Genomic Selection for
Complex Traits
in
Bread Wheat Breeding

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Abstract

Modern plant breeding methods and technologies in combination with improved crop management practices enabled the great achievement of more than double the yield of major cereal crops during the last hundred years. However, the annual increase of grain yield in wheat and other crops feeding the world is currently insufficient at the given rate of population growth and projection show that factors like climate change, pests and diseases are hampering the goal of achieving another doubling of yield that is needed until 2050. Hence, the development and applications of new technologies seems necessary to accelerate the genetic improvement of crop plants, and with the advent of genomics two decades ago the usage of molecular markers became one promising tool to support breeders in achieving this aim. Numerous research studies have shown the large potential of using genome-wide distributed markers for estimating genomic breeding values and genomically selecting superior individuals in a breeding population in recent years, but most studies utilized cross-validation within restricted germplasm sets and research investigating genomic selection across multiple breeding cycles of years in applied plant breeding programs were lacking. However, one main objective when applying genomic selection in line breeding programs is the prediction of genotype performance in yet untested years in order choose the most promising among the potential varietal candidates for thoroughly testing in resource-demanding multi-environment trials. Hence, employing the example of a commercial winter wheat breeding program the aims of this study were (i) to assess the accuracy of genomic breeding values of non-phenotyped breeding lines to genomically select them for grain yield, protein content and protein yield across yet untested years, (ii) compare conventional phenotypic selection with various genomic selection approaches, and (iii) integrating phenotypic information from preliminary yield trials into the genomic selection framework for enhancing line breeding schemes in general.

For this purpose all analyses of this study focus on a multi-family breeding population of 861 genotyped lines that was phenotyped in multi-environment trials under South-Eastern European conditions from 2010 to 2015, whereas different subpopulations were tested in each year as is characteristically of a line breeding program. The dataset was complemented

by a large quantity of additional phenotypic information of grain yield and protein content obtained in single location preliminary yield trials. First, within-year 5-fold cross-validation was compared to across-year predictions for 2010-2014, which revealed a substantial bias of the prediction accuracy that was less pronounced for protein content and most for protein yield. Cross-validation using the years as folds aimed to avoid this bias and reached a maximum prediction accuracy of r = 0.51 for protein content, r = 0.38 for grain yield and r = 0.16 for protein yield indicating the suitability of genomic selection for practical applications in commercial wheat line breeding programs. Independent validation with 2015 resulted furthermore no advantage of removing outlier years or field trials from the model training data, suggesting that careful consideration is necessary before any such outlier correction is undertaken. Implementing genomic selection wheat in line breeding programs at hand is economically feasible in parallel to single location preliminary yield trials, thus a comparison between conventional phenotypic selection based on preliminary yield trials and genomic selection was required for assessing the actual merit of the latter. The prediction accuracy using only phenotypic data was rather low (r = 0.21) for grain yield but could be improved by modelling genetic relationships in unreplicated preliminary yield trials (r = 0.33). Genomic selection models were nevertheless found to be superior to conventional phenotypic selection for predicting grain yield performance of lines across years (r = 0.39). The problem of predicting untested lines in untested years to predicting tested lines was subsequently simplified to predicting untested years by combining breeding values from preliminary yield trials and predictions from genomic selection models by an heritability index. This genomic assisted selection led to a 20% increase in prediction accuracy, which could be further enhanced by an appropriate marker selection for both grain yield (r = 0.48) and protein content (r = 0.63).

Given this strong advantage of genomic selection over conventional phenotypic selection both for low and high heritable traits the former could support wheat breeders in developing varieties that preferably combine high yield, quality, disease resistance and tolerance against abiotic stresses. The tremendous decrease in genotyping costs in recent years allow furthermore implementing genomic selection in line breeding schemes in an economically feasible way even in medium sized breeding programs as revealed in this study. Although appropriate strategies for fully harnessing the benefits of genomic selection for both variety development and population improvement might differ between breeding programs, the exchange of datasets and genomic selection knowledge across both the public and private sector would contribute significantly to the enormous task of doubling yield in wheat and other crops until 2050.

Zusammenfassung

Moderne Methoden und Techniken der Pflanzenzüchtung haben es in Kombination mit verbesserten pflanzenbaulichen Maßnahmen ermöglicht, den Ertrag der wichtigsten Getreidekulturen in den letzten hundert Jahren zu verdoppeln. Der jährliche Ertragsfortschritt im Weizen und in anderen weltweit angebauten Kulturen ist derzeit jedoch nicht in der Lage, mit dem weltweiten Populationswachstum und entsprechendem Bedarf Schritt zu halten, wobei Prognosen zeigen, dass Einflüsse wie Klimawandel, Schädlinge und Pflanzenkrankheiten der erneuten und notwendigen Verdoppelung des Ertrags bis 2050 im Wege stehen. Die Entwicklung und Anwendung neuer Technologien die das Potential haben den Zuchtfortschritt unserer Kulturpflanzen zu beschleunigen, wird somit immer notwendiger. Mit dem Aufschwung genomischer Methoden vor zwei Jahrzehnten versprach die Anwendung molekularer Marker ein wichtiges Werkzeug der Pflanzenzüchtung zu werden, um dieses Ziel zu erreichen. Zahlreiche Forschungsstudien haben das große Potential von genomweit verteilten Markern zur Schätzung genomischer Zuchtwerte aufgezeigt, welche genutzt werden können, um vielversprechende Zuchtstämme frühzeitig zu selektieren. Die Ergebnisse der meisten Studien basieren jedoch auf Kreuzvalidierungen mit eingeschränkter Genetik in wenigen Jahren, wohingegen Forschungsarbeiten zu genomischer Selektion in angewandten Züchtungsprogrammen über mehrere Züchtungszyklen und Jahre hinweg bisher fehlten. Eines der Hauptziele bei der Anwendung genomischer Selektion in der Züchtung von Liniensorten bei Weizen und anderen Kulturen ist jedoch die Vorhersage der Leistung über Jahre hinweg, um die Selektion der vielversprechendsten Sortenkandidaten für aufwendige mehrortigen Versuchsserien zu ermöglichen. Diese Punkte sollen am Beispiel eines Winterweizenzuchtprogramm in der vorliegenden Studie stärkere Beachtung finden wobei zunächst darauf abgezielt wurde (i) die Genauigkeit genomischer Zuchtwerte für Kornertrag, Proteingehalt und Proteinertrag über mehrere Jahre hinweg zu schätzen, (ii) daraufhin das herkömmliche phänotypische Selektionsverfahren mit genomischer Selektion zu vergleichen und schließlich (iii) die phänotypische Information von Ertragsvorversuchen in die Methodik der genomischen Selektion zu integrieren, um einen Beitrag zur allgemeinen Verbesserung von Linienzuchtschemen zu leisten.

Zum Erreichen dieser Ziele wurden zunächst die phänotypischen Daten einer Zuchtpopulation von 861 genotypisierten Weizenzuchtstämmen analysiert, welche in mehrortigen Versuchen in Österreich und Süd-Ost Europa über den Zeitraum 2010 bis 2015 erhoben wurden. Verschiedene Subpopulationen unterschiedlicher Größe wurden dabei, wie in einem Weizenzuchtprogram üblich, in unterschiedlichen Jahren geprüft. Der Datensatz wurde letztendlich durch zusätzliche Informationen über Kornertrag und Proteingehalt aus einortigen nicht-wiederholten Ertragsvorversuchen ergänzt. Zunächst wurde die Schätzung der Vorhersagegenauigkeit einer 5-fache Kreuzvalidierung innerhalb eines Jahres mit der Vorsagegenauigkeit über Jahre hinweg verglichen, wobei die Verwendung des Ersteren Verfahrens, für alle Jahre von 2010 bis 2014, zu einer deutlichen Überschätzung führte, die für den Proteingehalt weniger und für den Proteinertrag sehr stark ausgeprägt war. Eine Kreuzvalidierung welche jeweils ein einzelnes Jahr bzw. Subpopulation auslässt, welches dann durch alle anderen Jahre vorhergesagt wird, zielte darauf ab diese Überschätzung zu vermeiden und ergab eine maximale Vorhersagegenauigkeit von r = 0,51 für der Proteingehalt, r = 0,38 für den Kornertrag und r = 0,16 für den Proteinertrag. Die Ergebnisse zeigten somit einiges Potential genomischer Selektion für die Linienzüchtung von Winterweizen, was durch eine unabhängige Validierung mit Daten aus 2015 bestätigt werden konnte. Das Korrigieren von Ausreißerjahren oder -feldversuchen ergab keinen Vorteil und zeigte, dass mit solche Korrekturmaßnahmen vorsichtig umzugehen ist. Der Einsatz genomischer Selektion in der Linienzüchtung von Weizen ist im Allgemeinen parallel zu Ertragsvorversuchen realisierbar, womit ein Vergleich zwischen herkömmlicher phänotypischer Selektion und genomischer Selektion nötig ist, um den tatsächlichen Wert der Letzteren im praktischen Zuchtprozess zu bestimmen. Der in dieser Studie durchgeführte Vergleich zeigte dabei eine eher niedrige Vorhersagegenauigkeit für den Kornertrag basierend auf phänotypischen Daten (r = 0,21), welche jedoch durch die Modellierung genetischer Verwandtschaftsverhältnisse in den nicht-wiederholten Ertragsvorversuchen verbessert werden konnte (r = 0,33). Die Vorhersage auf Basis genomischer Selektionsmodelle war dennoch, bei der Vorhersage der Ertragsleistung der Zuchtlinien über die Jahre, deutlich überlegen (r = 0,39). Das Problem der Vorhersage nicht-geprüfter Zuchtlinien in nicht-geprüften Jahren wurde schließlich, durch die Kombination von Zuchtwerten aus

Ertragsvorversuchen und genomischen Vorhersagen mit einen Heritabilitätsindex, zur Vorhersage geprüfter Zuchtlinien in nicht-geprüften Jahren vereinfacht. Die daraus resultierende genomisch gestützte Selektion führte letztlich zu einer Steigerung der Vorhersagegenauigkeit um 20%, welche durch eine entsprechende Markerselektion sowohl für den Kornertrag (r = 0,48) als auch den Proteingehalt (r = 0,63) noch weiter gesteigert werden konnte.

Auf Grundlage des enormen Vorteils der genomischen Selektion im Vergleich mit herkömmlicher phänotypischer Selektion hat die Erstere großes Potential Weizenzüchter bei der Sortenentwicklung zu unterstützen um Kornertrag, Qualität, Krankheitsresistenz und Stresstoleranz effektiver zu kombinieren. Die stetige Abnahme der Genotypisierungskosten in den letzten Jahren macht es zudem möglich genomische Selektion auch in mittelständischen Zuchtbetrieben, wie in der vorliegenden Studie, einzusetzen. Gleichwohl sich der optimale Einsatz von genomischer Selektion zwischen verschiedenen Zuchtprogrammen unterscheiden mag, würde ein entsprechende Wissensaustausch sowohl zwischen dem öffentlichen als auch privaten Sektor maßgeblich dazu beitragen das Ziel zu erreichen den Ertrag im Weizen und anderen Kulturarten bis 2050 zu verdoppeln.

Introduction

Selection through the years

Wheat (*Triticum aestivum* L.) originated from the hybridization between the wild species *Triticum turgidum* and *Aegilops tauschii* around 10,000 years ago (Salamini et al. 2002). While *T. turgidum* itself is a hybrid between *T.uratu* and *Ae.speltoides* that are the original sources of the A and B wheat genomes respectively (Marcussen et al. 2014), the hybridization events with *Ae.tauschii* provided the D genome and resulted in the allopolyploid nature of wheat we know today (Becker 2010). The domestication of wheat took place in the Middle Eastern region known as the Fertile Crescent around ten thousand years ago (Feuillet et al. 2008), where the early plant breeders were farmers who genetically improved their landrace cultivars mostly by phenotypic mass selection for desired traits like non-brittle rachis, non-shattering of seeds, free threshing, and increased grain size (Murhpy 2007). The selection for these domestication traits improved the value of cultivation and use of wheat and its allopolyploid nature provided furthermore a broad adaptation to various growing environments, yet it took several thousand years before the cultivation of wheat spread in the Mediterranean Sea, Western Europe and South-East Asia beginning the near unparalleled success story of this crop (Harlan 1981).

Wheat is grown on 2.5 Billion hectares worldwide nowadays and the grain yield average was more than doubled during the last hundred years to 3 t ha^{-1} (FAOSTAT 2017) by improved crop management practices in combination with modern plant breeding methods and technologies. The basis for this development was laid by the beginning of scientific plant breeding in the end of the 19th century when the introduction of new selection methods like pedigree or bulk breeding largely replaced traditional mass selection. These advances were stimulated and accompanied by a growing interest and knowledge base concerning the inheritance of traits in plants, animals and humans among which the rediscovery of the Mendelian rules (Mendel 1866) by Erich von Tschermak-Seysenegg, Carl Correns and Hugo de Vries in 1900 constituted a milestone for the scientific community. The biological basis of the Mendelian rules was deciphered soon afterwards by

Walter Sutton who discovered the meiotic behaviour of the chromosomes (Sutton 1903), and was pivotal for the working group of Thomas Hunt Morgan for creating the first genetic maps. This ground breaking basic research focused strongly on qualitatively inherited traits, while others complemented these studies and built the link to quantitatively inherited traits by demonstrating the additive gene action as well as the influence of the environment on such traits (Johannsen 1903; Nillson-Eihle 1909).

The mathematical description of inheritance promoted furthermore a rapid evolution of population and quantitative genetics theory and found also great resonance in practical applications in wheat breeding, together with biometric approaches for field trial testing of new potential varieties. These developments led finally the foundation for the Green Revolution beginning in the 1940s with the development of stem rust resistance and short-strawed high yielding wheat varieties, the promotion of high agrochemical input such as mineral fertilizers and plant protection agents. Although the Green Revolution had also negative impacts such as the reduction of biodiversity in farmers´ fields many people benefitted from the developments and it is commonly accepted that it most likely prevented a hunger crisis in South-East Asia (Becker 2010).

The doubling of wheat grain yield in the last hundred years showed the tremendous progress that can be achieved with classical phenotypic selection and can be attributed to the knowledge and experience of the many involved breeders world-wide. However, the annual increase of grain yield in wheat and other crops feeding the world is currently insufficient at the given rate of population growth and projection show that factors like climate change, pests and diseases are hampering the goal of achieving another doubling of yield needed until 2050 (Ray et al. 2013). Hence, the development and applications of new technologies seems necessary to accelerate the genetic improvement of crop plants, and with the advent of genomics two decades ago the usage of molecular markers became one promising tool in helping to cope with the large challenges that agriculture is facing in the 21st century.

QTL mapping and marker-assisted selection

The application of a molecular marker-assisted selection requires first of all the detection of marker-trait associations, which is classically done by linkage mapping employing one or several genotyped bi-parental populations. These populations usually consist of either of recombinant inbred or doubled haploids lines in self-compatible plant species like wheat, which have to be tested in multiple environments i.e. locations and years to obtain reliable phenotypic data. The obtained phenotypic records can subsequently be connected with the genotypic data using statistical genomic models such as simple or composite interval mapping (Zeng 1993; Zeng 1994)to identify molecular markers that are putatively linked with quantitative trait loci (QTL) influencing the respective trait of interest.

Nevertheless, creating such bi-parental mapping populations is often time-consuming and the number of individuals within each population is limited, which is in most cases hindering the precise mapping of QTL especially for traits with low or medium heritability (Moreau et al. 1998; Melchinger et al. 2004). Another option for finding marker-trait associations is thus the usage of genome-wide association mapping within a diverse panel of genotypes (Yu et al. 2006; Kang et al. 2008; Kang et al. 2010; Zhang et al. 2010). Genome-wide distributed markers are thereby tested individually for their significant association with one or multiple traits exploiting linkage disequilibrium between markers and the underlying QTL as well as ancient recombination within a target species (Würschum 2012). The latter often leads to a higher mapping resolution in comparison with linkage mapping, and the usage of a diversity panel with current breeding material may additionally allow the identification of marker-trait associations in germplasm that is more relevant for an immediate deployment in applied plant breeding programs.

Although being theoretically very appealing, both linkage and disequilibrium mapping will identify numerous false positive marker-trait associations especially the ones declared significant near the detection threshold. Common strategies for dealing with this problem are the introduction of stricter detection thresholds and accounting for family relationships and a putative population structure in the mapping population e.g. by extending mixed linear models used for genome-wide association mapping with an additional principal component effect (Kang et al. 2008; Stich et al. 2008). A major consideration for finding interesting marker-trait associations is thus an appropriate balance between the false positive and false negative rate, and the K-model that is modelling kinship between individuals within the mapping population via a genetic relationship matrix is generally recommended for achieving this purpose (Stich et al. 2008; Bernardo 2013). Aside from specific experimental considerations, it is not feasible to detect low frequency alleles with genome-wide association mapping, which though are of special interest to breeders, and many studies merely validated already known QTL (Bernardo 2016a). Linkage mapping with bi-parental populations has a higher chance to find such rare variants (Bernardo 2016a), while de novo found marker-trait associations should be validated in a different genetic background i.e. a validation population that is independent from the discovery population, before the markers can be deployed in a breeding program and used for a routine marker-assisted selection to select genotypes with the desired combination of alleles at several loci. Simulations showed furthermore a large benefit of integrating marker-assisted selection into breeding programs (Knapp 1998; Lande and Thompson 1998; Moreau et al. 1998), empirical studies showed however that previous reported QTL effects were strongly overestimated when markers were retested in larger validation populations and different genetic background (Melchinger et al. 2004).

Epistatic interactions between loci and marker by environment interaction might additionally reduce the potential of candidate markers for a routine implementation. Hence, there are few successful applications of marker-assisted selection strategies (Kuchel et al. 2007; Bernardo 2008) but they can have a large merit if a marker is associated with a major QTL for a trait of high agronomic value (Miedaner 2011). Prominent examples are markers linked with barley yellow mosaic virus resistance genes in European barley germplasm (Werner et al. 2005) or marker-assisted selection for fusarium head blight resistance conferred by the major QTL *Fhb1* in Northern American wheat breeding programs (Steiner et al. 2017) where this fungal disease has a very high economic impact. Screening for such major QTL in wheat might also include the *Ppd* photoperiodic sensitivity loci for flowering date (Beales et al. 2007), the *Rht* dwarfing genes for plant height (Knopf et al. 2008), and the *Glu* loci for baking quality (Payne 1987) though these are often undertaken only for genotypes that are used as crossing paretnts in breeding programs. The latter mentioned markers that are associated with the composition of high molecular weight subunit composition at the *Glu-1* and *Glu-3* loci might also serve for predicting laborious to phenotype baking quality traits, however complementing small-scale test are often necessary to achieve a high prediction accuracy (Oury et al. 2010).

The strategy of marker-assisted selection to target mostly single QTL or genes of high interest is especially valuable when breeding for qualitative inherited traits, most traits in wheat breeding are however quantitatively inherited and controlled by many QTL with minor to medium effects. Indirect phenotypic selection is thus more often applied in wheat breeding programs even when major QTL are known e.g. when selecting for baking quality in wheat by using the highly heritable protein content or sedimentation values instead of single marker assays, although the many underlying major QTL for gliadin and glutenin and associated markers are known since the early 1990s (Payne 1987; Rogers et al. 1989). An additional issue were high genotyping costs that hampered a broad application of a marker-assisted selection strategy useful in the past, but in recent years genotyping costs have strongly declined due to advances in next-generation sequencing technologies making it nowadays economically feasible to a genotype large number of individuals every year with a high marker density covering the entire genome (Elshire et al. 2011; Poland et al. 2012b; Heslot et al. 2013b).

A hitchhiker´s guide to genomic selection

The exploitation of genome-wide distributed markers for assessing breeding values of selection candidates has originally been suggested by Meuwissen et al. (2001), who concluded that the usage of such genomic breeding values could substantially accelerate the rate of genetic improvement in both plant and animal breeding. The basic methodology of such a genomic selection approach is relatively simple, which led to its tremendous success in animal breeding (Hayes et al. 2009; García-Ruiz et al. 2016), and in recent years numerous studies have investigated the merit of introducing this procedure also for a routine application in plant breeding programs (Heffner et al. 2011a; Windhausen et al. 2012; Crossa et al. 2014; Lehermeier et al. 2014).

Firstly, a training population of thoroughly phenotyped individuals must be genotyped with a large number of genome-wide distributed markers. This training population is subsequently used to fit prediction models employing the marker genotypes as explanatory variables (Heffner et al. 2009; Jannink et al. 2010). These prediction models can subsequently be used to estimate genomic breeding values for genotyped selection candidates of whom no phenotypic information about major agronomic traits like grain yield is available yet by summing across all their estimated marker effects (Jannink et al. 2010). The genomic breeding values can in this way support breeders in their selection decisions, by providing them in earlier phases of variety development with more information about their breeding material at hand. A critical issue is thereby the reliability of this additional information for each selection candidate (Clark et al. 2012) that is closely linked to the prediction accuracy (He et al. 2016b). The latter is generally measured by the correlation between the observed and predicted genotype performance; as the observed genotype performance can only be determined after selection in the next growing session many scientific studies as well as practical users simulate a genomic selection using different cross-validation schemes in order to test the prediction models before recommending them to breeders. Classically a larger part e.g. 80% of the genotypes in a given dataset are thereby sampled into a training population that is used to fit genomic selection models for predicting a validation population of left-out genotypes, of whom the available phenotypic data is masked. The prediction accuracy can in this way be estimated as the correlation between observed and predicted performance in the validation population, which might be divided by the square root of the heritability (Dekkers 2007) for measuring the proportion of maximal achieved prediction accuracy in comparison to conventional phenotypic selection (Bernal-Vasquez and Möhring 2014). The above-described algorithm is usually repeated several hundred times with varying training by validation population combinations and sometimes different sampling strategies in order to correct to confounding effects with genotype by environment interaction, population structure, and family relationships (Windhausen et al. 2012; Ly et al. 2013; Storlie and Charmet 2013; Albrecht et al. 2014; Lehermeier et al. 2014).

The two latter are a major driving force for obtaining a high prediction accuracy, of which an important portion can be attributed to family differences (Windhausen et al. 2012; Würschum et al. 2017), whereas relatedness among genotypes can even be more important than actual targeting marker-trait associations (Habier et al. 2013; He et al. 2016a). Early research that focused on increasing the prediction accuracy assumed on the other hand that the genome-wide distributed markers used for genomic selection are for a large part in linkage disequilibrium with minor to major QTL and numerous models were developed with varying assumptions about the underlying genetic architecture (Heslot et al. 2012). Bayesian models assumed e.g. unique variances for each marker and some extensions like the *BayesB* selected markers by assigning zero variance to them, which aimed to estimate effects of major QTL more accurate (Heffner et al. 2009). However, at least in wheat breeding scenarios the Bayesian models did not give an advantage over frequentistic approaches (Heffner et al. 2011a; Heffner et al. 2011b; Rutkoski et al. 2012; Liu et al. 2016; He et al. 2016b; Moore et al. 2017) like the ridge-regression best linear unbiased prediction (RR-BLUP) or its equivalent the genomic best linear unbiased prediction (G-BLUP) (VanRaden 2008; Piepho 2009).

Further possibilities for estimating genomic breeding values are the usage models with different weighting or selection of markers i.e. predictor variables like heteroscedastic ridge regression (Shen et al. 2013; Hofheinz and Frisch 2014) and *BayesC* (Pérez and de los Campos 2010), non-parametric models from the machine learning methodology (Ogutu et al. 2011; Ogutu et al. 2012), and kernel methods like the Reproducing Kernel Hilbert Space (RHKS) (de los Campos et al. 2010; Crossa et al. 2014). Although substantial effort was put into developing these models a comparison of different datasets from several studies revealed that all models show on average the same performance (Heslot et al. 2012), and advantages of specific models are often differences often depending on traits and dataset specific properties (Arruda et al. 2015; Tayeh et al. 2015; Battenfield et al. 2016; Spindel et al. 2016). Hence, the basic G-BLUP has gained the largest popularity as a computational fast and robust method for most practical applications of genomic selection, and quite some research focused on its extensions to further increase the prediction accuracy.

One of these possibilities that was especially interesting for product development in line breeding is the modelling of additive x additive epistatic effects by including an additional epistatic relationship matrix into the prediction model (Jiang and Reif 2015). This extended genomic best linear unbiased prediction (EG-BLUP) has shown some potential to improve the prediction accuracy for both disease resistance and grain yield compared to the baseline G-BLUP model (Jiang and Reif 2015; Mirdita et al. 2015; He et al. 2016a; He et al. 2016b). Using prior biological information by modelling known major QTL as fixed effects into prediction models has turned out to be another valuable option for improving the G-BLUP model (Bernardo 2014a; Zhao et al. 2014). The upweighting of specific markers in this weighted best linear unbiased prediction (W-BLUP) (Zhao et al. 2014) has shown some merit in several empirical studies (Arruda et al. 2016; Boeven et al. 2016; Losert et al. 2016; Juliana et al. 2017; Moore et al. 2017), verifying simulations that suggested an advantage of this method if the underlying QTL explained more than 10% of the genetic variance (Bernardo 2014a). The idea was extended by Spindel et al. (2016) who employed de novo found marker-trait association that were identified by GWAS in order to increase the prediction accuracy of highly heritable traits like flowering date in rice. However, significant marker-traits associations for low heritable traits like grain yield are often not repeatable across years even in large mapping populations (He et al. 2016a), and modelling them as fixed effects might thus introducing an error putatively decreasing the prediction accuracy (Michel et al. 2017a). Nevertheless, several major QTL that have already been validated in different genetic backgrounds underpinning important traits in wheat breeding can readily be targeted with single-marker assays (Rasheed et al. 2016), and model prediction accuracy might benefit by integrating them into the genomic selection framework thereby effectively using the experience and information gained in numerous QTL mapping studies during the last decade.

An important driving force in precision of these QTL mapping studies was the trait heritability, and in genomic selection this is likewise one of the major entry points that can be influenced by breeders for increasing the prediction accuracy (Lorenz 2013; Riedelsheimer and Melchinger 2013; Longin et al. 2014; Marulanda et al. 2016). Testing in a larger number of trial locations is thereby a more appropriate strategy for enhancing the data quality than increasing the number of replicates per location given the same available resources (Möhring et al. 2014), due to capturing a larger proportion of the genotype by environment interaction and thus a higher correlation between the observed and true breeding value of the selection candidates in such multi-environment trials. The data quality of individual trials could be improved by using a sophisticated trial design that enables corrections for spatial trends in the field and at the same time reducing the number of replicates to spare phenotyping costs and enable testing in multiple locations (Cullis et al. 2006; Williams et al. 2011) given an optimal resource allocation (Lorenz 2013; Riedelsheimer and Melchinger 2013; Kleinknecht et al. 2016). The possibility to correct for spatial trends by moving averages or spatial models with according variance-covariance structures can furthermore improve the data quality from individual trials (Leiser et al. 2012; Lado et al. 2013; Bernal-Vasquez and Möhring 2014) and for all subsequent downstream analysis, which is a major prerequisite for applying genomic selection where the choice of environments can markedly influence the prediction accuracy (Heslot et al. 2013a).

The size and the composition of the training population tested within these environments is yet another consideration that might influence the success of genomic selection in a breeding program (Neyhart et al. 2017). An increase in training population size is commonly accepted to increase the average and stability of the prediction accuracy (Lorenz et al. 2012; Lund et al. 2016; Nielsen et al. 2016), however different approaches were previously proposed for an optimal training population design among others the *CDmean* (Rincent et al. 2012) that aims to minimize the prediction error variance while retaining genetic diversity and an extension with stratified sampling taking the structure of the entire population of a breeding program into account (Isidro et al. 2015). One major aim of these criterion-based training population design strategies is the uniform coverage of the target genetic space (Bustos-Korts et al. 2016), while (Marulanda et al. 2015) showed that the main influence at least in bi-parental populations is the total phenotypic variance to capture the effects of all QTL segregating within such a population.

Given the methods for deriving high quality phenotypic and genotypic data as well as computational fast and robust tools for estimation genomic breeding values, genomic selection has shown great promise for accelerating the genetic improvement of crops within all variety categories (Schnell 1982) such as clones (Ly et al. 2013; Habyarimana et al. 2017), population cultivars (Annicchiarico et al. 2015; Grinberg Nastasiya et al. 2016), hybrids (Ziyomo and Bernardo 2013; Albrecht et al. 2014; Auinger et al. 2016; Philipp et al. 2016) and lines (Jarquín et al. 2014b; Sallam et al. 2015; Spindel et al. 2015; Lado et al. 2016; He et al. 2016b; Duhnen et al. 2017). A pivotal consideration for the actual implementation of genomic selection into a breeding program is its accuracy when predicting across several years and in different genetic backgrounds. Wheat breeders are e.g. interested in being informed about the future performance of their selection candidates, which is tough complicated by genotype by environment interaction and according changes in the ranking of genotypes across locations and years (Lynch and Walsh 1998). A convenient option for handling these issues are finding repeatable patterns of genotype by environment interaction and breed for specific adaptation to so-called mega-environments (Atlin et al. 2000; Annicchiarico et al. 2005; Piepho and Möhring 2005). Using genomic selection in this framework did however result in hardly any benefit in prediction accuracy (Dawson et al. 2013; Lado et al. 2016) as the effect of changing weather conditions across years is often predominant and hardly predictable. Several studies tried thus to handle genotype by environment interaction by using environmental covariates and crop growth models (Heslot et al. 2014; Jarquín et al. 2014a; Cooper et al. 2016), which revealed even for the difficult task to predict the performance of untested genotypes within yet untested environments a small advantage. The underlying environmental covariates have though be assessed by thoroughly environtyping and the underlying models are often computational demanding but can deliver valuable information concerning the product placement of newly developed varieties within fitting target regions.

A more simplistic approach regards genotype performance in different environments as different correlated traits (Lynch and Walsh 1998) by modelling genotype by environment interaction similar to a multivariate model including a genomic relationship matrix (Burgueño et al. 2007; Schulz-Streeck et al. 2013). Implicitly modelling marker by environment in this way did not reveal an appreciable increase in accuracy when predicting untested genotypes in untested environments (Schulz-Streeck et al. 2013; Lopez-Cruz et al. 2015) but showed a substantial merit for predicting the performance of genotypes for field trials in which they were not included (Lopez-Cruz et al. 2015; Crossa et al. 2016; Cuevas et al. 2016; Pérez-Rodríguez et al. 2017). Hence, the usage of prior information about line performance in different environments seems worthwhile and further extension could include vegetation indices obtained from high-throughput phenotyping (HTP) platforms. They might be promising to deliver secondary correlated traits if grain yield cannot be measured directly, as is the case in very early generations of line breeding schemes (Rutkoski et al. 2016). The usefulness of multivariate models with secondary traits generally depends on their heritability and genetic correlation with the target trait (Bauer and Léon 2008; Jia and Jannink 2012), and the additional information can strongly increase the prediction accuracy in cases where information of the secondary trait is already available (Jia and Jannink 2012) e.g. for predicting baking quality associated dough rheology in wheat, where high quality phenotypic data for the protein content is much earlier available in the variety development process than e.g. dough rheological parameters (Michel et al. 2017a). Summing up, the usage of additional information coming either from prior knowledge of trait genetic architecture, correlated secondary traits or environments have the potential to increase prediction accuracy in various genomic selection applications. The primary requirements for the resulting prediction model are easy implementation, robustness, and computational efficiency in order to cope with the large training and selection populations in applied plant breeding programs.

Implementing genomic selection into a line breeding program

The first choice that a breeder must make when implementing genomic selection in his or her breeding program is currently the usage of a classical chip-based system versus genotyping-by-sequencing (GBS) for genotyping the breeding population. The latter uses methylation sensitive enzymes for complexity reduction thereby increasing the efficiency of sequencing by eliminating repetitive genome regions (Elshire et al. 2011), and has been successfully applied in numerous plant species for identifying single nucleotide polymorphisms (SNPs) (Poland et al. 2012a; Poland et al. 2012b). A couple of years ago, the costs of genotyping with GBS were significantly lower than for a chip-based system, however the costs for the latter technology have meanwhile strongly declined especially for major crops like wheat. This makes chip-based systems nowadays a reasonable alternative for routine genotyping of the many selection candidates every year for conducting genomic selection. Furthermore, breeders might prefer such a ‘key-lock-system’ with a representative set of SNP markers, some of them with prior known linkage to major QTL for important agronomic traits. Nevertheless, the deployment of a GBS-based routine for genotyping is still cheaper and it does have a smaller ascertainment bias than chips, where SNP markers with intermediate allele frequency will be overrepresented (Heslot et al. 2013b). While obtaining many genome-wide distributed markers that are rather a random sample of polymorphism in a breeding population (Elshire et al. 2011; Heslot et al. 2013b) the derived markers matrix contains usually lot of missing data points warranting further imputation (Huang et al. 2014).

Once a decision concerning the marker system is reached a breeder has to determine at which stage in his or her breeding program genomic selection should be implemented. Line breeding programs that are based on doubled haploids (DH) e.g. many barley breeding programs in Western Europe could genotype a broad population shortly after DH production and pre-select the most promising lines with regard to grain yield and quality for seed multiplication and subsequent yield trials by genomic selection (Longin et al. 2014). The pedigree method is on the other hand more prominent for breeding durum and bread wheat cultivars and genotyping is more likely to be conducted with more homozygous breeding lines after several generations of head row selection. Accordingly, the main application of genomic selection in line breeding is until now to support breeders in their decisions which subpopulation of lines from their selection candidates are advanced for further testing in multi-environment trials (Heffner et al. 2010; Spindel et al. 2015; Guzmán et al. 2016; Marulanda et al. 2016). This decision is of foremost importance in the development of new line varieties as multi-environment trials are costly and resource-demanding and merely the best selection candidates should preferably enter this testing stage. Genomic selection is for this reason currently often implemented in parallel to preliminary yield trials in line breeding programs. Hence, breeders might consider the replacement of traditional preliminary yield trials by genomic selection to spare phenotyping costs or even choose to integrate these trials into the genomic selection framework, as they deliver a first insight into the future performance of the putative varietal candidates (Endelman et al. 2014). Employing the example of an applied winter wheat breeding program the aims of this study were thus (i) to assess the accuracy of genomic breeding values of non-phenotyped breeding lines to genomically select them for grain yield, protein content and protein yield across yet untested years, (ii) compare conventional phenotypic selection with various genomic selection approaches, and (iii) integrating phenotypic information from preliminary yield trials into the genomic selection framework for enhancing line breeding schemes in general.

Genomic Selection across Multiple Breeding Cycles in Applied Bread Wheat Breeding

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ORIGINAL ARTICLE

Genomic selection across multiple breeding cycles in applied bread wheat breeding

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Abstract

Key message **We evaluated genomic selection across five breeding cycles of bread wheat breeding. Bias of within-cycle cross-validation and methods for improving the prediction accuracy were assessed.**

Abstract The prospect of genomic selection has been frequently shown by cross-validation studies using the same genetic material across multiple environments, but studies investigating genomic selection across multiple breeding cycles in applied bread wheat breeding are lacking. We estimated the prediction accuracy of grain yield, protein content and protein yield of 659 inbred lines across five independent breeding cycles and assessed the bias of within-cycle cross-validation. We investigated the influence of outliers on the prediction accuracy and predicted protein yield by its components traits. A high average heritability was estimated for protein content, followed by grain yield and protein yield. The bias of the prediction accuracy using populations from individual cycles using fivefold cross-validation was accordingly substantial for protein yield (17–712 %) and less pronounced for protein content (8–86 %). Cross-validation using the cycles as folds aimed to avoid this bias and reached a maximum prediction accuracy of $r_{GS} = 0.51$ for protein content, $r_{GS} = 0.38$ for grain yield and $r_{GS} = 0.16$ for protein yield. Dropping outlier cycles increased the prediction accuracy of grain yield to $r_{GS} = 0.41$ as estimated by cross-validation, while dropping outlier environments did not have a significant effect on the prediction accuracy. Independent validation suggests, on the other hand, that careful consideration is necessary before an outlier correction is undertaken, which removes lines from the training population. Predicting protein yield by multiplying genomic estimated breeding values of grain yield and protein content raised the prediction accuracy to $r_{GS} = 0.19$ for this derived trait.

Introduction

Originally suggested by Meuwissen et al. (2001), genomic selection showed great promise to strongly increase the rate of genetic improvement in both animal and plant breeding programs. This new method allowed a comparative larger gain from selection by estimating all marker effects simultaneously and subsequent selection of genetically superior individuals based on their genomic estimated breeding value (GEBV) (Bernardo and Yu 2007; Piyasatian

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et al. 2007), instead of using a few significant markers as in classical marker-assisted selection (Lande and Thompson 1990). Genomic selection was readily integrated into applied animal breeding programs due to a high accuracy of breeding values and a previously existing similar system, which employed pedigree instead of marker information (VanRaden 2008; Hayes et al. 2009). Notwithstanding, the relative simple methodology made genomic selection also interesting for applied plant breeding: a training population of phenotyped and genotyped individuals is utilized to establish a statistical model that predicts breeding values of non-phenotyped individuals from a selection or validation population by their genomic fingerprints (Heffner et al. 2009; Jannink et al. 2010).

Although methodologically simple the sparse knowledge about its functionality made it initially difficult to find starting points for increasing the prediction accuracy. Theoretical studies thus laid the foundation for optimizing breeding with genomic selection by trying to understand the underlying mechanics of this ‘green box’ approach. The driving forces of prediction accuracy that can be most readily influenced by plant breeders are the training population size and heritability (Muir 2007; Hayes et al. 2009), by adequately adjusting the resource allocation (Riedelsheimer and Melchinger 2013; Longin et al. 2015). Recent advances in sequencing technologies made it possible to apply cost effective genotyping methods such as genotyping-by-sequencing (GBS) in various crop species (Elshire et al. 2011; Poland et al. 2012; Huang et al. 2014) yielding an appropriate large number of markers for genomic selection (Hayes et al. 2009; Schulz-Streeck et al. 2011). The use of dense genome-wide markers increases the chance of markers being in linkage disequilibrium (LD) with QTL influencing the trait of interest (e.g. Meuwissen et al. 2001), and determines to some extent how well genetic relationship and genetic architecture are captured by the genomic selection model (Daetwyler et al. 2010; Heslot et al. 2013a). The importance of a close genetic relationship between training and validation populations to achieve a high prediction accuracy (Habier et al. 2013) has been verified numerous times in plant breeding studies, e.g. with sugar beet (Würschum et al. 2013); rapeseed (Würschum et al. 2014), maize (Zhao et al. 2012; Riedelsheimer et al. 2013; Albrecht et al. 2014; Lehermeier et al. 2014), and wheat (Charmet et al. 2014; Crossa et al. 2014), which motivated investigations for an optimal training population construction to reduce phenotyping costs (Rincent et al. 2012; Isidro et al. 2015).

Summing up, valuable insights into genomic selection have been gained in relative short time opening up numerous possibilities for its implementation into the framework of plant breeding (Heslot et al. 2015). Notwithstanding, most studies were conducted with the same genetic material across multiple environments or made use of historical phenotypic data (Dawson et al. 2013; Storlie and Charmet 2013; Ly et al. 2013; Rutkoski et al. 2015), while few have focused on the problem of predicting across breeding cycles in applied plant breeding programs. This issue was addressed for the first time in sugar beet breeding, where genomic selection showed great promise across two subsequent breeding cycles especially for highly heritable traits (Hofheinz et al. 2012). A population of parental lines was employed to predict GEBVs for five successive years in a recent study with six-row barley by Sallam et al. (2015), who reported varying degrees of prediction accuracy depending both on the progeny set and trait. We are not aware of any studies investigating genomic selection across multiple breeding cycles in applied bread wheat breeding; thus the objectives of this study were (1) to estimate the accuracy when predicting grain yield, protein content and protein yield of wheat inbred lines across multiple independent breeding cycles; (2) compare within-cycle and between-cycle prediction accuracy obtained from different cross-validation schemes; and (3) investigate model independent possibilities to increase the prediction accuracy.

Materials and methods

Plant material and phenotypic data

We analyzed five breeding cycles from a commercial winter wheat (*Triticum aestivum* L.) breeding program, where breeding cycles correspond to the different starting years. A total of 659 genotyped lines from multiple families, either $F_{4:6}$ or directly derived by the double haploid method, were tested in multi-environment trials from 2010 to 2014. A balanced subset of trial locations was selected for evaluating the merit of genomic selection across multiple breeding cycles. Within each breeding cycles a different set of 64–176 lines was tested orthogonally across all trial locations. Grain yield (dt ha^{-1}), protein content (%) and protein yield (dt ha^{-1}) were assessed in 2–8 trials per breeding cycles. Protein content was determined by near infrared spectroscopy (NIRS) directly at harvest and protein yield was derived by multiplication of grain yield and protein content on a plot basis. An additional independent set of 178 genotyped lines from the same breeding population was tested in 2015 employing the same phenotyping procedure as in 2010–2014. Trial locations spanned from Austria over Serbia, Croatia, Hungary, and Romania to the Central Anatolian High Plateau in Turkey, thus covering a large diversity of target environments. Trials were designed according to the standard procedure in plant breeding, where non-replicated earlier generation lines are tested along with replicated checks, which allowed correcting for spatial field trends and estimation of error variances.

Phenotypic analysis

We followed a two-stage analysis strategy of the phenotypic data, where each individual trial, i.e. location by breeding cycle combination was analyzed separately in the first stage. A baseline model without correction for spatial trend was compared by Akaike's Information Criterion (AIC) with models correcting for row and/or column effects, if feasible an autoregressive variance–covariance structure of the residuals was additionally integrated (Burgueno et al. 2000). The model with the smallest AIC was chosen to derive best linear unbiased estimates (BLUE) for each trial. The heritability was computed as suggested by Piepho and Möhring (2007) by $h^2 = \sigma_{\mathrm{G}}^2/(\sigma_{\mathrm{G}}^2 + \frac{1}{2}\mathrm{MVD})$, where σ_{G}^2 designates the genetic variance and MVD the mean variance of a difference of the BLUEs. The analysis at the first stage contained both checks and genotyped lines.

We retained only trials with a heritability larger than 0.3 and genotyped lines for the analysis across trials at the second stage. A linear mixed model of the form

$$y_{\mathrm{ij}} = \mu + g_{\mathrm{i}} + t_{\mathrm{j}} + gt_{\mathrm{ij}} + e \tag{1}$$

was fitted for all traits, where y_{ij} are the BLUEs from the first stage, μ is the grand mean, and g_{i} is the effect of the ith inbred line. The effect of the *j*th trial t_{j} was fixed, while the line by trial interaction effect gt_{ij} was random. The inverse of the squared standard errors of the means derived from the first stage of analysis were used as weights in this stage to take the varying accuracy of phenotypic records into account (Möhring and Piepho 2009). The residual variance was fixed to 1 for grain yield and 0.1 for protein content and protein yield, which allowed the separation of residual and line by trial interaction variances. Heritability estimates across trials were computed using the standard formula $h^2 = \sigma_{\mathrm{G}}^2/(\sigma_{\mathrm{G}}^2 + t^{-1}\sigma_{\mathrm{GT}}^2)$, dividing the line by trial interaction variance σ_{GT}^2 by the number of trials t. All phenotypic analyses were conducted using the statistical package ASReml 3 (VSN International 2015) for the R programming environment (R development core team 2015).

Genotypic data

Leaves for DNA extraction were sampled from $F_{4:5}$ plants in small observation plots after phenotypic pre-selection during early summer. DNA was extracted following the protocol by Saghai-Maroof et al. (1984). All 659 lines were genotyped with approximately 20 K SNP markers using the DarT genotyping-by-sequencing (GBS) approach (Diversity Array Technologies, 2015). Quality control was applied by filtering out markers with a call rate lower than 90 %, a minor allele frequency smaller than 0.05, and more than 10 % of missing data. Missing data of the remaining 9.5K SNP markers was imputed by the MVN-EM algorithm by Poland et al. (2012) which was specially designed for the use of GBS markers.

Genomic selection and genetic relationship

Genomic predictions of inbred lines were estimated using a ridge regression best linear unbiased prediction (RR-BLUP) model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e} \tag{2}$$

where $\mathbf{y}$ is an $N \times 1$ vector of BLUEs obtained in the phenotypic analysis, $\mathbf{b}$ is a vector of F fixed effects and $\mathbf{X}$ its corresponding $N \times F$ design matrix. $\mathbf{Z}$ is a $N \times M$ matrix, which coded the M markers as either $+1$ or -1 for homozygous loci and 0 for heterozygous loci. Random marker effects were assumed to follow a normal distribution $\mathbf{u} \sim N(0, \mathbf{I}\sigma_{\mathrm{u}}^2)$ and equally shrunken towards zero given the penalty parameter $\lambda^2 = \sigma_{\mathrm{e}}^2/\sigma_{\mathrm{u}}^2$ where σ_{e}^2 is the variance of the residuals which followed $\mathbf{e} \sim N(0, \mathbf{I}\sigma_{\mathrm{e}}^2)$. The kinship between lines was estimated by the genomic relationship matrix, which was computed according to Endelman and Jannick (2012):

$$\mathbf{K} = \mathbf{W}\mathbf{W}^{\mathrm{T}}/2\Sigma(p_{\mathrm{k}} - 1)p_{\mathrm{k}} \tag{3}$$

where $\mathbf{W}$ is a centered $N \times M$ marker matrix of the i lines with $W_{ik} = Z_{ik} - 2p_k$ and p_{k} being the allele frequency at the *k*th locus. The derived variance–covariance matrix was used to fit a genomic best linear unbiased prediction (G-BLUP) model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{g} + \mathbf{e} \tag{4}$$

where $\mathbf{g}$ is an $N \times 1$ vector of genotypic effects with $\mathbf{g} \sim N(0, \mathbf{K}\sigma_{\mathrm{G}}^2)$. Model (4) has been shown to be equivalent to (2) (VanRaden 2008; Piepho 2009) and allowed estimating the accuracy of each individual line by $r_{\mathrm{PEV}} = \sqrt{1 - (\mathrm{PEV}/\mathbf{G}_{\mathrm{ii}}\sigma_{\mathrm{G}}^2)}$ where PEV is the prediction error variance, σ_{G}^2 the genetic variance explained by the model and $\mathbf{G}_{\mathrm{ii}}$ is the diagonal element of the genomic relationship matrix for each line *i* (Clark et al. 2012). All models for genomic selection were implemented with the R package rrBLUP (Endelman 2011).

Validation and accuracy

At first we estimated the correlation between the accuracy of each individual line r_{PEV} and the genetic relationship to investigate this important driving force of prediction accuracy across several cycles of wheat breeding. The average genetic relationship of the most related lines from the training population was computed for each line in the validation population and correlated with r_{PEV}. The number of most related lines was varied between 1 and 500, and one breeding cycle was left out at a time using all other breeding cycles as training population. A fixed year effect was included into model (4) to account for the different yield levels of the studied breeding cycles. Prediction accuracy is generally defined as the Pearson correlation between predicted and true breeding values $r_{\mathrm{MT}} = r_{\mathrm{GEBV,TBV}}$. The true breeding values were unknown in our study; so we estimated prediction accuracy as the correlation between predicted and observed line performance $r_{\mathrm{GS}} = r_{\mathrm{GEBV,BLUE}}$. Across-cycle prediction accuracy was subsequently assessed by computing marker effect estimates with the RR-BLUP model of all possible pair-wise training and validation population combinations of the five breeding cycles. Three cross-validation schemes each with fivefolds and 100 replicates were employed to cover different aspects of the prediction accuracy r_{GS}:

1. Within-cycle prediction accuracy was computed by randomly dividing the data into equally sized folds using 80 % of lines within each breeding cycle as a training population and subsequent prediction of the left-out fold. This procedure was repeated for every fold and the resulting prediction accuracy was averaged for each of the 100 replicates.
2. The same training populations as in the within-cycle cross-validation were used to separately predict lines of each other breeding cycle. The average prediction accuracy was saved and utilized to estimate the bias of within-cycle versus between-cycle cross-validation.
3. Fivefold cross-validation, where the breeding cycles constituted the folds, was used to estimate the prediction accuracy across cycles. An equal number of lines were randomly sampled from each breeding cycle, simulating a breeding scenario where training populations for genomic selection models are an assembly of several mixed populations from multiple breeding cycles. Training population sizes varied between 16 and 256 lines. An additional fixed year effect was added to model (2) in order to account for the different yield and protein levels in 2010 to 2014.

Furthermore we studied two possibilities for increasing the prediction accuracy across breeding cycles. First outliers were identified by approximating the genetic correlation among environments by their pair-wise prediction accuracies (Heslot et al. 2013b), and breeding cycles or trials with a strongly deviating character were dropped from the training population. The influence of these outliers was subsequently investigated by comparing the prediction accuracy with the full and outlier corrected dataset, using the same across-cycle cross-validation approach as before. Training population size was kept constant by randomly choosing additional lines from each remaining breeding cycle in the outlier corrected cross-validation. In addition to cross-validation, the 178 lines from 2015 served as an independent validation population and were predicted by estimated marker effects using either the full or outlier corrected dataset.

Finally we investigated the possibility to increase the prediction accuracy of the derived trait protein yield by multiplying GEBVs of its component traits grain yield and protein yield. The prediction accuracy was estimated by the above described across-cycle cross-validation approach with 100 replicates for each training population size.

Results

Quantitative-genetic parameters

The plant material was tested in a broad spectrum of environments ranging from the Pannonian Basin to the Central Anatolian High Plateau. Despite the expected large genotype by environments interaction we observed a medium to high heritability in each individual breeding cycle for grain yield and protein content (Table 1). A relatively large number of trials having at least a heritability larger than 0.3 were pre-selected for this study to achieve valid and robust results. The excellent data quality was also reflected by the medium to high heritability for protein yield in all but one breeding cycle. Estimates of heritability were lower for protein yield than grain yield except for 2013, where it was 26 % larger. The protein content had on average the highest heritability followed by grain yield and protein yield.

Genetic relationship and prediction accuracy of genomic selection

The correlation between accuracy of each individual line and the genetic relationship was strongly dependent on the number of most related lines and the respective validation population (Fig. S1). Optimal correlations for grain yield were achieved using the 11–133 most related lines, while choosing the 70 most related lines led to significant correlations larger than $r = 0.80$ for all validation populations. Similar patterns were observed for protein content and protein yield. The average of the top 70 genetic relationship between lines range from 0.08 to 0.14 within the years, and was smaller between years with an overall average genetic correlation of 0.07 (Fig. S2).

Within-cycle prediction accuracy was compared to between-cycle prediction accuracy by fivefold cross-validation utilizing the same training populations for each

Table 1 Mean, variance components and heritability for grain yield (dt ha^{-1}), protein content (%) and protein yield (dt ha^{-1}) of genotyped lines across all trials in the respective breeding cycles 2010–2014

Trait	Parameter	Breeding cycles				
		2010	2011	2012	2013	2014
Grain yield	Trials	5	6	4	5	8
	σ^2_{G}	2.28 ± 1.28	4.60 ± 1.60	5.03 ± 1.25	6.64 ± 1.76	37.00 ± 4.71
	σ^2_{GT}	23.70 ± 1.83	23.67 ± 1.99	17.80 ± 1.21	40.98 ± 2.36	54.48 ± 2.26
	h^2	0.32	0.54	0.53	0.45	0.84
Protein content	Trials	4	2	3	4	2
	σ^2_{G}	0.23 ± 0.05	0.18 ± 0.05	0.35 ± 0.05	0.37 ± 0.06	0.33 ± 0.09
	σ^2_{GT}	0.36 ± 0.04	0.07 ± 0.03	0.27 ± 0.03	0.65 ± 0.05	0.65 ± 0.08
	h^2	0.72	0.84	0.80	0.69	0.50
Protein yield	Trials	4	2	4	4	3
	σ^2_{G}	0.04 ± 0.03	0.03 ± 0.07	0.05 ± 0.02	0.26 ± 0.05	0.76 ± 0.14
	σ^2_{GT}	0.41 ± 0.04	0.38 ± 0.09	0.34 ± 0.03	0.69 ± 0.05	1.30 ± 0.11
	h^2	0.30	0.14	0.37	0.60	0.64
	Lines	94	64	165	160	176

Genotypic variance (σ^2_{G}), genotype by trial interaction variance (σ^2_{GT}), and heritability (h^2)

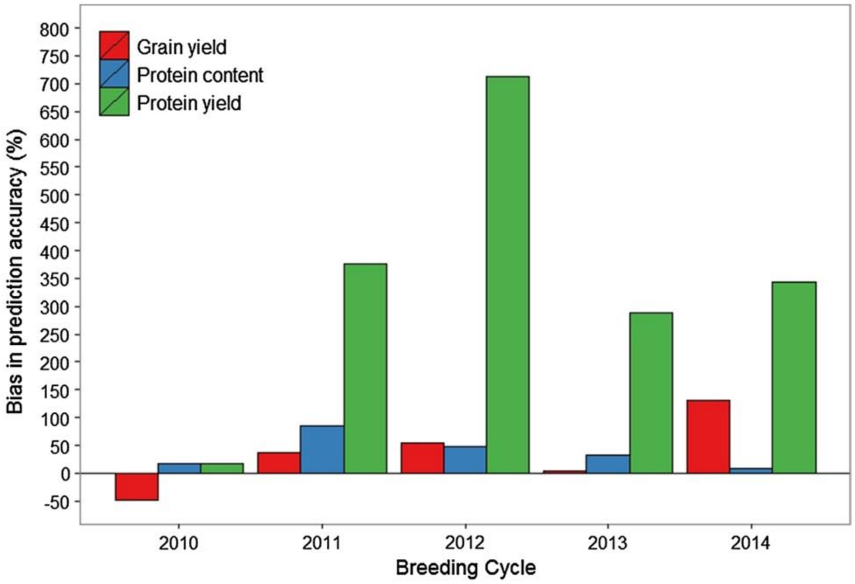

Fig. 1 Bias of the within- cycle prediction accuracy in comparison with the between-cycle prediction accuracy for grain yield, protein content and protein yield and using lines from the years 2010–2014 as training populations

cross-validation scheme. A strong upward bias of within-cycle prediction accuracy was observed for 10 out of 15 traits by cycle combinations and was less than 25 % in four instances (Fig. 1). The bias was especially pronounced for 2014, where the predictive ability of grain yield was overestimated by 130 % and even more for protein yield by 344 %. Protein yield had overall the largest bias ranging from 17 % up to 712 %, while the prediction accuracy of protein content was maximally overestimated by 86 %. Within-cycle cross-validation underestimated the prediction accuracy for grain yield by 47 % merely in one case.

The intention behind using the breeding cycles as folds in a fivefold cross-validation was the avoidance of this bias when estimating the prediction accuracy. Sampling an equal number of lines from each breeding cycle furthermore aimed to avoid a confounding effect between training population sizes and breeding cycle. According to expectation the prediction accuracy increased with the number of lines in the training population (Fig. S3). A maximum was reached for a training population size of 240 lines at $r_{\mathrm{GS}} = 0.51$ for protein content, $r_{\mathrm{GS}} = 0.38$ for grain yield and $r_{\mathrm{GS}} = 0.16$ for protein yield.

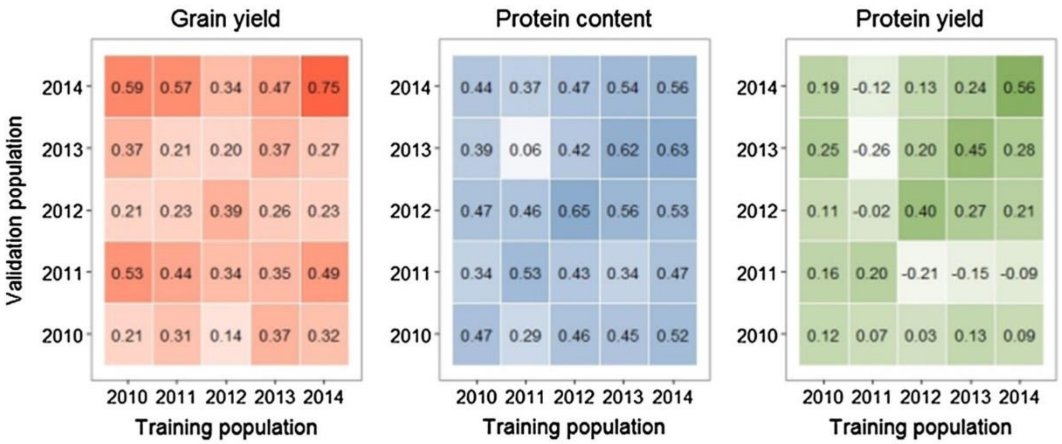

Fig. 2 Heatmap of the pair-wise prediction accuracy between breeding cycles on the off-diagonal and the result of the fivefold within-cycle cross-validation on the diagonal

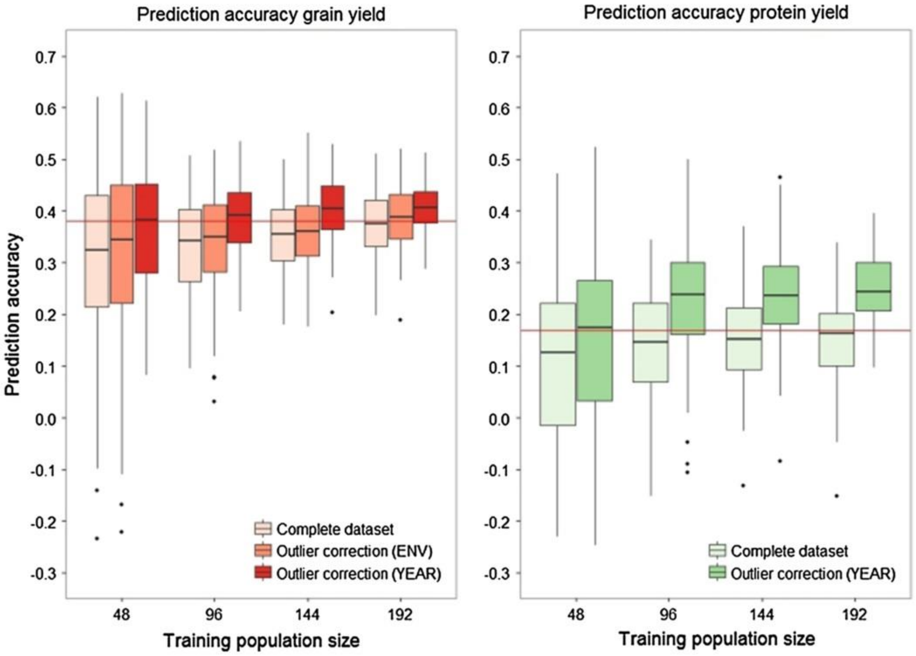

Fig. 3 Influence of removing outlier years or environments from the training set on the prediction accuracy. Results were obtained using across-cycle cross-validation with years as folds. The *horizontal red line* indicates the maximum prediction accuracy in the complete dataset

Outlier correction and estimation of derived traits

Pair-wise prediction accuracies furthermore provided an approximation of the genetic correlation between breeding cycles. It was assumed that a breeding cycle with an overall low predictive ability also had a low value of representativeness for the breeding program across several breeding cycles, and was thus considered an outlier. The breeding cycle 2012 clearly presents itself as such an outlier with regard to grain yield. It achieved on average a much lower prediction accuracy when utilized as a training population ($r_{GS} = 0.26$) in comparison with all other breeding cycles ($r_{GS} = 0.36$) (Fig. 2). Although the heritability was relatively high for 2012, the phenotypic data was most likely strongly influenced by frost damage and drought stress in some trials. The low predictability and prediction accuracy furthermore identified the breeding cycle 2011 as an outlier for protein yield.

The influence of these outliers was investigated by omitting the above described breeding cycles when selecting lines for the training population. Using this approach, the prediction accuracy of protein yield increased from

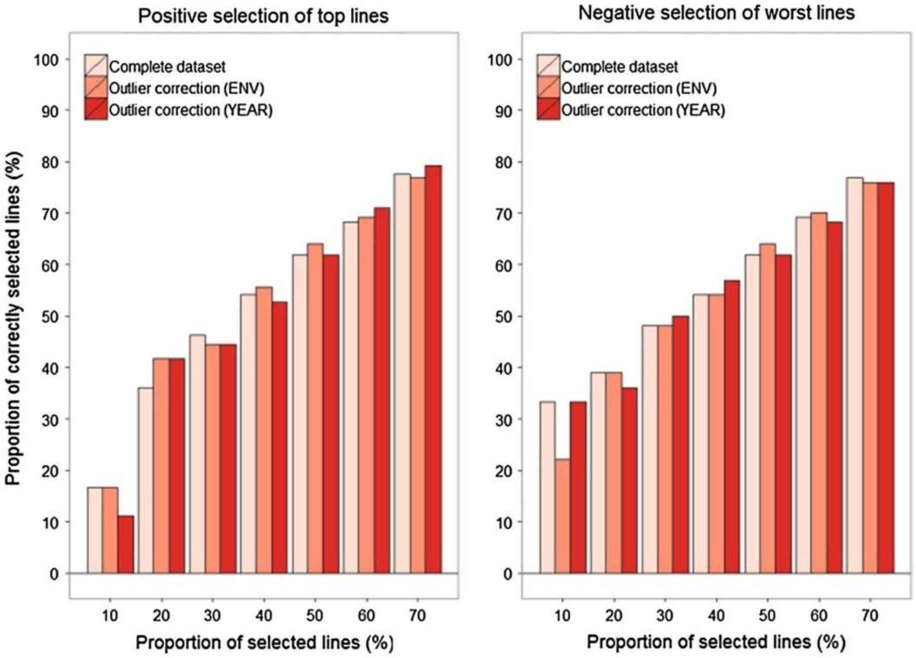

Fig. 4 Proportion of correctly selected lines when applying genomic selection for grain yield of either the best or worst lines in the independent validation population of the year 2015

$r_{GS} = 0.15$ to $r_{GS} = 0.25$ at a training population size of 192 lines (Fig. 3). A similar pattern was observed for grain yield at the same training population size, where the prediction accuracy raised from $r_{GS} = 0.38$ to $r_{GS} = 0.41$ when omitting phenotypic data from 2012. Strikingly a prediction accuracy of $r_{GS} = 0.41$ was estimated in the outlier corrected dataset using a training population size of 144 lines, surpassing the maximum of $r_{GS} = 0.38$ with a much larger training population of 256 lines in the complete dataset. One of the trials suffered severe drought stress and showed a negative phenotypic correlation with all other trials from 2012, and its mean prediction accuracy as a training population for all other trials was negative ($r_{GS} = -0.15$) and far below the average of all trials ($r_{GS} = 0.16$). Removing this trial from the phenotypic analysis of grain yield increased the heritability to $h^2 = 0.61$ and the average prediction accuracy of 2012 from $r_{GS} = 0.26$ to $r_{GS} = 0.30$, although the prediction accuracy obtained by cross-validation was not significantly higher than without outlier correction.

Grain yield was predicted with an accuracy of $r_{GS} = 0.34$ in the independent validation population of the breeding cycle 2015 ($h^2 = 0.57$). Dropping the drought stressed trial from the phenotypic analysis had no effect, while removing the entire breeding cycle 2012 decreased the prediction accuracy by 4 %. Removing 2011 from the training population decreased the prediction accuracy of protein yield by 3 % in the independent validation ($h^2 = 0.30$). These minor changes in prediction accuracy had only a slight influence when genomically selecting for the top or against the worst lines tested in multi-environment trials in 2015 (Fig. 4).

The prediction accuracy of protein yield was rather low, while its component traits grain yield and protein content were estimated more accurately. The low estimated prediction accuracy for protein yield was raised from $r_{GS} = 0.16$ to $r_{GS} = 0.19$ by multiplying GEBVs of its component traits, instead of modeling protein yield directly, which corresponds to an increase of 19 %.

Discussion

Genomic selection has received attention in plant breeding research and caused some excitement in the last years (e.g. Heffner et al. 2009; Heslot et al. 2015). Nevertheless, results from practical applications in the framework of applied breeding programs are still sparse. This study focused on the problems and prospects of genomic selection in bread wheat. Five breeding cycles from an ongoing commercial breeding program were used as a base for assessing and enhancing the potential of genomic selection in bread wheat breeding.

Model selection

Since the introduction of genomic selection models from both the Bayesian (e.g. Technow and Melchinger 2013) and Frequentist methodology (Piepho 2009; Schulz-Streeck and Piepho 2010; Hofheinz and Frisch 2014) as well as machine learning methods (Ogutu et al. 2011, 2012) have been applied in plant breeding. Although great effort was put into developing these models no method showed clear superiority over the others across species or traits (Heslot et al. 2012). Hence we chose RR-BLUP as a computationally fast and robust alternative in our study.

Bias of the prediction accuracy

Genomic selection of non-phenotyped genotypes based on RR-BLUP is strongly dependent on the relationship between training population and selection candidates (Habier et al. 2007; Hayes et al. 2009). Empirical studies from plant breeding show a strong decline in accuracy when predicting distantly related populations (Riedelsheimer et al. 2013; Albrecht et al. 2014) and higher accuracies within closely related families (Lehermeier et al. 2014). Hence, the genetic relationship between training and selection population might introduce a bias in the estimation of prediction accuracy depending on the cross-validation scheme (Ly et al. 2013; Würschum et al. 2013). Genotype by environment interaction effects furthermore led to overestimations of the prediction accuracy, when genotypes from the training and selection population were tested in the same environment or year (Storlie and Charmet 2013; Krchov et al. 2015).

Both effects play important roles when predicting selection candidates across cycles in an ongoing breeding program. Accordingly, Hofheinz et al. (2012) reported an upward bias of the within-cycle prediction accuracy computed by cross-validation in comparison with the actual prediction accuracy across two subsequent breeding cycles. This observation was verified by our analysis and especially pronounced for protein yield, while the bias was much smaller for protein content. The highly heritable traits fusarium head blight resistance and plant height showed likewise less variation in the prediction accuracy across several breeding cycles, than the complex and low heritable trait grain yield in a dynamic barley breeding population (Sallam et al. 2015). A similar relationship between heritability and across-cycle prediction accuracy was also found in sugar beet (Hofheinz et al. 2012). Highly heritable traits are expected to have a less complex genetic architecture (Combs and Bernardo 2013), thus haplotype structures and relatedness responsible for the prediction accuracy (Daetwyler et al. 2010) might be preserved across breeding cycles. These considerations are in accordance with the presented empirical results and suggest that prediction accuracy estimates for highly heritable traits are quite stable even across multiple breeding cycles.

Cross-validation results

Genomic selection is though especially interesting for low heritable traits and providing realistic estimates of trait-specific parameters is crucial for optimizing the resource allocations in an applied breeding program (Riedelsheimer and Melchinger 2013; Longin et al. 2015). Implementation of genomic selection in a breeding program faces the challenge of predicting a new set of genotypes with varying relatedness to previous generations or breeding cycles every year. Using breeding cycles as folds in cross-validation takes this problem into account, by sampling non-overlapping sets of genotypes from multiple breeding cycles as training populations and subsequent prediction of an independent breeding cycle. Estimates for grain yield derived from this across-cycle cross-validation scheme were on the upper bound of what has been reported before in mixed wheat populations (Heffner et al. 2011; Poland et al. 2012; Combs and Bernardo 2013; Storlie and Charmet 2013; Charmet et al. 2014; Isidro et al. 2015). Considering these studies together with our results a prediction accuracy between 0.3 (Longin et al. 2015) and 0.4 seems to be realistic for wheat grain yield across several breeding cycles. Interestingly these values correspond to the broad-sense heritability using variance components from Piepho et al. (2014) who analyzed long-term trends of bread wheat in the German official variety trials. A similar relationship between heritability and prediction accuracy across breeding cycles was previously observed by Hofheinz et al. (2012) for sugar content and molasses loss in sugar beet and several traits in barley (Sallam et al. 2015).

Particular with regard to the importance of phenotypic data (Bernal-Vasquez et al. 2014) a high estimate of the prediction accuracy was not unexpected in this study. First we selected trials with a high heritability from a larger population of target environments, as is common practice in plant breeding. Additionally, we selected only phenotypic records of lines that were tested orthogonally across all trials within a breeding cycle for building training populations. This allowed direct comparisons between all lines and consequently an expected higher efficiency than partial comparisons between lines or by using check varieties (Piepho et al. 2006). Even if a balanced subset cannot be extracted from existing data we recommend the use of the very best of trials to compute GEBVs for pending selection decisions as the data quality, measured by the heritability, is an important driving force of prediction accuracy in genomic selection (Jannink et al. 2010; Combs and Bernardo 2013).

Outlier correction and estimation of derived traits

A high heritability suggests that the phenotypic accuracy is high and gives good estimates of the underlying genotypes and true breeding values of the selection candidates (Visscher et al. 2008). Nevertheless, some years or trials have a low predictability despite of high heritability estimates (Dawson et al. 2013). Factors like biotic or abiotic stress caused by heavy rain, frost damage or drought can result in poor trial establishment and characterizes such environments as outliers (Heslot et al. 2013b). Although they give breeders the opportunity to select for special traits e.g. winter hardiness or resistance to a specific disease, dropping such outlier environments is justified when breeding for productivity and broad adaptation.

This outlier correction increased the prediction accuracy of grain yield, estimated by cross-validation, by 16 % in our study. We used pair-wise prediction accuracies and breeder´s knowledge as an ad hoc measure to identify outliers for grain yield in wheat, though implementation of a systematic search algorithm led to analogous results for grain yield in barley (Heslot et al. 2013b). Dropping low-quality data for protein yield had a similar effect by raising the prediction accuracy by 50 % underpinning again the importance of phenotypic data. Independent validation suggests on the other hand that careful consideration is necessary before an outlier correction is undertaken. Dropping all phenotypic records of a genotype might even have a detrimental effect on the prediction accuracy in some cases as a broad genetic base and maximizing the phenotypic variance are essentials for optimizing a training population (Rincent et al. 2012; Isidro et al. 2015).

Apart from outlier correction another convenient option to improve the prediction accuracy for the derived trait protein yield was its prediction by component traits. The low prediction accuracy of protein yield could be slightly raised by multiplying GEBVs of the medium predictable traits grain yield and protein content. This approach might also be beneficial for other derived traits in plant breeding with a low heritability or prediction accuracy.

Conclusions

Numerous genomic selection studies were conducted in recent years, pointing out its large potential and several applied plant breeding programs adopted this new technology with high expectations. Hence results from multiple genomically selected breeding cycles are becoming available now, bringing these expectations to a realistic level. Genomic selection certainly opened up new opportunities by predicting difficult or expensive to phenotype traits or the estimation of derived traits by GEBVs of its components. Furthermore the genomic selection framework helped to shed light on old problems, such as handling phenotypic data by approximating the genetic correlations among environments by their pair-wise prediction accuracy. Finally it also demands solutions to new problems such as optimizing training populations or redesigning breeding programs. Supported by the vast ongoing research, genomic selection is definitively becoming an integral part of modern bread wheat breeding and the future genetic improvement of crop plants.

Author contribution statement SM and CA analyzed the data and wrote the manuscript. HGR supported in the statistical analysis. FL, DE and HGU designed the field trials and collected the phenotypic data. FL and HB initiated and guided through the study. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical standard The authors declare that the experiments comply with the current laws of Austria.

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Genomic Assisted Selection for Enhancing Line Breeding: Merging Genomic and Phenotypic Selection in Winter Wheat Breeding Programs with Preliminary Yield Trials

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ORIGINAL ARTICLE

Genomic assisted selection for enhancing line breeding: merging genomic and phenotypic selection in winter wheat breeding programs with preliminary yield trials

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Abstract

Key message **Early generation genomic selection is superior to conventional phenotypic selection in line breeding and can be strongly improved by including additional information from preliminary yield trials.**

Abstract The selection of lines that enter resource-demanding multi-environment trials is a crucial decision in every line breeding program as a large amount of resources are allocated for thoroughly testing these potential varietal candidates. We compared conventional phenotypic selection with various genomic selection approaches across multiple years as well as the merit of integrating phenotypic information from preliminary yield trials into the genomic selection framework. The prediction accuracy using only phenotypic data was rather low ($r = 0.21$) for grain yield but could be improved by modeling genetic relationships in unreplicated preliminary yield trials ($r = 0.33$). Genomic selection models were nevertheless found to be superior to conventional phenotypic selection for predicting grain yield performance of lines across years ($r = 0.39$). We subsequently simplified the problem of predicting untested lines in untested years to predicting tested lines in untested years by combining breeding values from preliminary yield trials and predictions from genomic selection models by a heritability index. This genomic assisted selection led to a 20% increase in prediction accuracy, which could be further enhanced by an appropriate marker selection for both grain yield ($r = 0.48$) and protein content ($r = 0.63$). The easy to implement and robust genomic assisted selection gave thus a higher prediction accuracy than either conventional phenotypic or genomic selection alone. The proposed method took the complex inheritance of both low and high heritable traits into account and appears capable to support breeders in their selection decisions to develop enhanced varieties more efficiently.

Introduction

Selection and development of new varieties of autogamous crops relies on a number of different breeding schemes including the pedigree and bulk methods as well as breeding acceleration using doubled haploids or single seed descent with off-season generations. Notwithstanding, they all share a step of conventional phenotypic selection based on preliminary yield trials in their methodology. These preliminary yield trials are for the larger part unreplicated

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as merely a limited amount of seed is available from each selection candidate at this stage. Although the phenotypic data obtained in this way allow only preliminary predictions of their final values they strongly influence the selection of lines that enter the following more resource-demanding multi-environment trials, a crucial decision in every line breeding program as a large amount of resources are allocated for thoroughly testing these potential varietal candidates.

Genomic selection using genome-wide dense marker maps has been suggested as a more efficient alternative to conventional selection methods (Meuwissen et al. 2001) and several studies have shown its great potential in line breeding to enhance the selection for major agronomic traits like yield both in legumes (Jarquín et al. 2014; Burstin et al. 2015; Tayeh et al. 2015) and small grain cereals (Asoro et al. 2011; Sallam et al. 2015; Spindel et al. 2015; He et al. 2016; Michel et al. 2016). Additionally, genomic selection could support the accumulation of many small effect alleles to provide higher and more durable quantitative disease resistance (Lorenz et al. 2012; Ornella et al. 2012; Daetwyler et al. 2014; Arruda et al. 2015; Rutkoski et al. 2015b), which could be subsequently combined with labor-intensive and costly to assess quality traits (Heffner et al. 2011b; Schmidt et al. 2015).

The broad range of possible applications has led to different strategies concerning the implementation of genomic selection into line breeding schemes (Heffner et al. 2010; Longin et al. 2015; Spindel et al. 2015; Marulanda et al. 2016), though it is generally suggested that a genomic selection step is integrated before multi-environment trials are being conducted. Breeders might thus consider the replacement of traditional preliminary yield trials by genomic selection to spare phenotyping costs or even integrating them into the genomic selection framework as they deliver a first insight into the future performance of the putative varietal candidates (Endelman et al. 2014). An additional concern of genomic selection is the choice of lines that shall constitute the training population (Rincent et al. 2012; Isidro et al. 2015; Marulanda et al. 2015) especially if breeders conduct selection, which is not always optimal for genomic selection models (Zhao et al. 2012). Nevertheless, high quality phenotypic data for multiple traits is usually available for many advanced lines that were already tested in multi-environment trials and could possibly be used to build more suitable training populations. Hence, a comparison between conventional phenotypic selection based on preliminary yield trials and genomic selection together with an appropriate training population design is needed to shed more light on this issue for the optimization and enhancement of line breeding schemes. The objectives of this study were thus to investigate (i) the possibilities and merit of a posteriori training population designs, (ii) integrating phenotypic information from preliminary yield trials into the genomic selection framework and (iii) compare conventional phenotypic selection with various genomic selection approaches in line breeding schemes on the example of bread wheat.

Materials and methods

Plant material and phenotypic data

We analyzed a population of 861 genotyped lines from a commercial winter wheat (*Triticum aestivum* L.) breeding program that descend from multiple families and were either in the $F_{4:6}$ generation or directly derived by the double haploid method. Different subpopulations containing 64–192 lines were tested orthogonally in multi-environment trials from 2010 to 2015. Phenotypic data of these lines was thus of high quality, as they were thoroughly tested in all trial locations that spanned from Austria over Serbia, Croatia, Hungary, and Romania to the Central Anatolian High Plateau in Turkey. We also analyzed $F_{4:5}$ generation preliminary yield trials where all lines in the population were pretested in one location and year in Austria from 2011 to 2014 before multi-environment trials were conducted.

Unreplicated earlier generation lines were tested along with replicated check varieties in all trials. The replicated check varieties allowed correcting for spatial field trends according to standard procedure in plant breeding. The entire population of genotyped earlier generation lines from 2011 to 2014 comprised 1203 lines, with 731 lines being unique to the preliminary yield trials. The number of genotyped lines in these preliminary yield trials varied accordingly between 151 and 539 lines as this study also included historical data before genomic selection was routinely implemented into the winter wheat breeding program at hand. Phenotypic records included grain yield (dt ha^{-1}) and protein content (%), which was determined by near infrared spectroscopy (NIRS) directly at harvest.

Statistical analysis of phenotypic data

We followed a two stage analysis strategy of the phenotypic data, where each individual yield trial was analyzed separately in the first stage. Various models correcting for row and/or column effects as well as autoregressive variance–covariance structure of the residuals were introduced (Burgueño et al. 2000) and the best model was chosen by Akaike's information criterion (AIC) to calculate best linear unbiased estimates (BLUE) for each trial. The heritability was estimated by $h^2 = \sigma_G^2/(\sigma_G^2 + \frac{1}{2}\mathrm{MVD})$, where σ_G^2 designates the genetic variance and MVD the mean variance of a difference of the BLUEs (Piepho and Möhring 2007) and trials with a heritability larger than 0.3 were forwarded for further analysis.

Across trial analysis of the multi-environment trials were conducted separately for each year using a linear mixed model of the form:

$$y_{ij} = \mu + g_i + t_j + gt_{ij} + e \tag{1}$$

was fitted for all traits, where y_{ij} are the BLUEs from the first stage, μ is the grand mean, and g_i is the effect of the ith line. The effect of the *j*th trial t_j was fixed, while the line by trial interaction effect $gt_{ij}\frac{1}{2}$ was random. The residual variance was fixed and the inverse of the squared standard errors of the means derived from the first stage of analysis were used as weights in this stage to take the varying accuracy of phenotypic records into account (Möhring and Piepho 2009). Additionally, best linear unbiased predictions (BLUP) were derived for preliminary yield trials by modeling a random effect for the inbred lines in which the heritability was estimated by $h^2 = 1 - \left(\mathrm{VD}_{\mathrm{BLUP}}/2\sigma_G^2\right)$ with $\mathrm{VD}_{\mathrm{BLUP}}$ being the mean variance of a difference of the BLUPs (Cullis et al. 2006). The replicated check varieties were thereby used to estimate row and column effects as well as the error variance. The individual records of the unreplicated lines could in this way be adjusted accordingly, taking spatial trends in the preliminary yield trials into account. All phenotypic analyses were conducted using the statistical package ASReml 3 (VSN International, 2015) for the R programming environment (R development core team 2016).

Genotypic data

DNA was extracted following the protocol by Saghai-Maroof et al. (1984) using leaf samples that were collected from $F_{4:5}$ or doubled haploid lines by sampling minimum ten plants per line during early summer. All 861 lines tested in multi-environment trials as well as the 731 lines unique to preliminary yield trials were genotyped using the DarT genotyping-by-sequencing (GBS) approach (Diversity Array Technologies 2015). Quality control was applied by filtering out markers with a call rate lower than 90%, a minor allele frequency smaller than 0.05, and more than 10% of missing data. Missing data of the remaining 6.6 K SNP markers was imputed by an MVN-EM algorithm (Poland et al. 2012). The same marker data was again used for training genomic selection models with $F_{4:6}$ lines. The minor change in average heterozygosity was expected to introduce a small error which was nevertheless seen to be acceptable considering the cost-benefit ratio of re-genotyping all lines in the $F_{4:6}$ generation.

Genomic selection and estimation of breeding values in preliminary yield trials

Marker effects were estimated using a ridge regression best linear unbiased prediction (RR-BLUP):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \tag{2}$$

where **y** is an Nx1 vector of BLUEs obtained in the phenotypic analysis, **b** is a vector of F fixed effects and **X** its corresponding NxF design matrix. **Z** is a NxM matrix, which coded the M markers as either +1 or −1 for homozygous loci and 0 for heterozygous loci. Random marker effects were assumed to follow a normal distribution $\mathbf{u} \sim N\left(0, \mathbf{I}\sigma_u^2\right)$ with variance σ_{u}^2 and $\mathbf{e} \sim N\left(0, \mathbf{I}\sigma_e^2\right)$. The kinship between lines was estimated by the genomic relationship matrix, which was computed according to Endelman and Jannink (2012):

$$\mathbf{K} = \mathbf{WW}^{\mathrm{T}}/2\Sigma(p_k - 1)p_k \tag{3}$$

where **W** is a centered NxM marker matrix of the *i* lines with $W_{ik} = Z_{ik} - 2p_k$ and p_k being the allele frequency at the kth locus. The derived variance–covariance matrix was used to fit mixed linear models of the form:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zg} + \mathbf{e} \tag{4}$$

where **y** is an Nx1 vector of BLUEs obtained in the phenotypic analysis, **g** is an Nx1 vector of genotypic effects with $\mathbf{g} \sim N\left(0, \mathbf{K}\sigma_G^2\right)$ and the genetic variance σ_G^2 as well as its corresponding random effect design matrix **Z**. The shrinkage parameter was given by $\lambda^2 = \sigma_e^2/\sigma_g^2$ where σ_e^2 is the variance of the residuals that followed $\mathbf{e} \sim N\left(0, \mathbf{I}\sigma_e^2\right)$. The mixed linear models were completed by F fixed effects, which were contained in the vector **b** and its corresponding NxF design matrix **X**. Fixed effects included years in the case of prediction with multiple years and the grand mean for preliminary yield trials.

Breeding values for all the lines tested in preliminary yield trials were estimated by explicitly entering their phenotypic records i.e., BLUES for grain yield and protein content into model (4). In this way, genetic relationship between the lines were exploited to strengthen the predictiveness of preliminary yield trials although most selection candidates were tested unreplicated in just one plot (Endelman et al. 2014). We like to refer to this method as kinship enhanced best linear unbiased prediction of phenotypic breeding values (KBLUP) in this study to differentiate it from the genomic best linear unbiased prediction (GBLUP) model, where selection candidates are predicted purely on their relationship with a training population without any phenotypic records. Models for estimating marker effects by RR-BLUP were implemented using the R package rrBLUP (Endelman 2011), whereas the GBLUP and KBLUP models for predicting future line performance were fitted with the implementation of ASReml 3 (VSN International 2015) for R (R development core team 2016).

Cross-validation accuracy and training population design

We first investigated the merit of a posteriori designing a training population by picking a specific set from the entire available population of lines. The phenotypic variance of the training population is a major factor correlated with the prediction accuracy (Isidro et al. 2015; Marulanda et al. 2015), thus we aimed to maximize the phenotypic variance by sampling the highest and lowest performing lines from each respective year for entering into the training population.

The impact of this sampling method on the prediction accuracy was tested by 6-fold cross-validation, where the training and selection populations were built by randomly sampling 20–60 lines from each year and every year constituted a fold. GBLUP models were fitted with randomly sampled training populations and the benefit of maximizing the phenotypic variance was studied by equally sampling lines from the tails of the distribution e.g., the 30 highest and 30 lowest performing lines from a given year. The selection population was always equivalent in both cases and the training population size varied accordingly between 100 and 300 lines. This entire approach corresponds essentially to sampling both genotypes and environments for estimating a less upward biased prediction accuracy of genomic selection than obtained by sampling genotypes alone (Albrecht et al. 2014; Michel et al. 2016). Furthermore, the prediction accuracy of the full data set was estimated by leaving all lines from one year out as validation population and training a GBLUP model with all lines from the remaining 5 years at a time, which resulted in training population sizes of approximately 700 lines and validation populations that were on average composed of 140 lines. The benefit of a posteriori training population design was assessed by sampling 20–90% of the lines from each year in the training population, either randomly or with half of the lines coming again from either tail of the distribution.

Comparison between conventional phenotypic and genomic selection

The accuracy of conventional phenotypic selection was estimated by correlating the line performance in preliminary yield trials in 2011–2014 and BLUEs from multi-environment trials the following year. This estimate was based on 96–145 retested lines that formed the selection populations and despite a certain selection pressure still covered a broad range of both protein content and grain yield (Fig S1). Line performance per se was thereby predicted by classical BLUP as well as the above described KBLUP that took genetic relationships among lines within preliminary yield trials into account.

Pure genomic selection is on the other hand undertaken without prior knowledge of line performance from preliminary yield trials. We compared this approach with conventional phenotypic selection by predicting the performance of the same 96–145 retested lines but excluded all their phenotypic data from both the year of the preliminary yield trial and the multi-environment trials to fit GBLUP models. The influence of the training population constitution was studied by setting up a cross-validation scheme, using alternatively all possible three-way combinations of the remaining four years in which lines from the selection population did not occur (Fig S2). Hence, every one of the four selection populations was predicted by four different training populations. The training population size was fixed at 180 lines and constructed by sampling an equal number of 60 lines from each one of the training population years. The prediction accuracy of the different selection populations was finally obtained by correlating the genomic estimated breeding values (GEBV) with the BLUEs from the across trial analysis of the multi-environment trials.

Genomic assisted selection and marker selection

Although genomic selection is a relatively new approach the implementation of preliminary yield trials has been part of most line breeding schemes for a long time. We like to simplify the problem of predicting untested lines in untested years to predict tested lines in untested years in this study by integrating phenotypic information from preliminary yield trials into the genomic selection framework. Therefore, we first estimated the line breeding values by the KBLUP model for every preliminary yield trial and GEBVs from the GBLUP model for every one of the previously described training by selection population combinations. The heritability for the GBLUP model was estimated via the shrinkage parameter $\lambda^2 = \sigma_e^2/\sigma_g^2$ which could be written as:

$$\sigma_e^2/\sigma_g^2 = \left(1/h^2\right) - 1 \tag{5}$$

This approximation by Hofheinz et al. (2012) also allowed us to estimate the heritability h^2 for the unreplicated preliminary yield trials via both the genetic variance σ_g^2 and the residual variance σ_e^2 as computed by the KBLUP model. The estimated heritabilities were subsequently used as weights in a heritability index, which was built with predictions from both the GBLUP and KBLUP models:

$$\begin{aligned}\mathrm{GEBV}_{\mathrm{Index}} = {} & \mathrm{GBLUP}_{\mathrm{Scaled}} * w_{\mathrm{GBLUP}} \\ & + \mathrm{KBLUP}_{\mathrm{Scaled}} * w_{\mathrm{KBLUP}}\end{aligned} \tag{6}$$

where $\mathrm{GEBV}_{\mathrm{Index}}$ are the GEBVs obtained for genomic assisted selection, $\mathrm{GBLUP}_{\mathrm{Scaled}}$ and $\mathrm{KBLUP}_{\mathrm{Scaled}}$ are the scaled predictions from the GBLUP and KBLUP models, and the weights $\mathrm{w}_{\mathrm{GBLUP}}$ and $\mathrm{w}_{\mathrm{KBLUP}}$ are equivalent to the heritabilities computed by (5). The scaling of the prediction was done as appropriate for index selection by subtracting the mean of the predictions and subsequent division by the variance for each GEBV. It should be note that only the selection candidates were involved in the scaling process.

Prior knowledge of line performance from preliminary yield trials enabled furthermore a knowledge-based and more sophisticated selection of markers actually associated with the trait of interest. For this purpose, marker effects were first estimated by fitting RR-BLUP models separately for the preliminary yield trial and the training population of lines in each fold i.e., training by validation population combination of the employed cross-validation scheme. Markers whose effect showed a change of sign between these two models were considered to rather introduce errors into the prediction model and were removed from the marker and genomic relationship matrix before GEBVs were estimated by GBLUP. All phenotypic data involved in the validation of the models was explicitly excluded from this process. RR-BLUP models were also refitted with the selected markers to investigate the proportional change of markers with the same and different sign. We like to highlight at this point that this marker selection approach was only undertaken on the side of the training population from multi-environment trials as no beneficial effect of marker selection was observed when estimating breeding values in preliminary yield trials by KBLUP (data not shown). Assuming larger information content of the GBLUP model in this case the index weight was accordingly adjusted:

$$w_{\mathrm{GBLUP}} = h^2_{\mathrm{GBLUP}} / \left(1 - \left| r_{\mathrm{GBLUP;KBLUP}} \right|\right) \tag{7}$$

where w_{GBLUP} is the index weight, h^2_{GBLUP} the heritability estimated from the GBLUP model following (5) and $\left| r_{\mathrm{GBLUP;KBLUP}} \right|$ the absolute value of the correlation between predicted breeding values of lines in selection population based on multi-environment (GBLUP) and preliminary yield trial (KBLUP) data. The adjustment was undertaken as after the marker selection the heritability estimated in the GBLUP model by (5) was reduced, yet a dynamic index with a larger weight on the GBLUP that is based on phenotypic data obtained from several years and locations was seen to be beneficial.

Selection decision inferences and a one-year selection experiment

After this comparison between selection methods in terms of prediction accuracy we continued by studying their influence on actual selection decisions. An appropriate selection decision by either conventional phenotypic, genomic or genomic assisted selection could be made if lines from preliminary yield trials that are predicted to be among the highest performing lines would also show a superior performance in multi-environment trials. We recorded thus the 5–50% of lines from each training population combination (Fig S2) that were predicted to be among the highest and lowest performing ones by the different selection methods. A comparison was then made whether the conventional phenotypic, genomic or genomic assisted selection approach correctly identified the actual highest and lowest performing lines with a higher frequency averaged over all training by selection population combinations.

Finally, a selection experiment was conducted to test the efficiency of genomic selection compared to conventional phenotypic selection. A set of 60 lines was purely genomically selected in 2013, while the involved wheat breeder selected 70 lines using all available phenotypic information from preliminary yield trials and beyond without genomic information. Among the 60 genomically selected lines 10 lines were chosen for their excellent predicted grain yield, whereas the other 50 were advanced due to superior predicted performance based on a genomic selection index that took grain yield, protein yield as well as fusarium head blight and stripe rust resistance into account (Ametz 2015). The tested set was completed by the five worst performing lines according to the genomic selection index and 31 randomly sampled lines, which were all retested in the multi-environment trials of 2014.

Results

Maximizing the phenotypic variance of the training population

We found a classical relationship of higher prediction accuracy with increasing training population size using the 6-years as folds for cross-validation, while this effect was more pronounced for protein content than grain yield (Fig. 1a). The benefit of maximizing the phenotypic variance by sampling the highest and lowest performing lines as training population from each year was minimal in comparison to the full training population when leaving one year out as a validation population at a time (Fig. 1b), while for the 6-fold cross-validation an average increase in prediction accuracy of 7% was observed for both traits. A prediction accuracy of $r = 0.37$ could be reached for example using a randomly sampled training population of 300 lines but was already surpassed when we fitted prediction models with 150 lines from the two tails of the distribution ($r = 0.38$).

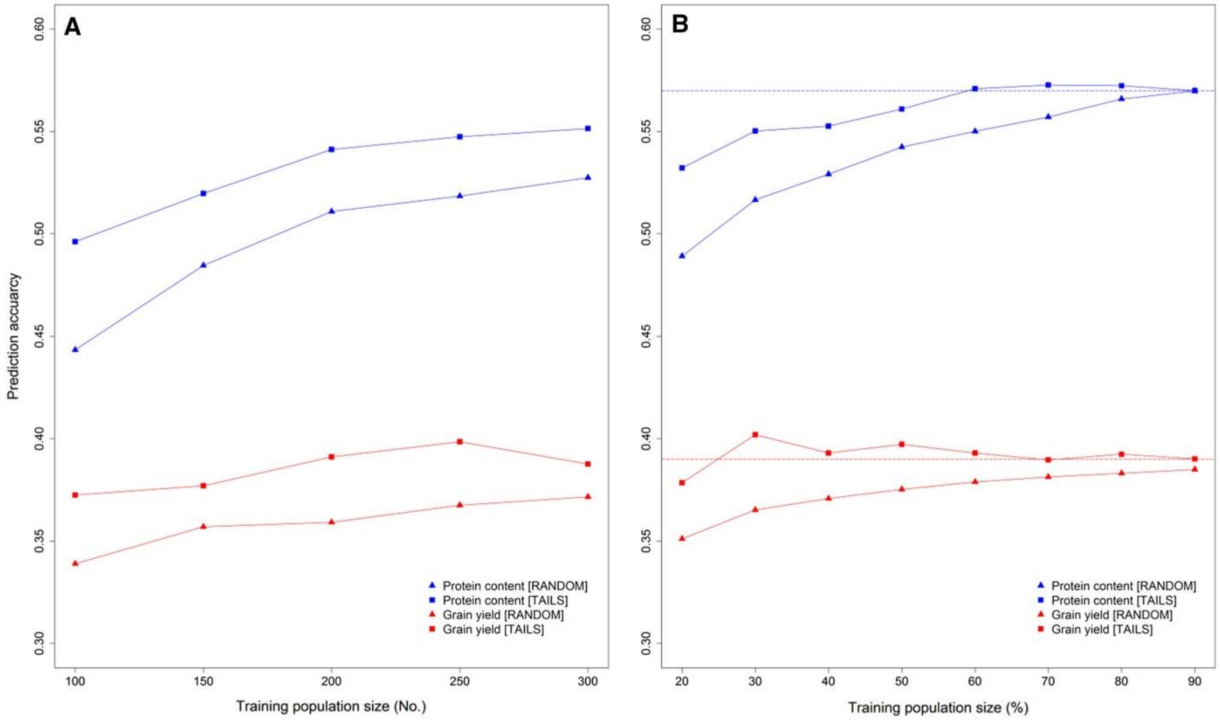

Fig. 1 Effect of the training population design on the prediction accuracy for grain yield and protein content. The *lines* in the training population were either randomly sampled or taken from the tails of the distribution, while the selection population was the same set of randomly sampled lines in both designs using a 6-fold cross-validation in which the years constituted the folds (**a**). Leaving all *lines* from 1 year out as validation population sampling 20–90% of the lines from each year in the training population either randomly or with half of the *lines* coming again from the tails of the distribution, where the *dotted horizontal line* designates the average accuracy when training with the entire set of lines of the remaining 5 years (**b**)

The impact of the training population design was also preserved at maximal training population sizes of 300 lines where the accuracy was $r = 0.55$ in comparison to $r = 0.53$ with a random sample for predicting the protein content. Likewise, grain yield was slightly (5%) better predicted using the highest and lowest performing lines for training ($r = 0.39$). The mean accuracies for both sampling methods were furthermore significant different according to a Wilcoxon rank sum test ($p < 0.01$), thus we chose to design training populations consisting of 60 lines from each year with 30 coming from either tail of the distribution to provide a high prediction accuracy with equally sized training populations for all folds in the comparison between conventional phenotypic and genomic selection.

Predicting the performance of tested and untested lines across years

It is of foremost importance in applied plant breeding programs to select the most promising lines which should enter resource demanding multi-environment trials with a high accuracy to develop successful varieties. We accordingly assessed the correlation between the predicted performance in the year of this selection decision and the actual performance in the following year, utilizing lines that were retested in multi-environment trials 2012–2015.

Classically, lines that will enter more thoroughly testing are selected purely on the basis of phenotypic information from preliminary yield trials. A rather low average prediction accuracy of $r = 0.21$ was found for grain yield using this method, while the highly heritable protein content could be predicted with a reasonable accuracy of $r = 0.45$ (Table 1). The predictive ability of preliminary yield trials could be further enhanced by introducing a genomic relationship to estimate breeding values employing the KBLUP model. Grain yield strongly profited from this method as the accuracy increased by 50% taking the genomic relationships among lines in the unreplicated preliminary yield trials into account.

Genomic selection on the other hand predicted the performance by the genetic relationship between thoroughly tested lines from multi-environment trials and the younger lines i.e., selection candidates without using

Table 1 Comparison between different selection methods by the prediction accuracy for grain yield and protein content across years, using multi-environment trials (MET), preliminary yield trials (PYT) and the genomic relationship matrix (GRM) as complementing information sources

Selection method	Model	Information source			Prediction accuracy	
		MET	PYT	GRM	Grain yield	Protein content
Phenotypic	BLUP		x		0.21 ± 0.09	0.45 ± 0.08
Phenotypic[†]	KBLUP		x	x	0.33 ± 0.27	0.52 ± 0.14
Genomic	GBLUP	x		x	0.39 ± 0.07	0.50 ± 0.06
Genomic assisted[‡]	GBLUP + KBLUP	x	x	x	0.46 ± 0.07	0.61 ± 0.04
Genomic assisted[§]	GBLUP + KBLUP	x	x	x	0.48 ± 0.05	0.63 ± 0.04

[†] Breeding values based on genetic relationships among lines in unreplicated preliminary yield trials

[‡] Genomic and phenotypic predictions were merged by a heritability index

[§] Markers were pre-selected before fitting the prediction models

any of their phenotypic records. Genomic selection was clearly superior to conventional phenotypic selection and nearly twice the accuracy ($r = 0.39$) could be achieved when predicting grain yield across years with the GBLUP model, whereas approximately the same accuracy was estimated using either GBLUP or KBLUP for protein content.

Both selection methods tackle though different problems: Genomic selection by the GBLUP model is predicting untested lines in untested years with high quality information, while the enhanced phenotypic selection by KBLUP is predicting preliminary tested lines in untested years. Merging the information sources by a heritability index gave a strong advantage over both methods alone, which was 18 and 40% over the GBLUP and KBLUP, respectively, for the low heritable trait grain yield. Even the highly heritable and well predicted protein content benefitted from using this genomic assisted selection approach, resulting in an average prediction accuracy of $r = 0.61$ which was 18–22% better than either the best phenotypic or genomic selection model.

Most astonishing though was the advantage over the conventional phenotypic selection (BLUP). With a prediction accuracy of $r = 0.46$ genomic assisted selection was 119% higher than conventional phenotypic selection for grain yield and gave with $r = 0.61$ also 36% more accurate predictions for the future performance of lines with respect to their protein content. Additionally, this approach gave a higher stability of the prediction accuracy than pure genomic selection by GBLUP as reflected by the lower standard error, and thus narrower confidence interval (Table 1).

Prior knowledge from preliminary yield trials gave furthermore the opportunity for a pre-selection of markers associated with the trait of interest in the selection population. Estimation of marker effects by RR-BLUP for both multi-environment and preliminary yield trials separately revealed that around 50% of the marker effects changed their sign between both models, and thus putatively introduced noise when predicting GEBVs (Fig. 2). Removing these markers from the computation of the genomic relationship matrix gave an additional slight increase in prediction accuracy when employing a genomic assisted selection (Table 1).

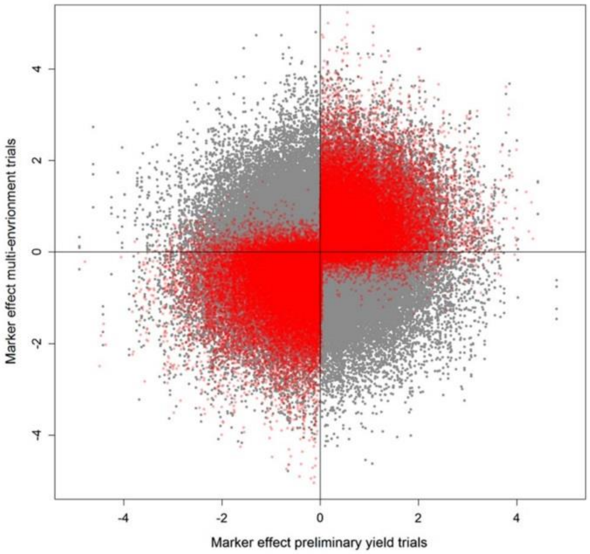

Fig. 2 Marker effect estimates before (*grey*) and after (*red*) pre-selection of markers. Marker effects were scaled and centered to allow a comparison between different training by selection population combinations

Interestingly, we found though merely an advantage for pre-selecting markers when it was conducted before fitting GBLUP models but not for the KBLUP which utilized phenotypic records from preliminary yield trials. A noteworthy observation was that after refitting RR-BLUP models with pre-selected markers, some marker effects still showed a change of sign (Fig. 2). Nevertheless, this percentage of putatively noisy markers decreased to 10% resulting in a majority of markers to estimate effects in the same direction.

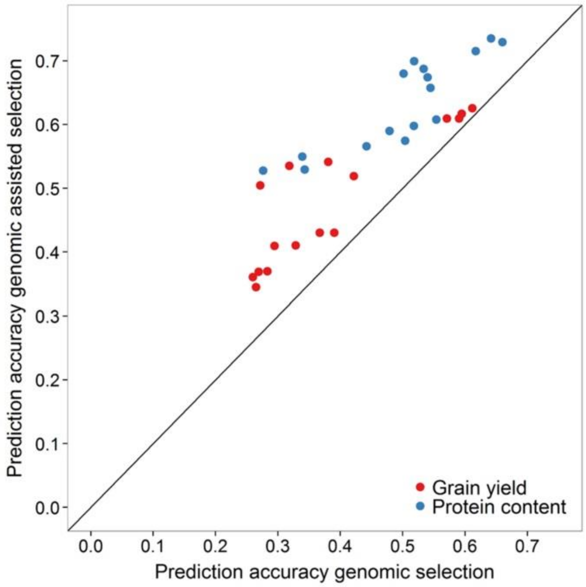

Fig. 3 Comparison between the prediction accuracy of genomic and genomic assisted selection for every training by selection population combination to predict grain yield and protein content across years

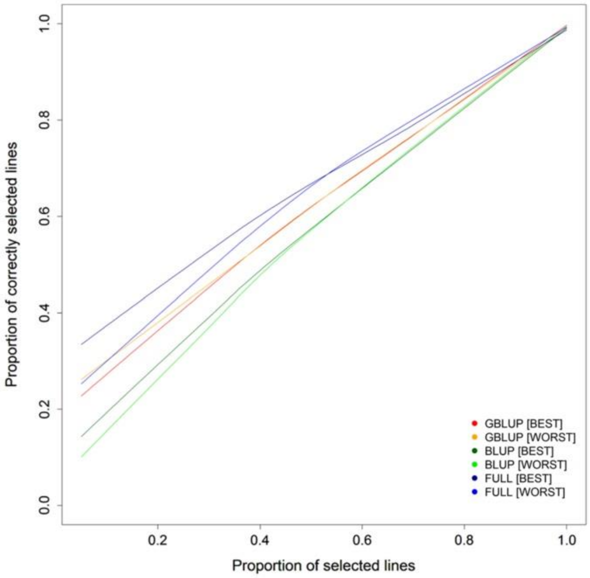

Fig. 4 Proportion of correctly selected best and worst performing lines with respect to grain yield by conventional phenotypic selection (BLUP), genomic selection (GBLUP) and genomic assisted selection with pre-selected markers (FULL) at varying selection intensity

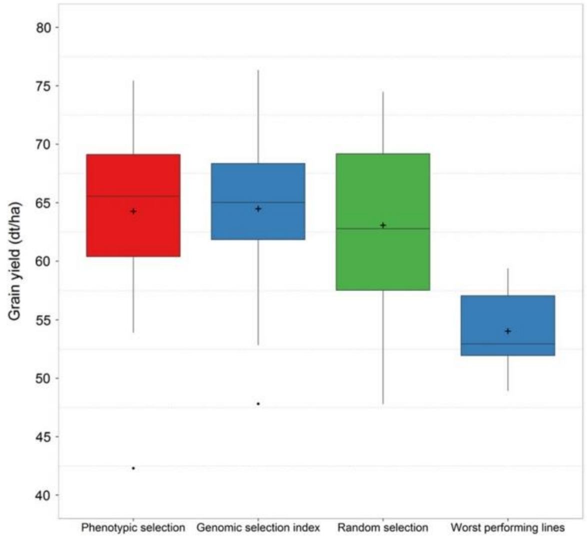

Fig. 5 Performance of lines chosen by different selection methods in the selection experiment during the vegetation period 2014

Genomic assisted selection with additional marker selection also turned out to be a robust approach, which gave constantly higher prediction accuracy than pure genomic selection for all validation by training population combinations (Fig. 3). According to a Wilcoxon rank sum test, the average prediction accuracy of this approach was also significantly higher both for grain yield ($p < 0.05$) and protein content ($p < 0.01$) than what could be achieved by predicting with standard GBLUP alone.

The influence of genomic assisted selection on selection decisions

The observed high and robust prediction accuracy of the genomic selection approaches promised a reasonably good identification of the highest performing lines in preliminary yield trials for further testing in multi-environment trials. We tested this prospect by examining whether or not the best 10–50% lines according to their prediction were indeed among the best in multi-environment trials. Genomic selection did especially well in this scenario at high selection intensities as applied in typical line breeding schemes and could be improved using a genomic assisted selection with marker selection (Fig. 4).

Assuming a breeder would select the best 200 from a total population of 1000 lines (20%), approximately 60 (30%) of these are correctly identified by conventional phenotypic selection but 90 (45%) by genomic assisted selection following the estimates in this study. It is moreover of interest to be informed about the worst lines to discard them by negative selection. This scenario gave nearly orthogonal results to the characterization of the highest performing lines, and the ability to identify the lines from the lower tail of the distribution was verified by the selection experiment (Fig. 5).

Conventional phenotypic selection by the breeder and the genomic selection index performed equally well and surpassed the grain yield of randomly selected lines by 3 dt ha^{-1}, which corresponded to a 3% gain by selection. This could be achieved even though the selection index gave a large weight to protein yield i.e., a trait with low prediction accuracy (Michel et al. 2016). Aside from grain yield, the breeder took also a multitude of morphological, quality as well as disease resistance traits into account that are associated with high and stable performance of the selected lines.

Discussion

This study focused on the prospect of enhancing the efficiency of selection decisions by implementing genomic selection into line breeding schemes. Integrating phenotypic information from preliminary yield trials into the genomic selection framework was combined with a posteriori training population design and resulted in a superior ***genomic assisted selection***. The practical application in commercial bread wheat served as a representative example of this new selection approach.

A two-tailed training population design

A main driving force of prediction accuracy in genomic selection is the relationship between training and selection population (Clark et al. 2012; Habier et al. 2013; Wientjes et al. 2013). Accordingly, genomic selection is expected to give more accurate predictions if lines included in the training population are closely related to (Asoro et al. 2011; Lehermeier et al. 2014; Lorenz and Smith 2015) or even come from the same population as the selection candidates (Windhausen et al. 2012; Charmet et al. 2014). The underlying population structure can be readily deciphered when multiple large bi-parental populations (Heffner et al. 2011a; Schulz-Streeck et al. 2012; Riedelsheimer et al. 2013; Lehermeier et al. 2014) or larger heterotic groups (Technow et al. 2013; Lehermeier et al. 2014; Spindel et al. 2015) are directly involved in the development of varietal candidates. Training and selection populations in line breeding schemes on the other hand, are usually pre-selected by usage of the pedigree method resulting in small families with varying degree of relatedness. Furthermore, breeders frequently introgress foreign material in their breeding pools and lines are often derived by crosses between introduced and their own germplasm, resulting in an unclear population structure in such mixed line breeding populations (Sallam et al. 2015; He et al. 2016; Michel et al. 2016). Simulation (Habier et al. 2013) and empirical (Lorenz and Smith 2015) studies clearly showed that adding distant relatives to prediction models can have detrimental effects on the accuracy, thus there is serious need for an appropriate training population design to achieve high prediction accuracies with genomic selection in line breeding.

A straightforward approach is the maximization of genetic diversity in the training population on the basis of marker data, which additionally enables to choose a subset of lines before phenotyping and saving costs for field trials (Huang et al. 2013). While this method is applicable to various genomic studies, the choice by the average expected reliability of contrast of lines (CDmean) was especially recommended for genomic selection (Rincent et al. 2012). It was further fine-tuned by Isidro et al. (2015) who integrated breeders' knowledge about the population structure into their choice of training populations. These approaches as well as the usage of a genetic algorithm based on reliability measures (Akdemir et al. 2015) have shown superior performance for a multitude of traits and crops in comparison to randomly choosing a training population (Rincent et al. 2012; Akdemir et al. 2015; Isidro et al. 2015; Rutkoski et al. 2015a; Tayeh et al. 2015). Marulanda et al. (2015) finally compared more than 21 indices corresponding to eight factors putatively correlated with prediction accuracy in a vast simulation study and found the phenotypic variance to be a major criterion for training population design. Hence, picking individuals from a two-tailed distribution to maximize the phenotypic variance as suggested by Isidro et al. (2015) seems to be a very suitable training population design strategy which was empirically verified in this study.

Notwithstanding, designing training populations a priori based on phenotypic variance might be difficult if the breeding material was not thoroughly tested yet. Moreover, in applied line breeding programs the major goal is to develop new and better performing varieties irrespective of any prediction accuracies. Selecting a posteriori training populations from the numerous potential line varieties in advanced generations might for this reason be a more convenient strategy. Such training populations should preferably include well phenotyped lines that are related to the current selection population and come from both tails of the distribution to ensure a large phenotypic variance. We also recommend to specifically tailoring them for each trait of interest separately, a procedure which is readily realized as the necessary phenotypic data is most cases already available. Even though the beneficial effect of a higher prediction accuracy due to a large phenotypic variance might diminish with increasing training population sizes (Marulanda et al. 2015), models will be computational less burdening but at the same time keeping a high prediction accuracy. Likewise, a two-tailed training population design could guide the choice which lines with historical phenotypic data should be sent to genotyping and might be very useful if few phenotypic records are available for labor-intensive and costly traits such as brewing quality in barley (Schmidt et al. 2015).

Attention should nevertheless be taken if selection is conducted before training populations are built, a common situation in all plant breeding programs that can lead to a strong bias in prediction accuracy of genomic selection approaches (Zhao et al. 2012). The accompanied loss in prediction accuracy could be substantial when carrying out unidirectional selection (Zhao et al. 2012) but usually a broad range of products is developed in line breeding; so even though the population mean is shifted upwards when going into the phase of testing experimental varieties in multi-environment trials a lot of variance from preliminary yield trials is still kept (Fig S1).

Merging conventional phenotypic and genomic selection

One of the most critical decisions in variety development is the selection of lines that should enter multi-environment trials. The limited phenotypic data that are available for this purpose in early generations led to the suggestion of supporting conventional phenotypic selection by marker assisted selection (Knapp 1998; Lande and Thompson 1990). The implementation of classical marker assisted selection was, however, of limited success for quantitatively inherited traits that are controlled by many loci, while with the advent of genomic selection handling these complex genetic architectures became a much more feasible task in recent years (Jannink et al. 2010; Crossa et al. 2014; Heslot et al. 2015). Although genomic selection has been found to be superior to conventional phenotypic selection and gave outstanding results in several selection experiments (Combs and Bernardo 2013; Beyene et al. 2015; Rutkoski et al. 2015b), genomic predictions rely strongly on genetic relationships and not on physical measurements on the selection candidates.

Hence, preliminary yield trials have the clear advantage of generating solid phenotypic data of which quality can be strongly improved by modeling genetic relationships among the tested lines (Endelman et al. 2014). Integrating pedigree or marker data into the estimation of breeding values has been shown to achieve much higher accuracies when selecting already phenotyped lines in several scenarios (Bauer et al. 2006; Oakey et al. 2007a; Viana et al. 2010; Endelman et al. 2014; Cowling et al. 2015), and was accordingly a very valuable option for enhancing the prediction of line performance across years in this study. The usage of this enhanced phenotypic data from preliminary yield trials for estimating breeding values tackled the problem of predicting tested lines in untested years, while genomic selection usually addresses the more challenging problem of predicting untested lines in untested years.

Merging the before-mentioned merits of genomic selection based on high quality phenotypic data from multi-environment trials with phenotypic selection in preliminary yield trials resulted in a genomic assisted selection that performed much better than either phenotypic or genomic selection alone. The benefits of this approach have also been indicated in bi-parental maize populations for predicting phenotyped doubled haploid lines across years (Lorenz 2013; Riedelsheimer and Melchinger 2013). Krchov et al. (2015) could empirically verify these prospects by combining genomic predictions and phenotypic records with the index weights suggested by Lande and Thompson (1990) for a more accurate prediction of grain yield and moisture in maize hybrids across years. A simple heritability index gave a 12% higher prediction accuracy than the former suggested method in our study, most likely as the additional modeling of a genomic relationship matrix significantly improved the phenotypic data from the preliminary yield trials. The attained genomic assisted selection method resulted furthermore in a higher prediction accuracy for both grain yield and protein content than the other selection approaches, highlighting its superior ability to address the complex inheritance of both low and high heritable traits.

Various marker selection approaches have been proposed for taking the genetic architecture of such traits into account (Heslot et al. 2012; Ogutu et al. 2012; Resende et al. 2012). These efforts are often obstructed by different genetic backgrounds (Schulz-Streeck et al. 2011, 2012) and linkage phase change between the training and selection population (Riedelsheimer et al. 2013; Lorenz and Smith 2015). The incorporation of preliminary yield trials into the genomic selection framework could promote a more targeted pre-selection of marker sets due to prior knowledge of the genetic variation in different selection populations. Hence, we tried to tailor the set of markers fitting the population of selection candidates to account for these altering genetic backgrounds by dropping markers whose effect changed in sign between the training and selection population. Although, we suggest here a rather rough approach that dropped half the markers from the corresponding matrix, the direct pulling of information from preliminary yield trials gave a high and stable average prediction accuracy in combination with genomic assisted selection.

High and stable prediction accuracies are obviously desirable but often very difficult to acquire due to the presence of huge genotype by environment interactions in plant breeding. The prediction of individual trials or locations across years is an especially difficult task (Dawson et al. 2013) and we observed a large variation in prediction accuracy for this undertaking in our study (Fig S3), fitting the results of other studies with autogamous crops (Heslot et al. 2014; Lado et al. 2016). Once multi-environment trials are being conducted, more options open up for enhancing the selection of variety parents like imputing untested lines in tested locations (Burgueño et al. 2011; Jarquín et al. 2014; Crossa et al. 2016; Lopez-Cruz et al. 2015) or enhancing the reliability of breeding values by a relationship matrix (Bauer et al. 2006; Oakey et al. 2007b; Bauer et al. 2009; Müller et al. 2015). Hence, predicting lines for the entire target population of environments might be a better strategy to select candidates that should enter multi-environment trials. These multi-environment trials could afterwards guide selection decisions in breeding for local adaptation to specific regions and variety registration.

Genomic assisted selection for more sophisticated breeder´s decisions

The chance of selecting the highest performing lines for multi-environment trials was much higher by genomic selection than conventional phenotypic selection in our study, and could be further increased by implementing genomic assisted selection. Depending on the breeding scheme it has been suggested to conduct positive genomic selection for the best lines (Bassi et al. 2016) or discarding the worst lines by negative selection (Longin et al. 2015), while we observed no difference of any genomic selection approach to correctly identify lines from either tail of the distribution. Nevertheless, these considerations are valid for single traits only and it is generally not recommended to sequentially select for one trait after another as a lower gain in selection is expected by such tandem selection (Hazel and Lush 1942). Different multivariate models have been developed to take this problem of simultaneous selection for several traits at the same time into account (Bauer and Léon 2008; Viana et al. 2010; Jia and Jannink 2012).

A computational less demanding alternative could be the usage of genomic selection indices (Ceron-Rojas et al. 2015; Schulthess et al. 2015), and even a simple index based on grain yield, protein content and disease resistance gave a similar gain as conventional phenotypic selection by the breeder in our selection experiment. Genomic selection approaches are thus enabling more sophisticated selection decisions but the knowledge and experience of breeders is still the best guarantee for success, while genomic selection indices can be an additional tool to ease their decisions given the multitude of traits to consider.

Conclusions

This study showed the strong advantage of genomic selection over conventional phenotypic selection in line breeding schemes on the example of bread wheat. The advantage was further enhanced by a posteriori selecting a training population that maximized the phenotypic variance and the integration phenotypic information from preliminary yield trials into the genomic selection framework. Conducting preliminary yield trials is a common procedure in most line breeding programs, thus we suggested exploiting their information by merging phenotypic and genomic selection for ***genomic assisted selection***. The easy to implement and robust genomic assisted selection gave a higher prediction accuracy than either one of the other methods alone and allowed a more sophisticated selection decision with regard to lines entering multi-environment trials. The proposed method took the complex inheritance of both low and high heritable traits into account and could support breeders in developing varieties that preferably combine high yield, quality, disease resistance and tolerance against abiotic stresses.

Author contribution statement SM wrote the manuscript, SM and CA analyzed the data. HGR supported in the statistical analysis. FL, DE, BA and HGU designed the field trials and collected the phenotypic data. FL and HB initiated and guided through the study. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical standard The authors declare that the experiments comply with the current laws of Austria.

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Discussion

This study showed some of the benefits that might arise by implementing genomic selection into a line breeding program taking the example of winter wheat. The prediction of line performance across multiple breeding cycles and years resulted in more realistic estimates of genomic selection in breeding programs than were available beforehand, thereby bridging the gap between more theoretical results with restricted germplasm from research studies and empirical results from applied plant breeding. The comparison between conventional phenotypic selection and genomic selection revealed furthermore the high potential of genomic selection if implemented in early generations of a line breeding scheme. Previous comparisons were often made with high quality data from multi-environment trials where genomic selection showed rather similar performance, while it was more than twice as good as phenotypic selection based on preliminary yield trials that is the actual competitor of genomic selection in line breeding.

Enhancing phenotypic selection by modelling genetic relationship among the selection candidates revealed to be an additional important component with substantial benefit given the large amount of marker data is generated in a line breeding program with genomic selection. This method was until now mostly ignored in the genomic selection literature, although being a convenient spin-off product and several studies already highlighted the importance of phenotypic data. Combining genomic breeding values with enhanced phenotypic performance estimates from preliminary yield trials further simplified the across-year prediction problem to predicting tested lines in untested years, and this usage of additional phenotypic information presented in this study could readily be converted to a routine procedure in applied plant breeding. A new two-tailed training population design was proposed to complement this method, which could find some use both in a streamlined variety development pipeline but also in a recurrent genomic selection, where a regular training population update is pivotal. The advantage of the suggested training population design has meanwhile been independently validated in a recent simulation study investigating recurrent genomic selection in barley (Neyhart et al. 2017), where the two-tailed training population design outperformed other previously proposed methods based on genetic distance and reliability. The selection experiment comparing response to selection of phenotypic selection by the breeder and a genomic selection index gave further insight into the response to selection that can be expected by both methods. Moreover, it demonstrated that genomic breeding values should be used as an additional information source to support breeders during variety development instead of conducting a pure genomic selection based on indices, and in this way come to an integrated selection decision in combination with observations in field.

Notwithstanding, a remaining key question is if the routine usage of genomic selection would be generally worthwhile when breeding line cultivars in wheat as well as other small grain cereals and legumes. Therefore, the cost-benefit ratio must be carefully evaluated by managers of breeding programs together with the involved breeders in order to find an economically feasible strategy for an optimal integration of a genomic selection step into the respective breeding scheme. The necessary investments for this endeavour include thereby the genotyping costs for several hundred to thousand lines every year as well as the associated logistics. However, genotyping costs have strongly declined in recent years and can be expected to further decline, while the fee of genotyping service providers are additionally dependent on the number of samples sent every season or in the foreseeable future. Furthermore, larger breeding programs might consider developing chip-based genotyping procedures in-house to further reduce their costs.

GBS is a cost-effective alternative to chip-arrays and several genotyping service providers are offering this technology at the moment. Its low technical error rate i.e. high reproducibility of allele calls highlights the robustness of GBS for obtaining genetic fingerprints of the numerous selection candidates in applied plant breeding, nevertheless the typical large proportion of missing marker data that associated with this method demands the usage of sophisticated imputation algorithms (Poland et al. 2012b; Rutkoski et al. 2013; Gorjanc et al. 2017). Map-based imputation have been shown as being highly accurate and can be recommended if reliable information about the marker order is available e.g. for GWAS studies (Howie et al. 2009; He et al. 2015). The applications of such map-based algorithms also improved the accuracy of genomic selection and can be generally recommended for GBS-like marker datasets. Map-independent algorithms for unordered markers like *missForest* (Stekhoven and Bühlmann 2012) show on the other hand a lower imputation accuracy, although they have a similar beneficial impact on genomic selection like map-based imputation and none of the available algorithms displays a clear advantage (Poland et al. 2012b; Rutkoski et al. 2013; He et al. 2015). Imputation algorithms have also revealed their merit when applied to marker datasets from chip-based genotyping, a major difference is though that the amount of missing data points is several magnitudes smaller (He et al. 2015). Research and development for this genotyping technology focuses on having a representative set of markers on the chip so that e.g. at least one SNP per haplotype group in the European winter wheat germplasm is present. This could improve the identification of candidate markers for agronomic traits by GWAS if complemented with the necessary large mapping population size and facilitate the selection of marker subsets for a genomic selection routine. Notwithstanding, such marker selection proved to be difficult if no prior phenotypic information of the selection candidates was available (Schulz-Streeck et al. 2011), and it would be especially difficult to find a marker subset that is representative for an entire breeding population.

With the wheat reference genome sequence being briefly before completion (IWGSC 2017), it might be possible in the future to obtain whole-genome sequence information of each selection candidate or at least from the most prolific crossing parents in a breeding population. Application of whole-genome sequencing as genotyping routine would facilitate an enormous increase in marker number that could theoretically be associated with an increase in prediction accuracy (Heffner et al. 2011a; Lorenz et al. 2012; Nielsen et al. 2016; Moore et al. 2017). Nonetheless, the advantage of an increasing marker number usually reaches a plateau after several thousand markers have entered prediction models (Jarquín et al. 2014b; Spindel et al. 2015). Empirical results from animal breeding where whole-genome sequence data is already available e.g. for dairy cows show accordingly rather low advantages for the prediction accuracy using such information for enhancing genomic selection (VanRaden et al. 2017), thereby verifying results from simulation studies (Pérez-Enciso et al. 2015). The latter studies already predicted a marginal benefit as a huge number of SNPs serving as predictor variables are in strong linkage disequilibrium in whole-genome sequence data, thus not delivering additional information to prediction models. Furthermore, the enormous computational load requires again the selection of marker subsets for any practical breeding applications but would then lead at least in animal breeding to a substantial economic benefit even with marginal increases in the reliability of genomic breeding values (VanRaden et al. 2017). Prior biological information would be ideal for fully harnessing the potential of whole-genome sequence data. The highest prediction accuracy could possibly be achieved when all casual polymorphisms would be known, while the next best information would be prior knowledge about underlying genes (Pérez-Enciso et al. 2015). Given the resources needed to clone a single gene even when phenotype is clear like for monogenic disease resistance (Thind et al. 2017), such scenarios are though unrealistic for quantitative traits like grain yield with complex genetic architecture at the moment, however the rapid advances biotechnology might open-up this information source for plant breeders in the future.

Restricted to the present possibilities, genomic selection is mainly used to assist selection for quantitative traits in early stages of breeding program (Heffner et al. 2010; Heslot and Mark 2015; Spindel et al. 2015; Guzmán et al. 2016). Consequently, the usage of genomic selection already represents a first paradigm shift in contrast to classical breeding schemes were such difficult and low heritable traits are selected at much later stages of variety development. Genomic selection might in this way guide decisions concerning which selection candidates will enter multi-environment trials, and the proposed genomic assisted selection performed much better for identifying the correct lines for this task in this study. Combined with the higher prediction accuracy, genomic assisted selection can thus be expected to additionally lead to higher realized responses to selection. Given the higher confidence of these predictions a breeder might also intensify selection to spare experimental plots in multi-environment trials for balancing out some of the necessary additional investments for genotyping. Lines with a superior genomic breeding value based on models that were fitted with phenotypic data from multiple years and numerous environments, and additionally show high performance if tested in multi-environment trials might also have a higher probability to be among the highest and stable performing lines in future yet untested years. Assessing this and similar studies (Sallam et al. 2015; Auinger et al. 2016; Sallam and Smith 2016; He et al. 2016b) one could infer that one stage of genomic selection is roughly equivalent to one year of multi-environment trials. Classical breeding schemes need at least two years of multi-environment trials before variety registration trials could be considered, thus assuming the same time is needed for deriving homogeneous material of a promising varietal candidate the data used to select them for official trials will have very high confidence as information from a lot more than 2-3 years are integrated with genomic selection.

Aside from improving major agronomic traits like yield, genomic selection would be useful for predicting laborious to phenotype traits in early generations. The genetic improvement of baking quality is e.g. a specific challenge in the development of new line cultivars in bread wheat breeding as the assessment of associated traits often involve time-consuming, labour-intensive, and costly testing and too less plant material i.e. grains are available from each selection candidate in early generations, forcing breeders to postpone thoroughly quality testing into later generations of variety development. Genomic selection showed great promise for pre-selecting lines with superior bread baking quality in early generations, several years ahead of labour-intensive, time-consuming, and costly quality analysis both in line and hybrid wheat breeding (Battenfield et al. 2016; Liu et al. 2016). The prediction models could finally guide in combination with rapid tests the choice of material that is send for baking quality test to the laboratory (Michel et al. 2017b), and depending on the accuracy of these models breeders might also consider the option of reducing the sample number for saving costs. Aside from quality improvement, breeding for biofortification is another difficult task, where genomic selection had great potential to enhance the concentration of zinc and iron in wheat to combat hidden hunger caused by deficiency of these micro-nutrients foremost in resource poor regions of the world (Velu et al. 2016). Abiotic stress tolerances comprise another set of difficult to phenotype traits, where genomic selection could greatly support breeding programs as e.g. drought stress must often be assessed under special labour-intensive and costly management conditions (Ziyomo and Bernardo 2013; Beyene et al. 2015; Vivek et al. 2016). Finally, some traits like frost tolerance (Zhao et al. 2013) or Fusarium head blight in central Europe (Jiang et al. 2014; Mirdita et al. 2015) cannot be observed every year making it necessary to conduct special tests (Sieber et al. 2014; Sieber et al. 2016) and establish nurseries for disease screenings. Depending on their importance in the respective breeding programs, these screenings are mostly conducted in advanced generations. Accordingly, a genomic selection approach would give a higher chance of selecting lines with desired trait combinations earlier in the breeding scheme, showing a better performance in the future through higher yield stability under abiotic and biotic stress conditions (Wang et al. 2015; Huang et al. 2016).

Apart from improving accuracy of selection, the usage of genomic estimated breeding values has been proposed for a couple of breeding acceleration methods (Bernardo 2010; Hickey et al. 2014; Neyhart et al. 2017). This represents the second and major paradigm shift in plant breeding with genomic selection, by dividing the program into separate product and population improvement cycles (Bernardo 2010; Hickey et al. 2014). The latter takes mostly additive effects i.e. actual breeding values into consideration, while for the former additive x additive epistasis plays an also important role as the final performance of newly developed varieties in line breeding is the sum of both additive and epistatic effects. Hence, modelling epistasis for training genomic selection models has been shown to give a significant advantage for predicting future performance of the varietal candidates (Jiang and Reif 2015). Considering both additive and epistatic effects in the phenotypic analysis of field trials enhanced by marker data could possible further improve phenotypic data quality (Moreau et al. 1999; Müller et al. 2015) especially if many lines are unreplicated as in preliminary yield trials (Endelman and Jannink 2012; Endelman et al. 2014). Great merit was observed for such practice in this study, and the usage of such high density marker could additionally enable the assessment of data quality in multi-environment trial series and the application of spatial models even for unreplicated trials. Resource allocations might accordingly be adjusted for achieving a higher response to selection by employing partially or unreplicated trials with testing in a higher number of environments (Möhring et al. 2014). Alternatively, breeders could not only consider reducing the number of replicates per trial but due to the increase in phenotypic data accuracy also reduce the number of trial locations to compensate for the additional genotyping costs with genomic selection. The improvement of phenotypic data is furthermore not restricted to the training population (Müller et al. 2015), but will likewise provide a more accurate reference for validating genomic selection models.

While the previous section referred to product development cycle, the population improvement cycle is generated by shortening the time between initial crossings to the next crossing for starting new breeding cycles. Classically, the product development and population improvement cycles are largely overlapping and successful lines which have shown superior performance entering official trials to be released as new varieties will often serve as crossing parents. Notwithstanding, some breeding lines with insufficient yield level might though show interesting characteristics such as disease resistance, and will thus be retained in the breeding population as crossing parents due to their high breeding value for some specific traits. The estimation of marker effects with models like RR-BLUP within the genomic selection framework has been suggested to predict superior progeny values for the multitude of parental combinations in order to support the planning of crosses (Zhong and Jannink 2007; Poland et al. 2012b). Superior progeny values enable therefore a crossing in earlier generations of line development for starting a new breeding cycle much sooner than it was possible beforehand. Promising lines of the current cycle are thereby further advanced in multi-environment trials and finally enter official variety registration trials. The superior progeny value is closely related to the usefulness concept (Utz et al. 2001), whose essential part is the mid-parent value that has been shown to correlate well with the population average of bi-parental crossing populations. Further extensions include predicting the segregation variance that is of great importance as from populations with large variance lines with higher performance may theoretically be selected than from a population with a higher average performance though smaller variance. However, the accurate estimation of this variance is very cumbersome (Bohn et al. 1999; Utz et al. 2001), and although some research focused on this problem resulted in promising approaches (Bernardo 2014b; Lian et al. 2015; Mohammadi et al. 2015; Lado et al. 2017) the issue is still unresolved. One of the major obstacles thereby is the empirical proof of concept of different segregation variance estimation methods, which would require a large effort e.g. at least several dozen unselected bi-parental population with 30-100 advanced recombinant inbred lines per population tested for grain yield in multi-environment trials.

Considering all the mentioned benefits that are standing against the costs, the question if genomic selection is worthwhile in line breeding cannot readily be answered. Although there are some entry points to reduce costs by intensifying selection, reducing sample size for quality analysis, and optimizing field trials designs as well as resource allocations, for most breeding programs genomic selection would likely be an additional investment.

Finally, the implementation of genomic selection is primarily a management decision, and it has to be decided if the cost-benefit ratio of genomic selection is favourable for the respective breeding program. Further considerations might involve at which stage and for what purpose i.e. negative versus positive selection genomic breeding values should be employed. Another issue that must be taking into account is if redesigning the entire breeding program is necessary or even adequate. Radical changes like this could e.g. involve the switch from a pedigree breeding scheme to a fully doubled haploid or single-seed descent based program, where a lot of mostly unselected lines are genotyping and genomically selection so that merely seed from the selected lines is multiplied for conducting multi-environment trials. The described very fast breeding scheme has a high potential but is also very costly as in addition to genotyping costs, facilities like greenhouses and laboratories have to be vastly extended.

Another approach would be creating larger bi-parental populations of which merely a part is phenotyped in field trials, while all lines are genotyped for estimating genomic breeding values. First suggested for hybrid breeding in maize, where less topcross progeny is then actually tested in the field (Krchov and Bernardo 2015), this strategy could also be used in wheat breeding to exploit the high prediction accuracy within bi-parental populations (Lorenzana and Bernardo 2009; Heffner et al. 2011b). Different options involving such a scheme have been proposed (Bassi et al. 2015); nevertheless a lot of lines have to be phenotyped and genotyped to achieve a sufficient prediction accuracy including lines that would probably be discarded a priori due to deficits like lodging, too late heading or plant type in head rows. High prediction accuracies within bi-parental populations can be achieved with small training populations by maximizing the phenotypic variance (Marulanda et al. 2015), however it is hardly feasible to priori determine which lines will show values at the lower and upper tails of the distribution. While this strategy might thus be interesting in maize breeding due to low correlations between per se and hybrid performance, it might be less suitable for line breeding where the per se performance is decisive. Accordingly, several breeding programs followed a rather conservative approach by conducting genomic selection in parallel to preliminary yield trials as is currently done e.g. in the CIMMYT spring wheat program (Guzmán et al. 2016) or in the winter wheat program investigated in this study. Hence, instead of radical redesigning the breeding program for genomic selection it was conveniently integrated in the existing variety development pipeline. The use of genome-wide distributed markers at various stages in the breeding program and taking advantage of various possibilities for enhancing selection as well as the above-described method for breeding acceleration makes genomic selection a valuable tool to support in their selection decisions, and accompanied by well-considered changes in resource allocation it has the potential to optimize breeding programs in an unprecedented way during modern plant breeding history.

The optimization of breeding programs with genomic selection also involves aiming for a high long-term response to selection, for which the appropriate management of genetic diversity in breeding programs is a central point (Cowling 2013). Although genomic selection has a similar performance as one-year phenotypic selection, the former targets directly marker alleles and has the potential to alter allele frequency more rapidly especially at loci with large effect (Sallam and Smith 2016). Accordingly, the loss of genetic variance is more pronounced when using genomic selection (Bastiaansen et al. 2012) due to the increased relationship between selected individuals (Jannink 2010). With respect to a long-term strategy, increasing the frequency of rare favourable alleles will be of high interest and upweighting these rare alleles in prediction models could counteract the loss of such alleles by phenotypic selection and led in the end to an overall higher response to selection (Jannink 2010; Liu et al. 2015). The routine genotyping of breeding lines when applying genomic selection has thus the convenient side-effect that it gives breeders the ability to actively monitor diversity in their breeding population on a molecular genetic level.

Aside from maintaining diversity, broadening the genetic base is of large interest in breeding programs. This generally includes the introduction of adapted elite plant material from other breeding programs by crossings with native germplasm according to breeders´ rights, but in a wider sense also introduction from non-adapted material in the framework of pre-breeding. Genomic selection has the potential to support these efforts by a more rapid introgression of exotic germplasm into the elite gene pool of applied breeding programs. Simulation studies thereby suggest that genomic selection should start in the F_2 generation instead of backcross generations when using a recurrent selection scheme for improving populations from elite x exotic crosses (Bernardo 2009), and F_2 training populations were even preferable in a backcross strategy to transfer favourable alleles for a quantitative inherited trait from exotic to elite germplasm (Bernardo 2016b). This approach could be verified by an empirical study aimed to development short statured maize population with higher density tolerance than currently available by crossing non-dwarf elite lines with semi-dwarf lines from an exotic population, where predicted and observed gains generally agreed resulting in a higher gain by genomic than by phenotypic selection (Combs and Bernardo 2013). The application of genomic selection for this endeavour has the additional advantage that it allows the usage of multiple glasshouse or off-season generations per year, making it an especially interesting method for accelerating pre-breeding programs. These programs will in this way be strengthened for effectively harnessing the genetic variation of landraces and other germplasm from gene banks (Gorjanc et al. 2016; Yu et al. 2016) that is currently severely underused, although there is some need to unlock their potential for achieving the aim of doubling the world-wide grain yield until 2050 (Longin and Reif 2014). The exchange of datasets and genomic selection knowledge worldwide (Spindel and McCouch 2016) across both the public and private sector would finally contribute significantly to this enormous task.

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Appendices

Supplementary material of Genomic Selection across Multiple Breeding Cycles in Applied Bread Wheat Breeding

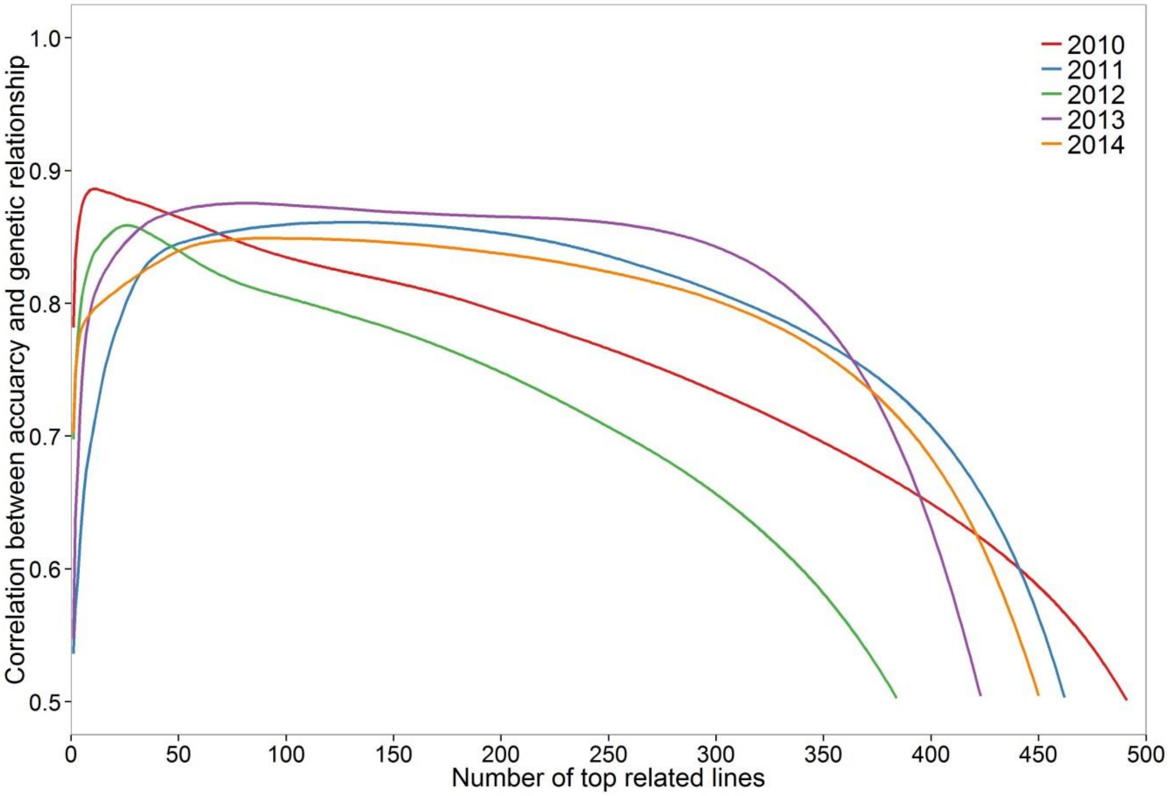


Fig. S1 Correlation between the accuracy of each individual line and the genetic relationship, varying the number of the most related lines from the training population. One breeding cycle was left out at a time using all other breeding cycles as training population.

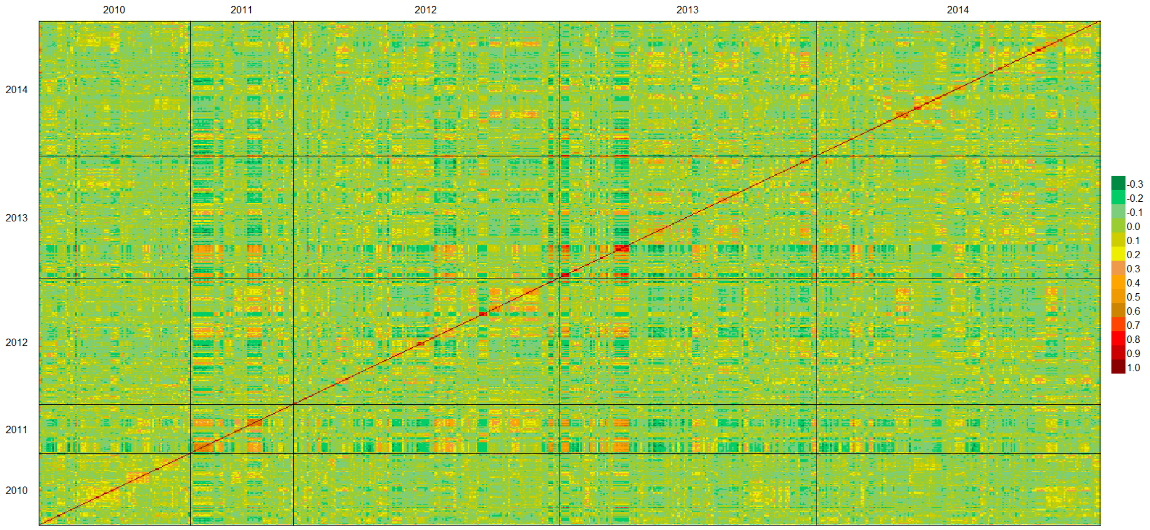


Fig. S2 Heatmap of the genomic relationship matrix displaying the genetic correlation among all lines.

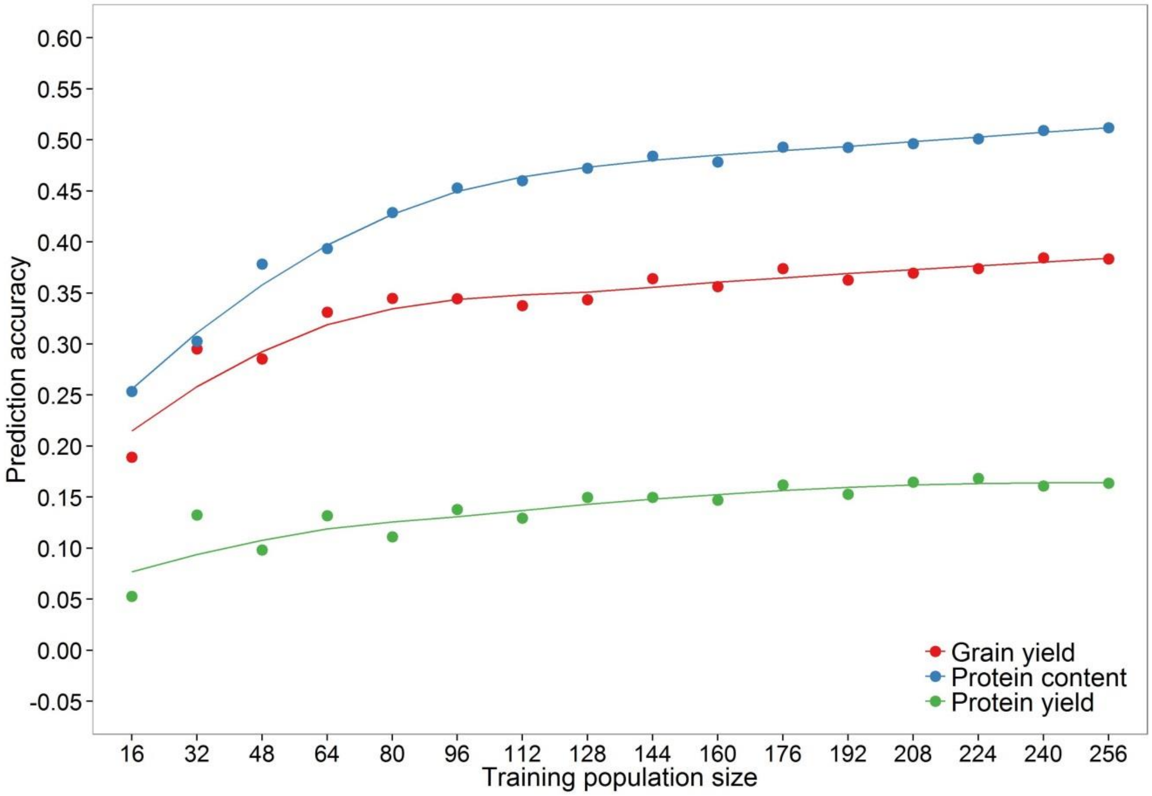


Fig. S3 Relationship between prediction accuracy and training population size for grain yield, protein content and protein yield using a 5-fold cross-validation with the breeding cycles 2010-2014 as folds.

Supplementary material of Genomic Assisted Selection for Enhancing Line Breeding: Merging Genomic and Phenotypic Selection in Winter Wheat Breeding Programs with Preliminary Yield Trials

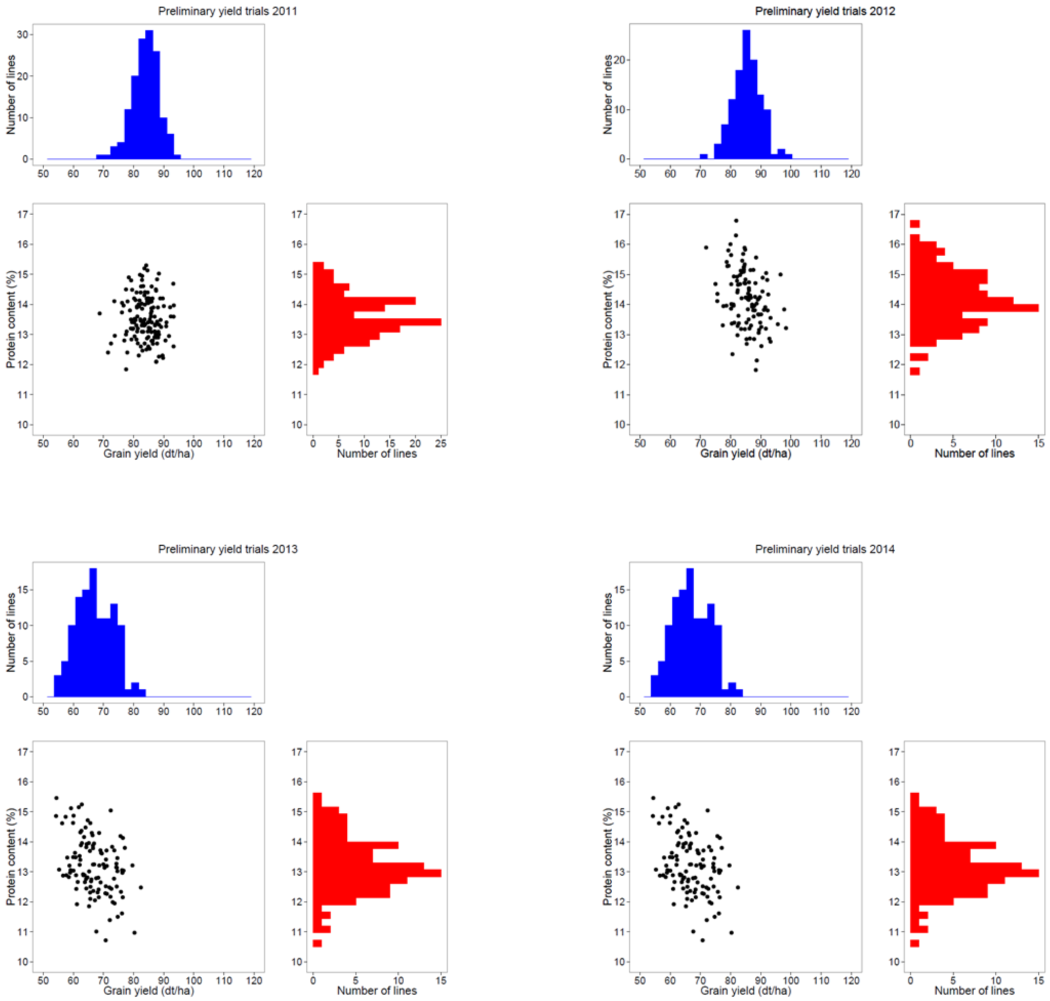
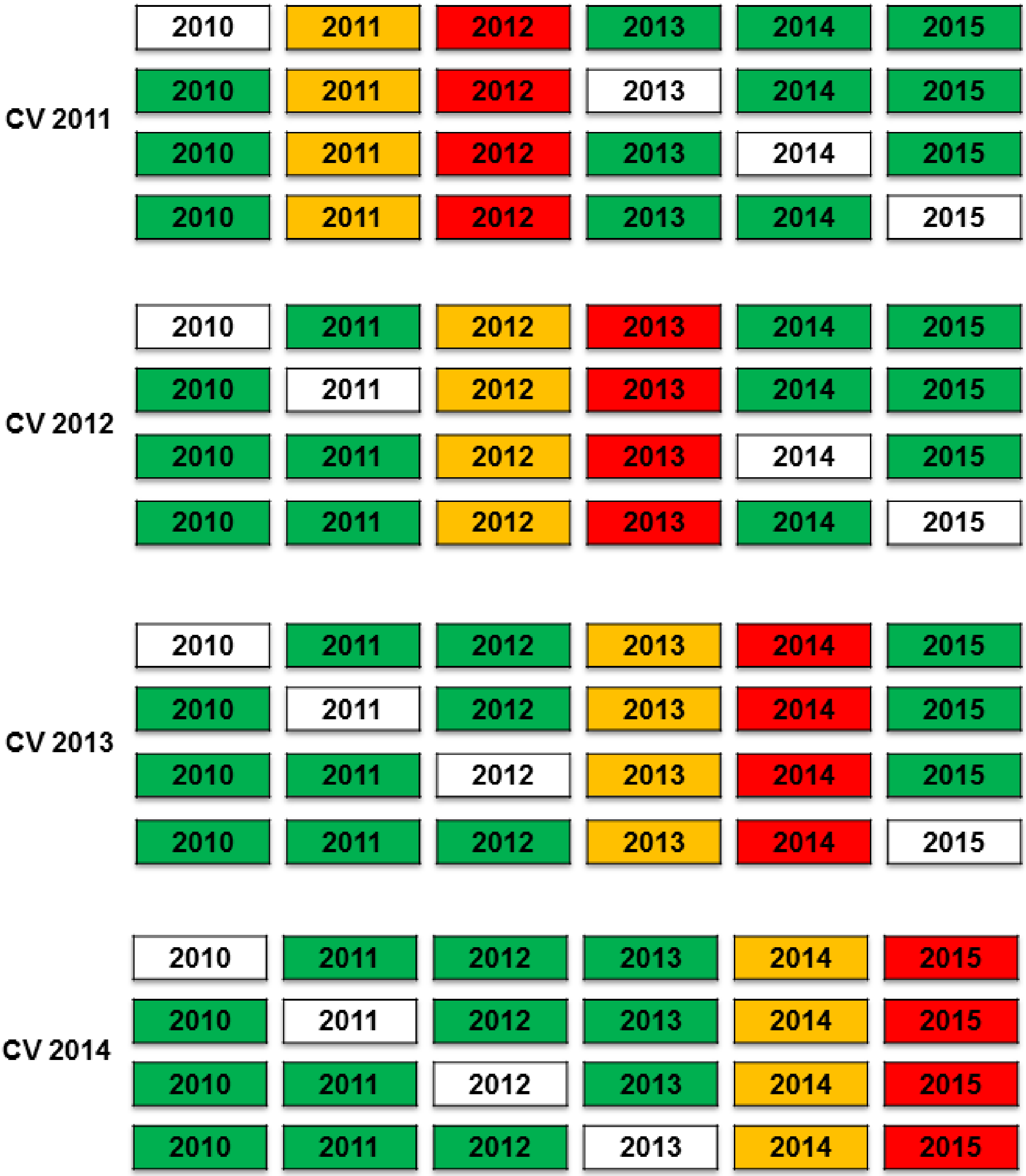


Fig. S1 Variation of grain yield and protein content in preliminary yield trials 2010–2014.



CV 2011	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
CV 2012	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
CV 2013	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
CV 2014	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015
	2010	2011	2012	2013	2014	2015

Fig. S2 Cross-validation scheme used for comparing the different selection methods. Genomic selection models were fitted with training populations of 180 lines, where 60 lines of this training population came from 3 different years (green). Phenotypic and genomic assisted selection included additional data from the year of a preliminary yield trial (orange). All models were validated with a validation population of lines retested in multi-environment trials following the year of a preliminary yield trial (red).

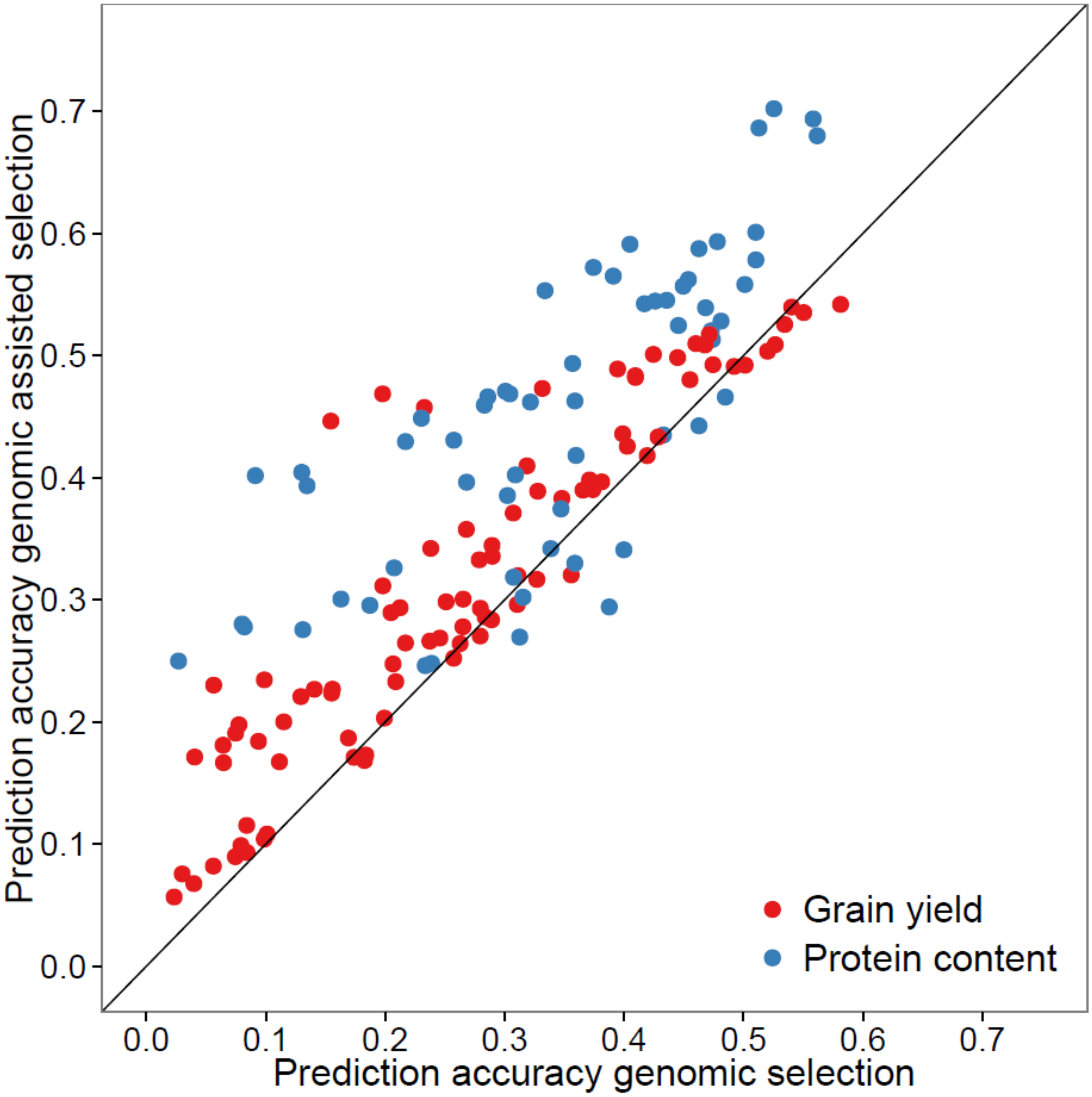


Fig. S3 Comparison between the prediction accuracy of genomic and genomic assisted selection for every training by selection population combination to predict grain yield and protein content of individual trials across years.

Declaration

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt, keine anderen als die angegebenen Hilfsmittel benutzt und alle aus ungedruckten Quellen, gedruckter Literatur oder aus dem Internet im Wortlaut oder im wesentlichen Inhalt übernommenen Formulierungen und Konzepte gemäß den Richtlinien wissenschaftlicher Arbeiten zitiert, durch Fußnoten gekennzeichnet bzw. mit genauer Quellenangabe kenntlich gemacht habe.

Tulln an der Donau, June 2017

Sebastian Michel