nd Table 1). Moreover, per se yield as well as PH were not significantly correlated with the female hybrid seed yield (Table 2). To rule out that the non-significant correlation between hybrid seed yield and per se yield was due to an incomplete environment overlap after discarding Böhnshausen 2020 data for the latter trait, we recomputed the BLUEs for hybrid seed yield but ignoring this particular environment and re-estimated the correlation. This new correlation estimate was again non-significantly different from zero (P-value ≥.05), confirming thus the lack of association between hybrid seed yield and per se yield. In case of PH, we cannot rule out that the pre-selection favouring short to medium genotypes caused the lack of correlation between this trait and female seed yield. In contrast, all studied phenological features of female plants presented significant pairwise correlations with the hybrid seed yield (P-value <.05). According to these pairwise correlations, female wheat plants heading or flowering later but having simultaneously short flowering periods were associated with higher hybrid seed yield. This motivated us to investigate the relationship between these traits and female hybrid seed yield in more detail by using variable selection to obtain a parsimonious model that can explain most phenotypic variation on hybrid seed yield. The final model explained 29.08% of the phenotypic variation on hybrid seed yield. Among significant components, the FT dur was highlighted as the only trait of female plants having a main influence, explaining 13.51 % of the variation on female hybrid seed yield (Table 3). In addition, the two-way interaction effects of FT begin with the FT dur and with HD of female plants were significant and explained 5.75% and 8.2% of the variation on female hybrid seed yield.

3.3 | Genome-wide association mapping suggests that female hybrid seed yield is a complex quantitative trait

The 100 diverse female lines were genotyped with 70,000 genome-wide SNP markers from the Illumina Infinium Chip array (Wang et al., 2014). After quality control, 10,194 high-quality polymorphic markers from the SNP array with available physical positions mapped to RefSeq V1.0 of Chinese Spring were retained for downstream analysis. A PCoA was applied to the modified RD distances derived from these makers to investigate the population structure (Figure 2). The first

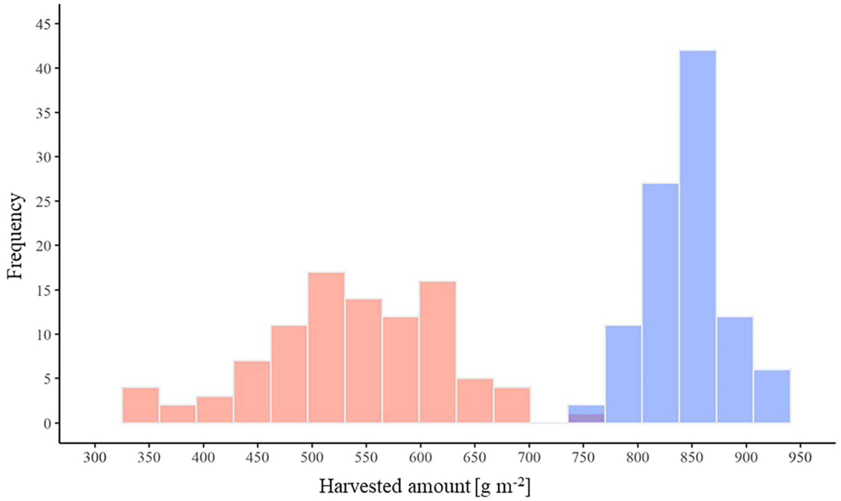


FIGURE 1 Histogram of the best linear unbiased estimations of the female hybrid seed yield (red bars) and the per se yield (blue bars) in g m^{-2} for 100 Central European winter wheat lines evaluated in four multi environmental field experiments. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Phenotypic (upper triangle) and genetic (lower triangle) correlation coefficients among the traits hybrid female seed yield (g m^{-2}), yield per se (g m^{-2}), heading date (days from 1st of January, HD), begin of flowering (days from 1st of January, FT begin), flowering duration (days, FT dur), end of flowering (days from 1st of January, FT end) and plant height (cm, PH) assessed for a population of 100 Central European female winter wheat lines in up to four environments. The significance of phenotypic correlations was evaluated through a *t*-test.

	Hybrid seed yield	Yield per se	HD	FT begin	FT dur	FT end	PH
Hybrid seed yield		.11	$.28^{**}$	$.35^{***}$	$-.37^{***}$	$.25^{*}$	.06
Yield per se	.18		−.09	−.13	0.12	−.09	.06
HD	.34	.14		$.91^{***}$	$-.64^{***}$	$.82^{***}$	$.47^{***}$
FT begin	.44	−.21	1		$-.74^{***}$	$.88^{***}$	$.32^{**}$
FT dur	−.75	.31	−1	−1		$-.35^{***}$	$-.3^{**}$
FT end	.35	−.16	1	1	−.77		$.26^{**}$
PH	.07	.09	0.54	0.38	−.16	.35	

Note: *, ** and *** indicates phenotypic correlations are significantly different from zero with *P*-value <.05, 0.01 and .001, respectively.

TABLE 3 Percentage of explained variation on female hybrid seed yield (partial R^2) and significance of the estimated main effects of flowering duration (FT), beginning of flowering (FT begin), heading date (HD) and the end of flowering (FT end) of female plants as well as their significant interactions. Model components were selected by applying a parsimonious model fitting method (please see Section 2 for further details).

Source of variation	Effect	Sum of squares	R^2 (%)	Significance
FT dur	156.8 g m^{-2} day^{-1}	115,860	13.51	***
FT begin	1038 g m^{-2} day^{-1}	10,839	1.26	
HD	1065 g m^{-2} day^{-1}	3010	0.35	
FT dur × FT begin	−1.052 g m^{-2} day^{-2}	49,317	5.75	**
FT begin × HD	−6.933 g m^{-2} day^{-2}	70,293	8.2	**
Residuals	–	608,121	70.92	

Note: *, ** and *** indicate ignificantly different from zero with *P*-value <.05, .01 and .001, respectively.

and second coordinates from the PCoA explained 10.25% and 7.72% of the total molecular variation, respectively, but no distinct family groupings that could graphically explain variation on female hybrid seed yield could be clearly observed. The lack of association between female hybrid seed yield variation and genome-wide genetic differences was also supported by a non-significant Mantel test between RD and Euclidean phenotypic distances ($r = -.0018$, P-value $= .5$). A null correlation between phenotypic and genomic similarities maximizes the expected detection power in GWAS (Myles et al., 2009), which motivated us to perform a genome-wide scan for marker–trait associations on female hybrid seed yield. According to the significant threshold of Gao et al. (2008), no significant (P-value ≥.05) major QTL was however detected for the hybrid seed yield variation observed among female lines (Figure 3). In contrast, GWAS for PH and phenological traits revealed some major QTL in our studied population (Figures S3–S7, Table S3). For PH, two significant markers were found on the distal region of the long arm of chromosome 5A (Figure S4) and explained up to 23.26% of the phenotypic variation. We presume that these detected signals on chromosome 5A correspond most likely to the *Rht9* dwarf locus, which maps ∼22 Mb distal from the associated markers (Ellis et al., 2005; Luján Basile et al., 2019). In addition, a total of seven

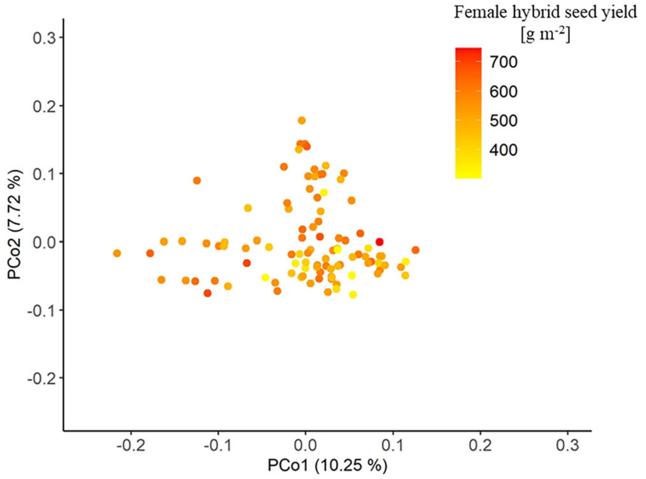
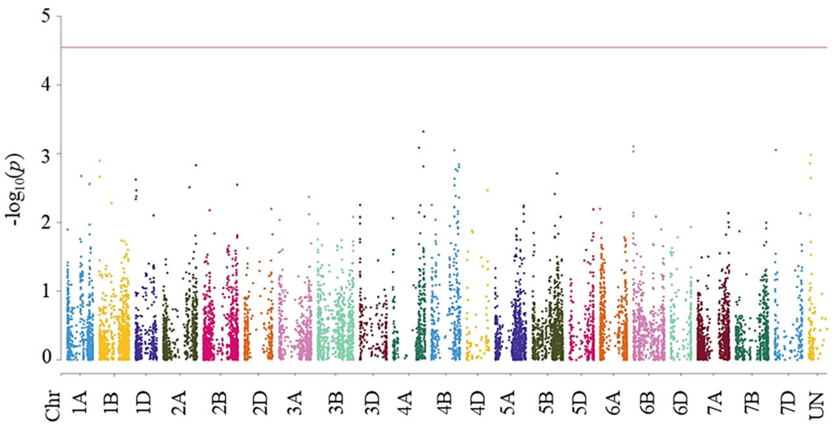


FIGURE 2 Principal coordinate analysis (PCoA) based on the modified Rogers' distances computed from 10,194 SNP markers among 100 Central European winter wheat lines used as female plants for hybrid seed production. The proportion of genetic variation explained by the first and second PCos are included within brackets, while the colour scale corresponds to the female hybrid seed yield in g m^{-2}. [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Manhattan plot of a genome-wide association analysis for the female hybrid seed yield (g m^{-2}) in a population of 100 Central European winter wheat lines used as female plants for hybrid seed production and genotyped with 10,194 SNPs. The significant threshold was set after Gao et al. (2008) considering a nominal alpha level of .05. The scale of *P*-values corresponds to $-\log_{10}(P\text{-value})$, while physical positions (bp) on chromosomes are according to RefSeq V1.0 of Chinese Spring (IWGSC, 2018) and were downloaded from: https://www.ebi.ac.uk/biostudies/arrayexpress/arrays/A-MTAB-6759. [Color figure can be viewed at wileyonlinelibrary.com]



markers—all of them mapping on the homologous chromosome group 2—were significantly associated with at least one of the assessed flowering traits (FT beg, FT end, FT dur) (Figures S5–S7). These significant markers could explain up to 36.95% of the variation on a single flowering trait (Table S3). Several past studies have already highlighted chromosomes 2A, 2B and 2D as carriers of important flowering-related genes in wheat, such as *Ppd*-1 (Royo et al., 2018; Zhang et al., 2022). Given that PH and flowering traits are described in the literature as traits of moderate to low complexity, their contrasting GWAS results with those for female hybrid seed yield are indicative of a more complex quantitative genetic architecture underlying the latter trait. In summary, our results suggest that the variation on hybrid seed yield of female plants is influenced by the net effect of many loci, each with a very small individual influence on trait variation.

4 | DISCUSSION

An efficient hybrid seed production system is an important prerequisite for establishing successful hybrid breeding programmes, but in the case of wheat, this is mostly limited by its self-pollinating nature. Therefore, both parents should contain fundamental traits, which support outcrossing and help to increase the hybrid seed yield on the female parent (Selva et al., 2020). Previous research has mainly focused on the optimization of the relevant traits for the male ideotype (Boeven et al., 2018; El Hanafi et al., 2022; Sade et al., 2022). In contrast, information about the properties of excellent female lines are little, while their routine large-scale assessment in breeding programmes is usually discarded, because of being difficult to measure and time consuming (Langer et al., 2014; Millan-Blanquez et al., 2022). This stimulated us to study the genetic component underlying the female hybrid seed yield and its prospects for targeted breeding.

4.1 | Improving female hybrid seed yield through its targeted phenotypic selection

In agreement with previous reports (Boeven et al., 2018; Pickett, 1993; Schneider et al., 2021), the effect of the environment influenced a large proportion of the female hybrid seed yield variation in our study (Table 1). Besides limiting photosynthesis and grain production, heat and drought stress, on the one hand, reduce the functionality of female reproductive organs and the viability of male pollen (De Storme & Geelen, 2014; Fábián et al., 2019; Imrie, 1966). On the other hand, high temperatures accompanied with high air moisture increase the risk of grain lost due to fungal diseases (Miedaner & Juroszek, 2021). We looked at the accumulated precipitation and temperature sums during the growing season of our experiments in more detail but found no clear trend that could explain the differences in average hybrid seed yields of female plants observed between environments (Table S2). Nevertheless, we expect that pervasive environment effects on hybrid seed yield are an overall limitation for all female plants in general and less genotype specific due to the minor relative importance of interactions between genotypes and environments (Tables 1 and S1). Moreover, the wide distribution for female hybrid seed yield (Figure 1) is in line with past observations (Boeven et al., 2018; Pickett, 1993; Schneider et al., 2021) and together with its high heritability (Table 1) holds the promise for an effective female hybrid seed yield improvement through targeted phenotypic selection. However, testing a large number (>1000) of potential female lines in crossing blocks is challenging, very costly and time consuming. Therefore, and similar as for per se yield, field testing and direct selection for female hybrid seed yield should be performed at later breeding stages, when population sizes have been already reduced to a manageable number and enough seeds are available to perform meaningful field trials. Population sizes could be reduced by performing indirect selection during early breeding stages based on highly heritable indicator traits correlated to hybrid seed yield of female plants. To this respect, further applied research on high-throughput non-destructive phenotyping methods, for example, automated imaging under controlled plant growing conditions, which highlight genotypes with the features of the female plant ideotype linked to high hybrid seed yield, is required. As we discuss later, early routine selection assisted by genomics could be also an alternative to reduce population sizes in breeding. Interestingly, the per se yield of female lines was not significantly correlated to their hybrid seed yield (Table 2), suggesting that selection for high-yielding varieties does not necessarily lead to an increase in grain yield in hybrid seed production. Nonetheless, hybrid wheat breeding programmes should at the end target both traits, because while female hybrid seed yield is of central importance for hybrid seed production itself, a high per se grain yield is required to maintain and multiply parent lines.

4.2 | The hybrid seed yield is highly influenced by the flowering period of female plants

In our experimental setup under continental environment and rainfed agricultural conditions of Central Europe, CHA-sterilized wheat plants heading or flowering later but having simultaneously short flowering periods were associated with higher female hybrid seed yield (Tables 2 and 3). In the absence of pollen, wheat plants make use of the second opening mechanism, in which the ovary increases its radial size and pushes the lemma and palea to open spike florets and favour pollination from external pollen sources (Okada et al., 2018). As indicated by the outcome of the fitted parsimonious model, the flowering duration of female plants has a positive main effect on their hybrid seed yield (Table 3), suggesting that male sterilized plants with high hybrid seed yield increase the likelihood of being pollinated by extending their flowering periods. This assumption goes in line with the conclusion of El Hanafi et al. (2020), who highlighted flowering duration of CHA-untreated plants as an important trait for the selection of suitable wheat female lines. Nevertheless, flowering duration of female plants and its start have a negative multiplicative interaction effect on the hybrid seed yield (Table 3), indicating that for hybrid seed production it is not only important for how long but also when female plants start to flower. With open spike florets in a late flowering context, the stigma of female plants becomes more exposed to hot and dry air and thus will progressively desiccate with prolonged flowering periods, which would lead in turn to a decreased stigma receptivity and lower final hybrid seed yield (Imrie, 1966; Millan-Blanquez et al., 2022). In addition, the negative multiplicative interaction effect on hybrid seed yield between the start of flowering and heading date of female plants (Table 3) highlights also the importance of the moment when the male sterilized plants are pollinated. Theoretically, the optimal timepoint is given by the synchronized flowering of pollen donor and receptor, that is, optimal nicking, accompanied by ideal weather conditions that ensure an effective pollination in crossing blocks (Selva et al., 2020). In our experiments, nicking was maximized by using a seed mix composed of different wheat male lines with excellent pollination properties and a wide range of flowering times. With this experimental setup, we expected to unmask the genotypic value of female plants that underlies hybrid seed yield. In practical breeding, this is however even more challenging because besides the pollinator qualities of the male lines, as well as the receptivity of the females, the overlapping flowering time of both parent lines should be also under consideration (Whitford et al., 2013). In this regard, the breeding of male and female components within maturity classes can substantially contribute to reduce some sources of seed yield lost during hybrid seed production.

4.3 | Potential confounding effects of CHAs

One of the main practical advantages of CHA treatments is their wide application range that allows to test many female lines without the need of more complex genetic fertility control systems (Easterly et al., 2019; Parodi & de los Angeles Gaju, 2009). Particularly for our study, the applied CHA—Croisor® 100—is a plant growth regulator and belongs to the new generation of CHAs, which are less harming for the environment, have a more effective sterilization effect and are less phytotoxic as compared to early CHA molecules/formulations (Farooq et al., 2023; Singh et al., 2015; Whitford et al., 2013). Genotype-specific effects for the effectiveness of male sterilization have been nevertheless described for Croisor® 100 in recent studies (Easterly et al., 2019; Schneider et al., 2021). Sterilization was controlled in our experiments by counting the number of kernels of plants that were bagged directly after the CHA treatment. Afterwards, plots in which the sterility did not work properly were removed from the analyses. By doing this, all genotypes were retained in the dataset across environments, and only for 3% of genotypes data had to be discarded for both replications in particular environments. This last observation suggests environment- and genotype-specific effects for the effectivity of sterilization. In addition, molecules with reduced phytotoxicity can still produce some level of plant damage/yield penalty for all plants in general or particularly for certain more susceptible genotypes. We further computed genotype-specific absolute and relative yield penalties by either only centring or centring and scaling, respectively, the BLUEs of hybrid seed yield with those of per se yield. Absolute yield penalties were slightly correlated with per se yield ($r = -.27$, P-value <.05; Figure S8), while no significant association was observed for relative yield penalties ($r = -.14$, P-value ≥.05; Figure S9). These results suggest that the negative effects of CHAs like Croisor® 100 are only slightly proportional to the per se yield level of the tested plant material. Although further confounding effects of CHAs cannot be fully discarded from our experiments, the estimation of their real magnitude lies out of the scope of our current work. In practice, hybrid wheat breeding programmes that rely on gametocides for hybrid seed production select female lines that show a highly repeatable sterilization response to the CHA treatment accompanied with high hybrid seed yield values (Easterly et al., 2019).

4.4 | Larger populations are needed to implement genomics-assisted breeding of hybrid seed yield of female plants

As far as we know, association/genetic mapping studies assessing the genetic architecture of potential female indicator traits for hybrid seed yield are scarce. A recent GWAS study investigated for the very first time the genetic architecture of potential indicator traits for female hybrid seed yield (El Hanafi et al., 2021). In their study, the authors reported 11 and three significant marker–trait associations for the indicator traits flower openness and the duration of floret opening, respectively. To the best of our knowledge, our work is the first study directly assessing the quantitative genetic architecture of the female hybrid seed yield. For GWAS, we computed the significance threshold to correct for multiple testing according to Gao et al. (2008), which offers a balance between detection power and false positives as compared to more liberal methods like false discovery rate (Benjamini & Hochberg, 1995) or highly conservative ones such as the classical Bonferroni correction. Moreover, the positive GWAS results for traits PH, FT begin, FT end and FT dur (Figures S4–S7), which are known for their relatively less complex genetic architecture (Ellis et al., 2005; Luján Basile et al., 2019; Pérez-Gianmarco et al., 2020; Royo et al., 2018; Zhang et al., 2022), further support the suitability of the chosen significant threshold to detect major QTL.

However, phenotypic variation influenced by numerous QTL, each of them with small effects, is more challenging to be reduced to the genetic level than variation that results from a small number of large-effect QTL (Myles et al., 2009). We attribute, therefore the lack of major significant QTL (Figure 3) to an underpowered population used for GWAS of female hybrid seed yield and to a hitherto polygenic genetic architecture underlying this particular trait. This is also consistent with the conclusions of Liu et al. (2013), who emphasize that smaller mapping populations with a high heritability for the trait of interest can nevertheless have a low QTL detection performance. In this regard, despite the relatively high heritability of .77 and the practically null correlation between phenotypic and genomic similarities (Figure 2)—which is expected to maximize power in GWAS (Myles et al., 2009)—could not counterbalance the limited population size of 100 genotypes for QTL detection of hybrid seed yield. Larger populations would be needed not only to disentangle the complex genetic architecture of the female hybrid seed yield but also to train genomic prediction models and evaluate the prospects of genomics-assisted selection by means of cross-validations (Jannink et al., 2010). Once mapping or training populations are established, significant markers and reliable genomic predictions could be used to routinely screen thousands of non-phenotyped but genotyped candidates in the long term and reduce therefore population sizes for later stages of breeding programmes.

AUTHOR CONTRIBUTIONS

Jochen C. Reif developed the concept of the study. Valentin Hinterberger designed the field experiments. Monika Spiller, Thierry Moittié, Mario Gils and Markus Wolf conducted the field experiments. Johannes Schneider performed statistical analyses with the support of Albert W. Schulthess and Valentin Hinterberger. Johannes Schneider and Albert W. Schulthess wrote the manuscript with the input of all other authors.

ACKNOWLEDGEMENTS

The study was performed under the frame of the Hyflor project financed by the Federal Ministry of Food and Agriculture (grant number FKZ2818401A18). A.W.S. was supported by the Federal Ministry of Education and Research through the GeneBank2.0 project (grant number FKZ031B0184B). Costs for open access publishing were partially funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, grant number 491250510). We would like to kindly thank Katja Cordes for their excellent technical support during the conduction of the field experiments. We are also very thankful to Dr Anne Fiebig, who provided additional insights on the obtention of physical positions of SNPs. We finally thank Prof Dr Thorsten Schnurbusch and Constanze Schmidt for the helpful discussion. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Phenotypic performances of the studied plant material estimated across experiments are included as supplementary material in the Excel sheet 'BLUEs_accross_experiments.xlsx' (Data S1). Molecular marker profiles were deposited into the e!DAL-PGP repository (Arend et al., 2016) and can be accessed here: https://doi.org/10.5447/ipk/2023/14. All original contributions of this work are included either in the manuscript or as supplementary material. Further inquiries can be directed to the authors.

ORCID

Johannes Schneider https://orcid.org/0000-0002-6623-3654
Valentin Hinterberger https://orcid.org/0000-0001-5515-7636
Monika Spiller https://orcid.org/0000-0002-2180-5380
Jochen C. Reif https://orcid.org/0000-0002-6742-265X
Albert W. Schulthess https://orcid.org/0000-0002-9365-0939

REFERENCES

Anscombe, F. J., & Tukey, J. W. (1963). The examination and analysis of residuals. *Technometrics*, *5*, 141–160. https://doi.org/10.1080/00401706.1963.10490071

Arend, D., Junker, A., Scholz, U., Schüler, D., Wylie, J., & Lange, M. (2016). PGP repository: A plant phenomics and genomics data publication infrastructure. *Database*, *2016*, baw033. https://doi.org/10.1093/database/baw033

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, *57*, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Boeven, P. H. G., Longin, C. F. H., Leiser, W. L., Kollers, S., Ebmeyer, E., & Würschum, T. (2016). Genetic architecture of male floral traits required for hybrid wheat breeding. *Theoretical and Applied Genetics*, *129*, 2343–2357. https://doi.org/10.1007/s00122-016-2771-6

Boeven, P. H. G., Würschum, T., Rudloff, J., Ebmeyer, E., & Longin, C. F. H. (2018). Hybrid seed set in wheat is a complex trait but can be improved indirectly by selection for male floral traits. *Euphytica*, *214*, 110. https://doi.org/10.1007/s10681-018-2188-1

Burdon, R. D. (1977). Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genetica*, *26*, 168–175.

Butler, D. G., Cullis, B. R., Gogel, B. J., Thompson, R. (2018). ASReml-R reference-Manual V4. Available at: https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-R-Reference-Manual-4.pdf

De Storme, N., & Geelen, D. (2014). The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant, Cell & Environment*, *37*, 1–18. https://doi.org/10.1111/pce.12142

Easterly, A. C., Stroup, W. W., Garst, N., Belamkar, V., Sarazin, J. B., Moittié, T., Ibrahim, A. M. H., Rudd, J. C., Souza, E., & Baenziger, P. S. (2019). Determining the efficacy of a hybridizing agent in wheat (*Triticum aestivum* L.). *Scientific Reports*, *9*, 20173. https://doi.org/10.1038/s41598-019-56664-9

El Hanafi, S., Bendaou, N., Kehel, Z., Sanchez-Garcia, M., & Tadesse, W. (2020). Phenotypic evaluation of elite spring bread wheat genotypes for hybrid potential traits. *Euphytica*, *216*, 168. https://doi.org/10.1007/s10681-020-02695-7

El Hanafi, S., Cherkaoui, S., Kehel, Z., Al-Abdallat, A., & Tadesse, W. (2021). Genome-wide association and prediction of male and female floral hybrid potential traits in elite spring bread wheat genotypes. *Plants (Basel)*, *10*, 895. https://doi.org/10.3390/plants10050895

El Hanafi, S., Cherkaoui, S., Kehel, Z., Sanchez-Garcia, M., Sarazin, J., Baenziger, S., & Tadesse, W. (2022). Hybrid seed set in relation with male floral traits, estimation of heterosis and combining abilities for yield and its components in wheat (*Triticum aestivum* L.). *Plants (Basel)*, *11*, 508. https://doi.org/10.3390/plants11040508

Ellis, M. H., Rebetzke, G. J., Azanza, F., Richards, R. A., & Spielmeyer, W. (2005). Molecular mapping of gibberellin-responsive dwarfing genes in bread wheat. *Theoretical and Applied Genetics*, *111*, 423–430. https://doi.org/10.1007/s00122-005-2008-6

Endelman, J. B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *Plant Genome*, *4*, 250–255. https://doi.org/10.3835/plantgenome2011.08.0024

Fábián, A., Sáfrán, E., Szabó-Eitel, G., Barnabás, B., & Jäger, K. (2019). Stigma functionality and fertility are reduced by heat and drought co-stress in wheat. *Frontiers in Plant Science*, *10*, 244. https://doi.org/10.3389/fpls.2019.00244

Farooq, A., Khan, U. M., Khan, M. A., Ali, Z., Maqbool, R., & Sajjad, M. (2023). Male sterility systems and their applications in hybrid wheat breeding. *Cereal Research Communications*. https://doi.org/10.1007/s42976-023-00376-4

Fróna, D., Szenderák, J., & Harangi-Rákos, M. (2019). The challenge of feeding the world. *Sustainability*, *11*, 5816. https://doi.org/10.3390/su11205816

Gao, X., Starmer, J., & Martin, E. R. (2008). A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genetic Epidemiology*, *32*, 361–369. https://doi.org/10.1002/gepi.20310

Gupta, P. K., Balyan, H. S., Gahlaut, V., Saripalli, G., Pal, B., Basnet, B. R., & Joshi, A. K. (2019). Hybrid wheat: Past, present and future. *Theoretical and Applied Genetics*, *132*, 2463–2483. https://doi.org/10.1007/s00122-019-03397-y

Imrie, B. C. (1966). Stigma receptivity in cytoplasmic male sterile wheat. *Australian Journal of Experimental Agriculture and Animal Husbandry*, *6*, 175–178. https://doi.org/10.1071/EA9660175

Jannink, J. L., Lorenz, A. J., & Iwata, H. (2010). Genomic selection in plant breeding: From theory to practice. *Briefings in Functional Genomics*, *9*, 166–177. https://doi.org/10.1093/bfgp/elq001

Langer, S. M., Longin, C. F. H., & Würschum, T. (2014). Phenotypic evaluation of floral and flowering traits with relevance for hybrid breeding in wheat (*Triticum aestivum* L.). *Plant Breeding*, *133*, 433–441. https://doi.org/10.1111/pbr.12192

Liu, G., Zhao, Y., Mirdita, V., & Reif, J. C. (2017). Efficient strategies to assess yield stability in winter wheat. *Theoretical and Applied Genetics*, *130*, 1587–1599. https://doi.org/10.1007/s00122-017-2912-6

Liu, W., Maurer, H. P., Melchinger, A. E., Utz, H. F., Tucker, M. R., Ranc, N., Della Porta, G., & Würschum, T. (2013). Optimum design of family structure and allocation of resources in association mapping with lines from multiple crosses. *Heredity*, *110*, 71–79. https://doi.org/10.1038/hdy.2012.63

Longin, C. F. H., Mühleisen, J., Maurer, H. P., Zhang, H., Gowda, M., & Reif, J. C. (2012). Hybrid breeding in autogamous cereals. *Theoretical and Applied Genetics*, *125*, 1087–1096. https://doi.org/10.1007/s00122-012-1967-7

Longin, C. F. H., Reif, J. C., & Würschum, T. (2014). Long-term perspective of hybrid versus line breeding in wheat based on quantitative genetic theory. *Theoretical and Applied Genetics*, *127*, 1635–1641. https://doi.org/10.1007/s00122-014-2325-8

Luján Basile, S. M., Ramírez, I. A., Crescente, J. M., Belén Conde, M., Demichelis, M., Abbate, P., Rogers, W. J., Pontaroli, A. C., Helguera, M., & Vanzetti, L. S. (2019). Haplotype block analysis of an Argentinean hexaploid wheat collection and GWAS for yield components and adaptation. *BMC Plant Biology*, *19*, 553. https://doi.org/10.1186/s12870-019-2015-4

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, *27*, 209–220.

Melchinger, A. E., Messmer, M. M., Lee, M., Woodman, W. L., & Lamkey, K. R. (1991). Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. *Crop Science*, *31*, 669–678. https://doi.org/10.2135/cropsci1991.0011183X003100030025x

Miedaner, T., & Juroszek, P. (2021). Climate change will influence disease resistance breeding in wheat in northwestern Europe. *Theoretical and Applied Genetics*, *134*, 1771–1785. https://doi.org/10.1007/s00122-021-03807-0

Millan-Blanquez, M., Hartley, M., Bird, N., Manes, Y., Uauy, C., & Boden, S. A. (2022). A scalable phenotyping approach for female floral organ development and senescence in the absence of pollination in wheat. *Development*, *149*, dev200889. https://doi.org/10.1242/dev.200889

Mühleisen, J., Piepho, H. P., Maurer, H. P., Longin, C. F. H., & Reif, J. C. (2014). Yield stability of hybrids versus lines in wheat, barley, and triticale. *Theoretical and Applied Genetics*, *127*, 309–316. https://doi.org/10.1007/s00122-013-2219-1

Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S. (2009). Association mapping: Critical considerations shift from genotyping to experimental design. *Plant Cell*, *21*, 2194–2202. https://doi.org/10.1105/tpc.109.068437

Okada, T., Jayasinghe, J. E. A. R. M., Nansamba, M., Baes, M., Warner, P., Kouidri, A., Correia, D., Nguyen, V., Whitford, R., & Baumann, U. (2018). Unfertilized ovary pushes wheat flower open for cross-pollination. *Journal of Experimental Botany*, *69*, 399–412. https://doi.org/10.1093/jxb/erx410

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Daniel Borcard, D., Gustavo Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2022). Vegan: Community ecology package. *R package version 2.6-4*. Vienna, Austria: R Foundation for Statistical Computing. Available at online: https://CRAN.R-project.org/package=vegan

Parodi, C., & de los Angeles Gaju, M. (2009). Male sterility induced by the chemical hybridizing agent clofencet on wheat, *Triticum aestivum* and *T. turgidum* var. *durum*. *Ciencia e Investigación Agraria*, *36*, 267–276. https://doi.org/10.4067/S0718-16202009000200011

Pérez-Gianmarco, T., Severini, A. D., & González, F. G. (2020). Photoperiod-sensitivity genes (*Ppd-1*): Quantifying their effect on the photoperiod model in wheat. *Journal of Experimental Botany*, *71*, 1185–1198. https://doi.org/10.1093/jxb/erz483

Pickett, A. A. (1993). *Hybrid wheat: Results and problems*. Paul Parey Scientific Publishers.

R Core Team. (2020). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Royo, C., Ammar, K., Alfaro, C., Dreisigacker, S., García del Moral, L. F., & Villegas, D. (2018). Effect of Ppd-1 photoperiod sensitivity genes on dry matter production and allocation in durum wheat. *Field Crops Research*, *221*, 358–367. https://doi.org/10.1016/j.fcr.2017.06.005

Sade, B., Ibrahim, A. M. H., Subramanian, N., Rudd, J., Liu, S., Opena, G., … Baenziger, S. (2022). Assessment of floral characteristics for hybrid wheat (*Triticum aestivum* L.) production in Texas. *Agrosystems, Geosciences & Environment*, *5*, e20228. https://doi.org/10.1002/agg2.20228

Sarkans, U., Gostev, M., Athar, A., Behrangi, E., Melnichuk, O., Ali, A., Minguet, J., Rada, J. C., Snow, C., Tikhonov, A., Brazma, A., & McEntyre, J. (2018). The BioStudies database-one stop shop for all data supporting a life sciences study. *Nucleic Acids Research*, *46*, D1266–D1270. https://doi.org/10.1093/nar/gkx965

Schneider, J., Berkner, M. O., Philipp, N., Schulthess, A. W., & Reif, J. C. (2021). Assessing the suitability of elite lines for hybrid seed production and as testers in wide crosses with wheat genetic resources. *Frontiers in Plant Science*, *12*, 689825. https://doi.org/10.3389/fpls.2021.689825

Schulthess, A. W., Kale, S. M., Zhao, Y., Gogna, A., Rembe, M., Phillip, N., Liu, F., Beukert, U., Serfling, A., Himmelbach, A., Oppermann, M., Weise, S., Boeven, P. H. G., Schacht, J., Longin, C. F. H., Kollers, S., Pfeiffer, N., Korzun, V., Fiebig, A., … Reif, J. C. (2022). Large-scale genotyping and phenotyping of a worldwide winter wheat genebank for its use in prebreeding. *Scientific Data*, *9*, 784. https://doi.org/10.1038/s41597-022-01891-5

Selva, C., Riboni, M., Baumann, U., Würschum, T., Whitford, R., & Tucker, M. R. (2020). Hybrid breeding in wheat: How shaping floral biology can offer new perspectives. *Functional Plant Biology*, *47*, 675–694. https://doi.org/10.1071/FP19372

Singh, S. K., Chatrath, R., & Mishra, B. (2010). Perspective of hybrid wheat research: A review. *Indian Journal of Agricultural Sciences*, *80*, 1013–1027.

Singh, S. K., & Joshi, A. K. (2003). Variability and character association for various floral characters in wheat [*Triticum aestivum* (L.) em Thell.]. *Indian Journal of Genetics and Plant Breeding*, *63*, 153–154.

Singh, S. P., Srivastava, R., & Kumar, J. (2015). Male sterility systems in wheat and opportunities for hybrid wheat development. *Acta Physiologiae Plantarum*, *37*, 1713. https://doi.org/10.1007/s11738-014-1713-7

Soleimani, B., Lehnert, H., Keilwagen, J., Plieske, J., Ordon, F., Naseri Rad, S., Ganal, M., Beier, S., & Perovic, D. (2020). Comparison between core set selection methods using different Illumina marker platforms: A case study of assessment of diversity in wheat. *Frontiers in Plant Science*, *11*, 1040. https://doi.org/10.3389/fpls.2020.01040

Stein, N., Herren, G., & Keller, B. (2001). A new DNA extraction method for high-throughput marker analysis in a large-genome species such as *Triticum aestivum*. *Plant Breeding*, *120*, 354–356. https://doi.org/10.1046/j.1439-0523.2001.00615.x

Stekhoven, D. J., & Bühlmann, P. (2012). MissForest-non-parametric missing value imputation for mixed-type data. *Bioinformatics*, *28*, 112–118. https://doi.org/10.1093/bioinformatics/btr597

Ter Steeg, E. M. S., Struik, P. C., Visser, R. G. F., & Lindhout, P. (2022). Crucial factors for the feasibility of commercial hybrid breeding in food crops. *Nature Plants*, *8*, 463–473. https://doi.org/10.1038/s41477-022-01142-w

Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, *327*, 818–822. https://doi.org/10.1126/science.1183700

The International Wheat Genome Sequencing Consortium [IWGSC]. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, *361*, eaar7191.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., … Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*, 787–796. https://doi.org/10.1111/pbi.12183

Whitford, R., Fleury, D., Reif, J. C., Garcia, M., Okada, T., Korzun, V., & Langridge, P. (2013). Hybrid breeding in wheat: Technologies to improve hybrid wheat seed production. *Journal of Experimental Botany*, *64*, 5411–5428. https://doi.org/10.1093/jxb/ert333

Wright, S. (1978). *Evolution and the genetics of populations* (Vol. 4 Variability Within and Among Natural Populations). University of Chicago Press.

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*, 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zhang, J., Islam, M. D. S., Zhao, Y., Anwar, M., Alhabbar, Z., She, M., Yang, R., Juhasz, A., Tang, G., Chen, J., Liu, H., Jiang, Y., Zhai, S., Hu, X., Rong, J., Zhang, Y., Qin, Y., Liu, Q., Yu, Z., … Ma, W. (2022). Non-escaping frost tolerant QTL linked genetic loci at reproductive stage in six wheat DH populations. *The Crop Journal*, *10*, 147–165. https://doi.org/10.1016/j.cj.2021.02.015

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Schneider, J., Hinterberger, V., Spiller, M., Moittié, T., Gils, M., Wolf, M., Reif, J. C., & Schulthess, A. W. (2024). First insights into the quantitative genetic composition of the female seed yield for an efficient hybrid seed production in wheat (*Triticum aestivum* L.). *Plant Breeding*, *143*(2), 131–141. https://doi.org/10.1111/pbr.13160