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Genomic Selection for Disease Traits in Wheat

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*Dedicated
to Leonor*

*Dedicado
a Leonor*

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Declaration

I hereby declare that I am the sole author of this work; no assistance other than that permitted has been used and all quotes and concepts taken from unpublished sources, published literature or the internet in wording or in basic content have been identified by footnotes or with precise source citations.

Tulln an der Donau

April 2021

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Abstract

Fusarium head blight (FHB) is one of the most hazardous diseases affecting small grain cereal grains including both bread and durum wheat, with the latter being especially susceptible. Aside from agronomic management, the most sustainable approach to counter fight against FHB is the development and growth of resistant varieties. Resistance to FHB in wheat is highly complex and quantitatively controlled by just a few medium to large effect QTL and numerous of loci with small effects, which makes it a perfect target for genomic prediction (GP) approaches. Selection of candidates for disease resistance based on genomic estimations or genomic selection (GS) have shown promising results.

A precedent study analysed a European multi-environment trial featuring a diversity panel of 186 durum wheat lines in the context of genome-wide associations revealing the negative correlations of FHB severity and both plant height (PH) and heading date (HD). Therefore, in the present work it was proposed to evaluate alternatives for the genomic prediction of FHB resistance involving the correlated traits PH and HD with three different approaches: (1) correcting the FHB severity scores using PH and HD followed by using the corrected phenotypic data for single-trait GS (STGS), (2) using PH and HD for multi-trait GS (MTGS), and (3) using selection indices to modulate the restrictions for FHB severity, PH and HD. The multi-trait GP model with HD gave a significant boost in prediction ability although with the concomitant larger response towards earliness, while the usage of restricted indexes led to reduction in prediction abilities for FHB but also a stricter control of an unfavourable response for PH and HD.

Anther retention (AR) is an agronomically neutral floral trait linked to the FHB resistance, and breeding against it is recommended as indirect selection strategy for resistant wheat genotypes. It is also known that the type of association between earliness and FHB susceptibility is highly dependent on weather conditions around anthesis and even more recent changes in climate patterns are believed to have contributed to more frequent FHB epidemics. The latter statements motivated the following aims: (1) to incorporate thermal measurements in the correction of FHB severity scores, and (2) to evaluate trait-assisted genomic prediction models incorporating anther retention. The first aim was achieved by using a best subset multi-linear regression analysis combining flowering time (FT) and accumulated thermal time (ATT) variables. Phenotypic accuracy for corrected scores was up to three times higher with the suggested correction. Analogously, GP model accuracies were on average higher when the corrected FHB severity scores were predicted compared with the uncorrected scores. Better performances were also found for MT.GS models with AR as assisting trait. Another important feature of the genomic predictions based on corrected scores was the higher consistency when lines from different trials were combined in larger training sets.

Genomic-based selection for FHB resistance has thus a high potential and is already being applied in many practical breeding schemes, as it facilitates saving both time and field space when conducted in early generations. The across-cycle GP must be carefully assessed since several studies already showed its lower accuracy, and therefore training population composition is a critical factor. In addition, specific and when possible, effective actions must be taken to balance responses to selection for FHB resistance and correlated traits that consider the cause and strength of unfavourable trade-offs. Finally, the importance to expand the research for the multitude of traits involved in the resistance to FHB open-up further possibilities like bringing the latest technologies like phenomics and high-throughput phenotyping to the field.

Zusammenfassung

Ährenfusariosen (FUS) gehören zu den schwerwiegendsten Krankheiten welche kleinkörnige Getreidearten wie Brot- und Hartweizen befallen, wobei die Letztere als besonders anfällig gilt. Neben diversen agronomischen Maßnahmen stellen die Züchtung und der Anbau von resistenten Sorten die nachhaltigsten Maßnahmen zur Bekämpfung von Ährenfusariosen dar. Die genetische Resistenz gegen Ährenfusariosen unterliegt einem quantitativen Erbgang und wird durch zahlreiche chromosomale Regionen kontrolliert von denen nur wenige einen mittleren bis großen Resistenzeffekt bewirken. Der Großteil der Resistenz wird durch chromosomale Regionen mit geringem Effekt bewirkt, was die Verbesserung der Ährenfusariosenresistenz zu einem perfekten Anwendungsgebiet zur Nutzung genomischer Vorhersagen für die züchterische Selektion macht.

In einer vorangegangenen genomweiten Assoziationskartierung zeigte sich eine deutlich negative Korrelation zwischen Ährenfusariosenresistenz (FUS) und der Wuchshöhe (WUH) sowie dem Zeitpunkt des Ährenschiebens (AE) in einer mehrortig geprüfte Hartweizenpopulation von 186 Linien. In der vorliegenden Arbeit wurden daher verschiedene Methoden der Genomischen Vorhersage von FUS unter Einbezug der korrelierten Merkmale AE und WUH untersucht: Genomische Vorhersagen mit (1) phänotypisch adjustierten Beobachtungen von FUS für AE und WUH, (2) Multivariate Analyseverfahren und (3) Selektionsindices für FUS mit Restriktionen für den korrelierten Selektionserfolg von AE und WUH. Die Multivariate Analyseverfahren resultierten in einem signifikant höherem Selektionserfolg für FUS, welcher jedoch mit einem ungünstigen Selektionserfolg für Spätreife verbunden war. Die Verwendung von Restriktionsindices führte zu geringeren Vorhersagegenauigkeiten für FUS, jedoch konnte die ungewünschte indirekte Selektion für Spätreife und größere Wuchshöhe reduziert werden.

Die Antherenretention (AR) ist ein agronomisch neutrales Merkmal das eng mit Ährenfusariosenresistenz korreliert ist, wobei offenblühende Genotypen eine eher geringere Anfälligkeit ausweisen. Darüber hinaus wurde bereits in der Vergangenheit die Hypothese aufgestellt, dass die oft-berichtete Assoziation zwischen Frühreife und Ährenfusariosenresistenz stark von den Wetterbedingungen während der Blüte beeinflusst ist. Die Beiden genannten Faktoren waren ausschlaggebend um (1) die Inbezugnahme von thermischen Variablen zu bei der Auswertung von Ährenfusariosenbonituren zu untersuchen und (2) die Möglichkeit die Genomische Selektion für Ährenfusariosenresistenz mit Multivariate Analyseverfahren unter Einbezug der Antherenretention zu evaluieren. Die Genauigkeit der Phänotypischen Daten für Ährenfusariosenresistenz konnte durch die Korrektur für das Blühdatum und die thermischen Variablen durch multivariate Regressionsmodelle verdreifacht werden. Die Vorhersagegenauigkeit der genomische

Modelle konnte analog verbessert werden, wobei die vorgeschlagene Methode vor allem der Kombination von Trainingspopulationen aus verschiedenen Feldversuchen und der Nutzung der Antherenretention in multivariaten Vorhersagemodellen förderlich war.

Die Genomische Selektion für Ährenfusariosenresistenz hat somit großes Potential und wird bereits in zahlreichen Zuchtprogrammen routinemäßig angewandt, da sich aus dieser Methode viele Vorteile wie eine deutliche Zeitersparnis und ein geringerer Aufwand für Feldversuche ergeben. Die genomische Vorhersage über Züchtungszyklen hinweg muss jedoch genau untersucht werden da hier geringere Genauigkeiten zu erwarten sind. Die Zusammenstellung der Trainingspopulation und der Einbezug von korrelierten Variablen bei komplexen Merkmalen wie der Ährenfusariosenresistenz sind somit entscheidende Faktoren für eine erfolgreiche Anwendung der Genomischen Selektion im praktischen Zuchtbetrieb. Die vorliegende Arbeit dient daher als Ausgangspunkt für ein weiteres Studium des Weizen-Ährenfusariosen-Pathosystems mit weiteren Methoden welche sich durch die technologischen Fortschritte, wie die Verfügbarkeit günstigerer Hochdurchsatzphänotypisierungsplattformen, eröffnet haben.

Resumen

Fusarium de la espiga de trigo (FET o en inglés Fusarium Head Blight) es una de las enfermedades más peligrosas que afecta a los cereales de grano pequeño, incluyendo trigo harinero y trigo duro, siendo este último especialmente susceptible a dicha enfermedad. Además del manejo agronómico, el enfoque más sostenible de lucha contra el FET es el desarrollo y cultivo de variedades resistentes. La resistencia a FET en trigo es altamente compleja y cuantitativamente controlada por pocos QTL de efecto moderado y numerosos loci con efectos pequeños, lo que lo convierte en una diana perfecta para aproximaciones de predicción genómica (PG o en inglés genomic prediction). La selección de genotipos candidatos para resistencia a enfermedades basada en estimaciones genómicas, a saber, selección genómica (SG o inglés genomic selection), ha mostrado resultados prometedores.

En un estudio precedente se analizó un ensayo multi-ambiente en Europa con un panel de diversidad compuesto por 186 líneas de trigo duro en el contexto de asociaciones del genoma completo (en inglés, GWA Genome-wide association) se revelaron correlaciones negativas entre la severidad de FET y los rasgos de: altura de planta (AP) y fecha de espigado (FE). Por lo tanto, en el presente trabajo se propuso evaluar alternativas de predicción genómica para resistencia a FET que involucren los rasgos asociados AP y FE con tres diferentes aproximaciones: (1) corregir las mediciones de severidad de FET usando AP y FE seguido del uso en predicciones genómicas de rasgos fenotípicos sencillos o simples (PG.RS), (2) emplear AP y ET en predicciones genómicas para rasgos múltiples (PG.RM), y (3) uso de índices de selección que modulen las restricciones para severidad de FET, AP y FE. El modelo de PG de rasgos múltiples con FE dio un impulso significativo en la habilidad de predicción aunque con la concomitante mayor de respuesta hacia madurez, mientras que con el uso de índices de restricción se redujo la habilidad de predicción para severidad de FET, pero también un control más estricto de la respuesta desfavorable para AP y FE.

La retención de anteras (RA) es un rasgo floral agronómicamente neutral ligado a la resistencia a FET y la mejora en su contra es recomendada como estrategia de selección indirecta para genotipos de trigo resistentes. Es también conocido, por otro lado, que el tipo de asociación entre madurez y susceptibilidad a FET es altamente dependiente de las condiciones meteorológicas alrededor de la antesis y que incluso los recientes cambios en los patrones climáticos habrían contribuido a brotes epidémicos más frecuentes de FET. Las anteriores afirmaciones motivaron los siguientes objetivos: (1) incorporar mediciones de temperatura en la corrección de la severidad de FET y (2) evaluar modelos de predicción genómica asistidos para el rasgo RA. El primer objetivo se consiguió con el empleo de un análisis de regresión lineal múltiple combinando las variables fecha de antesis (FA) y de tiempo térmico acumulado (TTA). Las precisiones fenotípicas para las mediciones corregidas

fueron hasta tres veces superiores con el método de corrección propuesto. Análogamente, las precisiones de los modelos de PG fueron en promedio superiores cuando las mediciones corregidas de severidad a FET fueron predichas, comparadas con las mediciones no corregidas. También fueron detectados mejores desempeños por parte de los modelos de PG de rasgo múltiple asistidos con RA. Otra característica importante de los modelos de predicción genómica basados en mediciones corregidas fue su mayor consistencia cuando líneas de diferentes ensayos fueron combinadas en sets de entrenamiento aumentados.

La selección genómica para resistencia a FET tiene entonces un gran potencial y está siendo aplicada en varios esquemas prácticos de mejoramiento ya que permite el ahorro de tiempo y espacio cuando es llevado a cabo en generaciones tempranas. Los modelos de PG interciclo deben ser evaluados cuidadosamente ya que varios estudios mostraron su baja precisión y por ende la composición de las poblaciones de entrenamiento de los modelos es un factor crítico. Adicionalmente, acciones efectivas deben tomarse cuando sean posibles para mantener el balance entre la respuesta a la selección para resistencia a FET y los rasgos correlacionados que se consideren la causa y magnitud de dichas desfavorables asociaciones. Finalmente, la importancia de expandir la investigación para una multitud de rasgos involucrados en la resistencia a FET abre las puertas a futuras posibilidades de llevar al campo tecnologías de vanguardia como la fenómica y el fenotipado de alto rendimiento (high-throughput phenotyping).

Introduction

The crops

Bread wheat (*Triticum aestivum* L.) is one of the top-three most important crops in the world, providing the calorific energy for around 20% of the world population (<http://www.fao.org/faostat>). It is the first and second source of protein and calories in developing countries [1–3]. In the year 2019–2020, global wheat production was 765.41 million tonnes over an area of 218.5 million ha (Statista 2019).

Durum wheat (*Triticum turgidum* L. var *durum* Desf.) is the 10th worldwide cultivated cereal with a yearly average production of 40 million tonnes in 2016/17 [4]. Durum is cultivated in a mix of variable and harsh environmental conditions, but primarily in three different regions: the Mediterranean basin, the Northern Plains in North America, and the south west of the United States and Mexico [5]. Durum is mainly produced for making pasta while couscous and bulgur are likewise important food products in North Africa and the Middle East. Couscous and pasta are products made from durum semolina resulting from milling of the hard-textured durum wheat kernel, whereas bulgur is obtained by cracking parboiled durum grains. Durum production reached 16 million hectares globally in 2020, which represented 5% of total wheat production [4].

The trait: Fusarium Head Blight resistance

Causative agents, epidemiology

In wheat, Fusarium Head Blight (FHB) is attributable to a number of *Fusarium* species such as the *F. graminearum* species complex (FGSC) which is the worldwide most important agent [6]. The latter is together with *F. culmorum*, *F. avenaceum* and *F. poae* among the abundant for wheat in Europe [7]. Additionally, *Fusarium asiaticum* was reported to produce FHB in durum wheat in Japan [8].

The mixed reproduction system that combines sexual and asexual reproduction allows genetic recombination, and the propagation of clones within a genome with low levels of repetitive elements leads to its rapid adaptation to selection pressures [9, 10]. *F. graminearum* also can display several pathogenicity factors to ultimately invade a host under various circumstances thus helping to explain the partial levels of resistance displayed in wheat [11, 12].

Fusarium belongs to the *Ascomycota* phylum. It can infect kernels, spikelets, or the full head of small grain cereals. Infection bodies are derived mainly from residues of previous crops. Infection begins when either the airborne perithecial bodies that colonized the host spikes release the sexual spores (ascospores) or via a splash dispersion of the asexually produced

spores (macroconidia). Germination takes place when the germ tubes differentiate to hyphae in a process that can take 6-12 hours on wheat [13]. Anthesis is the period of the highest host susceptibility on wheat since the swelling of lodicules that open the floret make the host vulnerable to infection. Once florets get opened, germ tubes may grow into the inner cavity. Another important factor is the formation of choline and betaine during anthesis as both have been reported as growth stimulants for *F. graminearum*. After a short biotrophic period the pathogen shifts to a necrotrophic phase that increases its proliferation. This phase is associated with an amplified production of mycotoxins to overcome the rachis and enable a spread throughout the spike tissue. Promoted by high humidity, hyphae may even grow on the outside of spike surface and infect distal florets. The expression of FHB appears as the bleaching of portions of the spike and kernels that finally become chalk-like tombstones.

FHB disease: epidemics, impact and control

One of the earliest descriptions of the FHB causing agent and the disease symptoms dates to the end of 19th century during an epidemic in the UK [14]. Few years later in 1890, the first FHB reports appeared in the USA [15–17]. During the first half of the last century, first reports of FHB appeared in Canada (1919) and in southern China (1936) where seven severe and 14 medium outbreaks have been reported throughout the 20th century. A severe outbreak affected Ireland in 1954 [18]. The FHB epidemic in 1993 that affected the wheat growing states of Minnesota, North Dakota and South Dakota in the USA and the Canadian province Manitoba resulted in yield losses of 4.8 million tonnes and an economic loss of over 1 billion US dollars [19]. In the beginning of the 21st century, important outbreaks were reported in Australia [20], Brazil [21], and China [22]. The severe epidemic of 2014-2016 in North America, with disease incidences up to 80% in some production fields in Georgia, caused significant yield losses in the Canadian province of Saskatchewan as well [23]

Usually the overall economic impact arises from the sum of both direct and secondary losses. Wheat yield losses were reported to range between 2% and 54% during 1997-2011 in a state-case study [24]. Additional studies have confirmed the strong correlation of FHB severity and yield reduction [25, 26]. Moreover, in terms of global economic impact *F. graminearum* has been considered as the fourth most threatening plant-pathogenic fungus in the world [27].

Additionally, the greatest impact of FHB is the contamination by various mycotoxins, particularly trichothecenes (e.g. DON and its derivatives) and the mycoestrogen zearalenone (e.g. ZEA). The consumption of zearalenone contaminated grains can cause intestinal irritation, vomiting, skin and immunological problems in mammals and feed refusal in livestock

[28, 29]. Therefore, legal maximum limits for the presence of mycotoxins in feed and food have been set in Europe and a range of countries worldwide [30]

Host-pathogen interactions are typically controlled by qualitative and quantitative resistance mechanisms. The former bases its mechanisms on resistance genes coding for proteins that trigger the so called effector-triggered immunity while the hypothesized mechanisms for the latter include among others the regulation of morphology traits, basal defence, production of anti-fungal compounds, and defence signal transduction [31, 32]. Resistance against FHB is however mostly quantitative in nature [33] and reflects controlled by many (hundreds to thousands) genes with small to moderate effects highly influenced by environment, plant organ and developmental stage. The magnitude of quantitative disease resistance QDR varies continuously from very small to nearly full resistance in visual assessments of symptoms. This complex inheritance and the fact that QDR is typically non-race specific provides a higher durability than qualitative DR [34].

Several types of resistance against FHB have been described, i.e. type I: resistance to initial infection, type II: resistance to spread from the initial point of infection, type III: resistance to kernel infection, type IV: tolerance to FHB yield loss, type V: resistance to DON accumulation, type VI: resistance grain constituents' modifications [35, 36]. Under field conditions disease severity scoring like an estimation of the average percentage of symptomatic spikelets in a spike in a given plot reflects both type I and II resistance. Given that initial infections may occur in several spikelets within a single spike, genotypes with fewer initially infected spikelets will most likely have higher type I resistance [37]. In bread wheat, genetic variation for FHB resistance is large and a range of resistance sources from “exotic” and “native” wheat germplasm are known [38]. Contrastingly, durum wheat resistance source are scarce and most durum wheat germplasm is susceptible to FHB [39, 40].

Breeding for FHB resistance

Chemical fungicides and agronomic practices have proven to be only partially effective for controlling FHB [24, 41], with genetic resistance offering the most promising approach for limiting the economic and ecological impacts of the disease [42, 43].

Although breeding for resistance to pests and diseases is usually considered as the most cost effective and sustainable approach against significant economic losses, factors such shifts in climate and the subsequent changes in weather patterns, cropping system responses, and pest and pathogen populations might result in altering breeding targets and goals faster and more frequently [44].

The main goal of breeders is to transfer resistance to FHB into lines with desirable quality and agronomic performances. The quantitative nature of this resistance in wheat task a challenging endeavour if only conventional breeding methods are used since with more than 550 quantitative trait loci (QTL) have been reported in the literature [45, 46]. Additionally, seven of these QTL have been fine mapped [47]. *Fhb1* was the first FHB resistance gene to be cloned but its true identity and function remains controversial. *Fhb2* does not seem to provide high resistance by itself [33]. In adapted European germplasm [45, 48] low to medium effect loci have been found, that might be accumulated to provide a higher resistance level by recurrent selection to accumulate numerous resistance loci. Difficulties might arise however with such an approach as FHB resistance is prone to complex GxE interactions [45], which oftentimes result in low to medium heritabilities.

Correlated traits

Morphological and developmental characteristics such as plant height, days to heading or flowering, anther extrusion (AE), head compactness [49, 50], content and composition of cell wall polymers [51], spike length, spike inclination, spike density, and spikelet number [52] are among the traits affecting FHB resistance.

Contrarily to earliness, the relation of FHB resistance with plant height, stating that the taller the plants the more resistant they tend to be, has been consistently demonstrated by numerous studies with wheat [35, 49, 53–59]. Plant height in wheat is controlled by reduced height *Rht* genes, and since the Green Revolution until present days selection towards their semi-dwarfing alleles has been used to achieve high yielding cultivars in many breeding programs [60].

Furthermore, co-localizations of PH and FHB resistance loci were found on chromosomes 2D (*Rht8*) [61], 4B (*Rht1* or *Rht-B1*) [62–64] and 4D (*Rht2* or *Rht-D1*) [64, 65]. On the other hand, it has been recently shown that a major dwarfing gibberellin-sensitive gene, *Rht24* located in chromosome 6A, did not increase FHB severity in a winter wheat population [66]. Some others mechanisms besides tight linkage might explain associations among these traits e.g. differences in stature per se, which might confer a disease escape that has been sometimes attributed to be a major explanation for the PH-FHB resistance trade-off in durum wheat [67, 68]. More recently [59] conjectured that spikes of taller plants dry faster and will be less affected by the disease. Finally, other possible explanation can arise from pleiotropic effects of the *Rht* genes *Rht-B1* and *Rht-D1* in wheat, causing a point-nonsense mutation in DELLA domain proteins, responsible for ultimately both shorten plant height [69–71] and possibly

allied with some physiological responses to FHB infection like cell death [60] or further pleiotropic instances like reduction of peduncle length or anther extrusion diminishment [72].

The latter trait anther extrusion (AE) shows a complex and quantitative inheritance [73] has also revealed to be associated with the FHB pathosystem in several studies [50, 72, 74]. Some *Rht* genes (*RhtD1*, *RhtB1*) were found to co-localized and negatively affect AE unlike *Rht24* which has been suggested for use in male lines for hybrid wheat breeding [75] as it appears not to have adverse effects on FHB resistance nor on maturity [66].

Genomic selection

Genomic selection (GS) is a modified form of marker-assisted selection (MAS) meaning that instead of using a restricted amount of markers genome-wide distributed markers are taken into account [76, 77]. A major assumption of this method is that each QTL associated with the trait of interest is targeted by at least one marker by linkage disequilibrium (LD) between them. GS implementations start training a given GP model with a training set of genotyped and phenotyped individuals and infer so-called genomic estimated breeding values (GEBV) for a validation set that has only been genotyped. Breeders can then make use of those GEBVs to select the best or discard the poorest performing individuals or breeding lines [78]. Although the seminal GS paper [77] suggested the usage of high dense SNP markers technologies for animal breeding, some previous reports already suggested the merit of employing molecular markers to predict the performance of hybrid maize [79] or animals [80].

Prediction accuracy of GP models is equivalent to the accuracy in the breeders' equation and therefore proportional to genetic gain [76]. Several factors are governing GS accuracy as recently reviewed by [81] e.g. marker density, statistical modelling, training population size, trait heritability, relatedness between training and validation sets, population structure, and retraining the GP models after several selection cycles. Regarding the GP model of choice, a plethora of parametric or non and semiparametric models have been developed in recent years. Parametric models under equal marker variance assumption include ridge regression-best linear unbiased prediction (RR-BLUP) [77] and GBLUP [82], while models from the Bayesian alphabet are also considered to be parametric but some of them assume heterogeneous marker effect variances following a prior defined distribution. Semiparametric models comprise e.g. kernel regression and reproducing kernel Hilbert spaces (RKHS) [83] while nonparametric include random forest or the variety of deep learning models. The main advantage of the latter two model classes is that they also allow to capture non-additive effects. Aside from accuracy, several studies have additionally compared GS and phenotypic selection in terms of response to selection. For instance, it took the same to two rounds of GS to perform equivalent to one round of phenotypic selection when breeding for resistance to

stem rust in wheat [84]. Responses 18 and 43 percent greater were achieved for GS compared to MAS in maize [85] and no significant differences in the response to selection were found between GS, PS and MAS in oats [86].

Given the complex and quantitative genetic architecture of FHB resistance, GS seems an ideal candidate tool for its study since phenotyping for FHB and mycotoxins is expensive and laborious. GS models are on the other hand able to inform breeders about selection candidates' performance in early breeding stages when FHB phenotyping is less feasible due to the large sample sizes even if PS can be more accurate than GS in some cases. Steiner et al [40] showed a 43% advantage of GS over PS using in a two-stage selection scheme. In a panel of 322 genotypes panel four models (RR-BLUP, Lasso, RKHS and RF) and two sets of markers (whole set and a subset of FHB related markers) were investigated to predict six FHB related traits [87]. Arruda et al [88] tested several training set sizes and marker subsets in a soft red winter wheat population and found that the best prediction accuracies were achieved with a density marker between 1500 and 3500 SNPs and a RR-BLUP model. The RR-BLUP model has been one of the most common used models and others like Lasso or Bayes are less common with the lower computational demand one of the reasons for the broader adoption of the former [89]. To improve predictions GP models can furthermore use loci identified by GWAS as fixed effect. Arruda [90] reported 15% higher accuracies for this GS plus de novo GWAS approach, although an artefact might be introduced if both TS and VS are used to identify QTLs. The expected transformation in the coming years include areas such: (1) prediction of the progeny's potential (2) prediction beyond additive effects (3) usage and exploit of pleiotropy and high-throughput phenotyping HTP (4) inclusion of the genetic architecture into GS models (5) utilization of big data and machine learning approaches [91].

Genomic selection for multiple traits

A necessity in crop breeding is the selection for multiple traits from several categories i.e. resistance to biotic and abiotic stress, grain quality as well as yield components. Multi-trait selection poses the challenge of balancing different breeding objectives and different strategies have been suggested for this purpose: (i) tandem selection i.e. different traits are sequentially selected in different generations [92] (ii) independent culling of traits each under different thresholds in the same generation (iii) index selection by switch weighted linear combinations between traits based on e.g. their economic importance of each trait and (iv) Pareto optimal solutions [93].

Selection indices (SI) can be either directly incorporated into the GS framework as new traits or in an indirect manner where SI are calculated based on multi-trait GS (MTGS) models. A study in rye [94] showed that better predictabilities might be obtained when directly

incorporating SI. Breeding goals in this context become crucial as e.g. maximizing one trait while keeping minimum standards on the others can also be addressed by specific restriction indexes [95]. Additional optimal solutions have come from nonlinear indexes [96] or more recently the look-ahead selection (LAS) algorithm which simultaneously maximizes the focal trait while constraining the indicator traits within flexible ranges at a given resource allocation, mating plan and selection strategy as exemplified in a study in maize [97].

Some complex traits being selected by breeders are genetically correlated and oftentimes share a common genetic architecture. Although GS models were at first developed to calculate GEBVS of a single trait, the advantages of MT.GS models with correlated traits were soon demonstrated in animal breeding [98]. Jia [99] showed first evidence of how the prediction of low heritable traits can benefit from correlated disease resistance traits with higher heritabilities. Additionally, the study of Fernandes et al [100] was among the first reports showing that if the secondary trait is available for both testing and training lines the accuracies could be improved up to 50% versus the single trait versions. The latter cross validation strategy has been called CV2 or trait assisted genomic selection. Bayesian and GBLUP models mostly have been extended to multi-trait versions and applied in several studies in crops as reviewed by Tong and Nikoloski [101]. Deep learning approaches have been also attempted e.g. in a wheat population multi-trait DL models outperformed the single trait versions but failed to do it with the single trait GBLUP [102, 103]. Various studies in wheat [104, 105], sorghum [100], and barley [106] have given proofs that predictabilities were boosted by using the trait-assisted GS. Predictabilities in a CV2 scenario may be biased since the usage the records of assisting traits in the testing population and Runcie and Cheng [107] proposed an alternative CV2* scenario using the phenotypic data of the close relatives of the genotypes in the testing population.

Phenomics has brought also new heritable correlated measurements or completely new traits like hyperspectral imaging. Algorithms capable to detect wheat spikes from images collected by Ground Mobil imaging open new perspectives on future application of deep learning models to detect infected spikes [108]. In this sense, high throughput phenotyping (HTP) has significantly enlarged data for additional traits in early stages of selection aiming to increase selection intensity in a combined phenomic-genomic breeding strategy [109, 110].

Publication 1

Genomic predictions for Fusarium head blight resistance in a diverse durum wheat panel: an effective incorporation of plant height and heading date as covariates

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Genomic predictions for *Fusarium* head blight resistance in a diverse durum wheat panel: an effective incorporation of plant height and heading date as covariates

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Abstract Selection for multiple traits is a highly challenging task for breeders due to potential unfavorable associations between characters. *Fusarium* head blight FHB, being one of the most relevant diseases affecting durum wheat frequently shows in this respect an unfavorable correlation with morpho-agronomical traits like plant height (PH) and heading date (HD). In this study, we used a cross-validation scheme to assess the prediction ability of the genomic predictions (GP) for FHB severity relying on genomic best linear unbiased prediction models in a diverse panel of 178 durum wheat lines evaluated across five environments. Additionally, we compared three types of approaches to include HD and PH as covariates into the analysis: (1) correcting FHB severity values before training GP models, (2) tuning the GP model parameters that included multi-trait alternatives, and (3)

adjusting the genomic-based predictions by restriction indexes. Models that weighted genomic estimated breeding values (GEBV) by restriction indexes as well as models that predicted FHBms values corrected by regression-based methods were efficient alternatives in diminishing the HD trade-off, nonetheless they were also associated with large reductions in prediction ability for FHB severity. After a simulated round of genomic selection, considering HD as fixed effect in the GP model were the most suitable alternative to select a higher proportion of genotypes moderately resistant with lower-than-average HD and PH estimations. Hence, an appropriate GP model given unfavorable association between characters should combine high predictabilities and adequate reduction of undesired trade-offs.

Keywords *Fusarium* head blight · Genomic prediction · Durum wheat · Covariates · Restriction indexes

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Introduction

Fusarium head blight FHB, also called scab, is one of the most calamitous diseases affecting cereals such as wheat, barley, maize and oat (Walter et al. 2010; Beres et al. 2018). FHB is mainly caused by *Fusarium graminearum* and *Fusarium culmorum* with the former being considered as the fourth most economic

and scientifically important plant fungal pathogen worldwide (Dean et al. 2012). The disease' effects comprise losses in grain yield and quality as well as contamination by mycotoxins like deoxynivalenol (DON) and its acetylated derivatives, which in turn are a serious threat for human and animal health and malting purposes (Darwish et al. 2014; Dweba et al. 2017; Fung and Clark 2004). In comparison to hexaploid bread wheat (*Triticum aestivum* L.), the tetraploid durum wheat (*Triticum turgidum* L. ssp. *durum*) is particularly susceptible to the Fusarium species complex which makes the development of resistant varieties a major breeding goal. Durum wheat is a cereal crop with an annual global production of 39 million of tons corresponding to 5% of the total wheat production (Kadkol and Sissons 2016). Besides bread and bulgur, other essential foodstuffs like couscous and pasta, consumed by hundreds millions people worldwide, are prepared based on semolina that is the granular milled product of durum grains (Fiedler et al. 2017; Tuberosa and Pozniak 2014). A certain level of control over FHB can be achieved with a series of cultural, biological and chemical control measures plus cultivar resistance (Pirgozliev et al. 2003). Moreover, and within a Genotype \times Environment \times Management (G \times E \times M) interaction framework, the development and adoption of cultivars tolerant to FHB is and will remain to be the cornerstone of any strategy aiming to manage this disease (Beres et al. 2018).

FHB resistance (FHB_r), is a trait whose quantitative inheritance is controlled by many loci each with small effect which leads to a slow genetic gain per unit of time. More than 500 QTL have been described for FHB resistance on all 21 chromosomes of hexaploid wheat (Buerstmayr et al. 2009; Jia et al. 2018; Liu et al. 2009; Venske et al. 2019), which is not the case in tetraploid wheats where only a small number of loci are reported (Prat et al. 2014). For the latter wheat species FHB_r loci have been mapped on all chromosomes except 1A, 1B and 5A, though all of them possess only small or moderate effects compared to the major resistance loci in hexaploid wheat e.g. *Fhb1* located on chromosome 3BS, *Fhb2* located on 6BS and *Qfhs.ifa-5A* all derived from the Chinese resistance cultivar "Sumai 3" (Prat et al. 2014; Zhao et al. 2018). In addition, within the durum gene pool there is a narrow genetic variation for FHB_r in both the mostly susceptible elite cultivars (Buerstmayr et al. 2003; Miedaner and Longin 2013) and landraces, while

among the latter only few have shown some elevated level of resistance (Huhn et al. 2012; Talas et al. 2011). Other wild wheat tetraploids relatives (*T. turgidum* ssp. *dicoccoides*, *T. turgidum* ssp. *dicoccum*, and *T. turgidum* ssp. *carthlicum*) have shown moderate to high resistance levels (Oliver et al. 2007, 2008) although the incorporation of loci from such sources into elite germplasm faces some obstacles like linkage drag, unfavorable epistatic interactions or pleiotropic effects (Kumar et al. 2018; Prat et al. 2014; Zhu et al. 2016). Nevertheless, a couple of studies in this direction have been conducted and reported successful pyramiding of resistance genes from hexaploid wheat together with native durum loci (Prat et al. 2014; Zhao et al. 2018).

Marker assisted selection (MAS) has proved to be a suitable and effective instrument for breeders when targeting a trait with small number of large effect genes like the stripe (yellow) rust resistance gene Yr15 in durum wheat (Yaniv et al. 2015). The actual implementation of MAS for quantitative inherited traits with an underlying complex genetic architecture is though less feasible, while genomic prediction (GP) has overcome such limitations since it uses genome-wide markers to estimate the breeding values of unobserved traits in a panel of selection candidates targeting also the multitude of minor QTL (Meuwissen et al. 2001). Precisely, GP models involving FHB resistance in hexaploid wheat (Arruda et al. 2015, 2016b; Mirdita et al. 2015; Rutkoski et al. 2012; Schulthess et al. 2017; Dong et al. 2018) and in durum wheat (Miedaner et al. 2017; Steiner et al. 2018) have shown higher prediction accuracy and larger selection gain than traditional MAS approaches. With few exceptions (Schulthess et al. 2017; Steiner et al. 2018), suggestions and proofs of the advantages of including correlated traits in GP models have been barely explored for FHB_r in wheat, even though the well-known associations with others traits like heading date or plant height are obvious. In this regard, earlier flowering genotypes are generally more susceptible to FHB and most of the published evidence support the existence of such negative and significant effect in both bread wheat (Gervais et al. 2003; He et al. 2016; McCartney et al. 2016; Paillard et al. 2004; Schmolke et al. 2005; Yi et al. 2018) and durum wheat (Buerstmayr et al. 2012; Miedaner et al. 2017; Miedaner and Longin 2013; Prat et al. 2017). Loci affecting both traits were found to overlap specifically

on chromosomes 4A, 5B, 6A, 6D and 7B in various of the mentioned studies, although the correlation FHB-heading date might be also highly dependent on environmental factors like temperature, rainfall and humidity (Miedaner and Longin 2013). Another trait studied in the host response to FHB is plant height where previous findings suggest a clear negative and significant trade-off between shortness and resistance to FHB in bread and durum wheat (Buerstmayr et al. 2012; Miedaner and Longin 2013; Prat et al. 2017; Talas et al. 2011). One of the most relevant height-reducing mutant alleles, *Rht-B1b*, has been intensively introgressed into elite durum wheat germplasm since the 1960s' and important loci for FHB have been mapped at the *Rht-B1* position on chromosome 4B, (Buerstmayr et al. 2012; Miedaner et al. 2017; Prat et al. 2017; Steiner et al. 2018) suggesting a pleiotropic effect of the latter on susceptibility to FHB (Srinivasachary et al. 2009).

In view of the findings described by Miedaner et al. (2017) regarding to the potential and improvements needed to implement GP methods for FHB, the scope of such endeavor has thus to be widened to deal with the multiple involved traits. Hence, the main objectives of this study were (1) to evaluate the implementation of different approaches in order to include the records of two covariates namely heading date and plant height into GP models and (2) to assess the performance of the developed GP models in terms of prediction accuracy and selection gain for all involved traits.

Materials and methods

Plant material

The international diversity panel in matter contained 170 winter and 14 spring types, including modern and old cultivars as well as current breeding lines. Field trials were conducted at the experimental stations Heidfeldhof (Hoh) and Oberer Lindenhof (Oli) of the University of Hohenheim, Stuttgart, Germany both in the cropping season 2013 and 2014 as well as at the experimental site of the Department of Agrobiotechnology Tulln in Austria (Tul) during 2014. The combination of location and year provided five environments (Hoh13, Hoh14, Oli13, Oli14, Tul14), in which experiments were laid out as α -lattice designs

with three replications in the locations Hoh, Oli and two replications in Tul.

The *Fusarium* inoculation procedure and its details can be found in the studies of Miedaner et al. (1996) and Miedaner et al. (2017). In short, the whole experiment was inoculated by 2×10^5 conidiospores ml^{-1} with a machine-driven small-plot field sprayer (Hoh, Oli) or a motor-driven backpack sprayer (Tul) several times during flowering. The inoculation dates were spread across the whole flowering period of the experiment in a way that each genotype was inoculated at least once at full flowering. In Tul, the crop canopy was kept moist by mist irrigation during 20 hr after inoculations. Fusarium head blight (FHB) severity was scored several times by the visual evaluation of all spikelets in a plot rating from 1 to 9, where 1 stands for no visible symptoms and 2 to 9 respectively means: < 5%, 5–15%, 15–25%, 25–45%, 45–65%, 65–85%, 85–95% and > 95%. Mean FHB severity (FHBms) will be the term referred in this study to the arithmetic mean of six individual plot ratings measured from the beginning of the symptoms development (11–15 days after inoculation) and repeated in a 3-days interval until the beginning of the yellow ripening stage. Heading date (HD) was noted as the day in the year when 75% of the ears of the plot emerged to 75% and plant height (PH) as the cm from the ground to the middle of the ear at EC70 stage.

Phenotypic data analysis

A two-step approach, as described by Möhring and Piepho (2009), was implemented in order to obtain the Best Linear Unbiased Estimates (BLUE) of each genotype for each of the traits. Firstly, each environment was analyzed by a linear mixed model of the form:

$$y_{ikm} = \mu + g_i + r_k + b_m + e_{ikm} \quad (1)$$

where y_{ikm} is the phenotypic observation of a trait of interest, μ is the overall mean, g_i is the effect of the i th genotype, r_k is the effect of the k th replicate, b_m is the effect of the m th block nested within the k th replicate, and e_{ikm} is the residual effect. All the effects except g_i were considered as random. In a second step, BLUEs were calculated across the multi-environment trials using the linear mixed model:

$$y_{ij} = \mu + g_i + E_j + e_{ij} \quad (2)$$

where y_{ij} is the BLUEs from the first step, E_j is the effect of the j th environment and e_{ij} is the residual term accounting for Genotype \times Environment interaction and the residual effect. The effect of genotypes was considered here as fixed as in the first model.

Variance components were estimated by restricted maximum likelihood (REML) to estimate the repeatability and entry-mean heritability, (H^2), within each environment and across them as described by Piepho and Möhring (2007) (Eq. 19) using instead the model described in section 2.2 in Miedaner et al. (2017). The models were implemented in the package sommer for R (Covarrubias-Pazarán 2016). Accessions' id and the estimated BLUEs for each environment can be found as supplementary material.

Correction of the FHBms estimators

Three methods were taken to correct BLUE of FHBms in each of the environments and across them. The first approach, ADJ.A, considered the residuals from the linear regression of FHBms on the covariates HD and PH, used both separate and simultaneously; in a general form following the equation:

$$y = 1\beta_0 + x_{HD}\beta_{HD} + x_{PH}\beta_{PH} + e \quad (3)$$

where y is a vector of BLUEs for FHBms (dependent variable), $1\beta_0$ is the intercept, while x_{HD} and x_{PH} are the BLUEs of the covariates HD and PH estimated within each environment by model (1), β_{HD} and β_{PH} are the regression coefficients and e are the residuals. Finally, the mean of the FHBms was added to the vector of residuals to be used as response variable in the subsequent analysis.

In the second method, denoted as ADJ.B, we investigated the approach suggested by Emrich et al. (2008), Miedaner et al. (2006), and included plant height and heading date as fixed covariates when calculating BLUEs resulting in an extension of model (1) to:

$$y_{ikm} = \mu + g_i + r_k + b_m + \beta_{HD}c1_{ikm} + \beta_{PH}c2_{ikm} + e_{ikm} \quad (4)$$

where $c1_{ikm}$ and $c2_{ikm}$ modeled the plot basis observations of HD and PH, same as in above method β_{HD} and β_{PH} are the regression coefficients, while

analogues to the adjustments made by model (3) either both traits were considered simultaneously or alone. The corrected FHBms values obtained from this method were defined as the effect g_i from model (4) plus the overall mean of the FHBms.

The third correction method ADJ.C was adopted from Rapp et al. (2018), and consisted in the combination of the target trait and the covariate(s) in a multi-variate mixed model, following this equation:

$$y_{ijkm}^T = \mu^T + g_i^T + E_j^T + (gE)_{ij}^T + r_{jk}^T + b_{jkm}^T + e_{ijkm}^T \quad (5)$$

where each of effects had the same nomenclature as used in models (1) and (2), except for the superscript T which denotes that each term is an array of $n \times t$ dimension being t the number of traits involved. The initial assumption is that all the effects are considered as random and each follows a multi-variate normal MVN distribution, like the one for the genetic effects g_{FHBms} , g_{HD} , g_{PH} :

$$\begin{pmatrix} g_{FHBms} \\ g_{HD} \\ g_{PH} \end{pmatrix} \sim MVN \left[\begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_{FHBms}^2 & r_1\sigma_{FHBms}\sigma_{HD} & r_2\sigma_{FHBms}\sigma_{PH} \\ r_1\sigma_{FHBms}\sigma_{HD} & \sigma_{HD}^2 & r_3\sigma_{HD}\sigma_{PH} \\ r_2\sigma_{FHBms}\sigma_{PH} & r_3\sigma_{HD}\sigma_{PH} & \sigma_{PH}^2 \end{pmatrix} \right] \quad (6)$$

where diagonal σ^2 terms stands for the respective genetic variances; the genetic correlation of FHBms with HD is: r_1 , with PH: r_2 and between covariates: r_3 . Then, the residuals from the model (5) were taken, similarly as in Thorwarth et al. (2018), as the corrected values for FHBms according to:

$$FHBms^{ADJ.C} = g_{FHBms} - \frac{r_1\sigma_{FHBms}}{\sigma_{HD}}g_{HD} - \frac{r_2\sigma_{FHBms}}{\sigma_{PH}}g_{PH} \quad (7)$$

Genotypic data

After a refinement step to exclude markers either with a minor allele frequency (MAF) of $< 5\%$ or with a percentage of missing data higher than 20%, as stated in Sieber et al. (2017); a final set of 23,542 DArT (Diversity Arrays Technology Yarralunla, Australia) markers and 7070 single nucleotide polymorphism (SNPs) markers were yielded. Missing data was imputed as the average score of each marker by the "mean" method included in rrBLUP R package

(Endelman 2011). Marker data is available for downloading as supplementary material.

Genomic predictions

Genomic best-linear unbiased predictions were obtained from the following model:

$$y = X\beta + Zg + e \quad (8)$$

where y contains the calculated FHBms estimates with or without any adjustment; β is the vector of the fixed effects containing the overall mean; g is the vector of genomic estimated breeding values (GEBV) [$g \sim N(0, G\sigma_g^2)$] with g^2 accounting for the genotypic variance estimated by REML and G stands for the genomic relationship matrix obtained according to the description of VanRaden (2008): $G = ZZ'/2 \sum p_j(1 - p_j)$ where Z is a $n \times m$ matrix of m markers and n individuals with elements $z_{ij} = x_{ij} - 2p_j + 1$ and x_{ij} being the value of a given allele for the i th genotype at the j th locus, and p_j the allele frequency of the j th marker. X and Z are the design matrices for fixed and random effects respectively and, e is a vector containing the residuals [$e \sim N(0, \sigma_e^2)$].

The prediction ability (PA) was calculated by a five-fold cross validation (CV) scheme as the correlation between the GEBVs and the corresponding BLUEs. Briefly, the panel was randomly divided in five equally sized subsets, then four out of those were used as the training set and the left one as the validation set. The procedure was repeated 40 times totaling 200 cross validation runs. Mean comparisons between the PAs under any model were estimated with Tukey's HSD (honest significant difference) test (Tukey 1949) with an alpha level of 5%.

Tuning GBLUP model parameters

The following GBLUP model reformulations included the approach taken by Song et al. (2017), where the covariates effects were directly integrated and considered as fixed in the GBLUP model (8). This approach will be referred later on as G + COV method. In addition, multi-trait GBLUP models (MT.GP) were executed following this general equation:

$$y_t = X_t\beta_t + M_t g_t + e \quad (9)$$

where y_t is the array for t traits, namely FHBms, HD and/or PH, containing the BLUE values; g_t is the array of genetic effects GEBVs [$g \sim MVN(0, \sum_g \otimes G)$] with as the complete unstructured variance-covariance

matrix $\begin{pmatrix} \sigma_{g1}^2 & cov_{g1,2} & cov_{g1,3} \\ cov_{g1,2} & \sigma_{g2}^2 & cov_{g2,3} \\ cov_{g1,3} & cov_{g2,3} & \sigma_{g3}^2 \end{pmatrix}$ alongside with

its design matrix M_t . The former terms σ_{g1}^2 , σ_{g2}^2 and σ_{g3}^2 are the genetic variances of FHBms, HD and PH; $cov_{g1,2}$, $cov_{g1,3}$ are the covariance of FHBms with HD and PH respectively, and $cov_{g2,3}$ is the covariance between the covariates. The residual array follows a distribution: [$e \sim MVN(0, \sum_e \otimes I_n)$] where I_n stands for an $n \times n$ identity matrix and for the variance-covariance matrix similar to the previous one but accounting for the residuals between traits.

Lastly we evaluated the so called trait assisted genomic prediction (TA.GP) (Fernandes et al. 2018; Michel et al. 2017) in which for each CV run, unlike as in the MT.GP model framework described above, the covariate estimators of the validation set were included into the training set and represented pre-existing information about the genotype performance.

Index selection

Genomic selection indexes were afterwards constructed out of the GEBVs obtained in the MT.GP models. Briefly, we used a *desired gains index* developed by Pešek and Baker (1969) but restricting gains for the covariates and thus allowing only response to selection towards the target trait.

$$GEBV_{index} = g_1 b_1 + g_2 b_2 + g_3 b_3 \quad (10)$$

where g_1 , g_2 and g_3 are the GEBVs obtained from multi-trait model (9), b is the array containing the indexes for each trait obtained as $b = \sum_g^{-1} \times a$, with a being the vector with the respective weights keeping always a_{FHBms} as 1 and a_{HD} , a_{PH} equal to zero. The genomic variance-covariance matrix Σ_g was derived from model (9). Finally, an alternative Σ_g matrix was derived by calculating the variances and covariances between the GEBVs obtained from the single trait models ST.GP from model (8). Table 1 condense all the GP approaches previously mentioned in the preceding sections.

Table 1 Summary of the different genomic prediction alternatives of FHB mean severity attempted in this study

Approach	Method/model	Description
Correction methods	ADJ.A	Residual method
	ADJ.B	Covariate(s) as fixed factor(s) on plot basis mode
	ADJ.C	Multi-trait model on plot basis
Tuning GP model parameters	G + COV	GBLUP with covariate(s) as fixed factor(s)
	MT.GP	Multi-trait GBLUP
	TA.GP	Trait-assisted GBLUP
Selection Index	IDX.ST	Restricted index derived from single trait GP models
	IDX.MT	Restricted index derived from MT.GP models

Expected selection gain

The differences between the mean of BLUE estimators of the best 5, 10, 15 and 20% performing lines and the overall mean for a given validation set were calculated in each CV run to estimate the expected selection gain (G_e). The mentioned differences were standardized, in order to make comparisons between traits, according to:

$$G_e = \frac{\mu_{Trait}^{sel} - \mu_{Trait}^{all}}{\sigma_{Trait}} \quad (11)$$

where μ_{Trait}^{sel} is the trait mean of the selected genotypes ranked based on the FHBms GEBVs and μ_{Trait}^{all} stands for the overall mean of the respective trait. When any correction approach was applied to perform the predictions, subscript *Trait* referred always to the non-adjusted estimations of FHBms. The standardized G_e was also obtained choosing lines based on the ranking on the phenotypic estimation of FHBms (phenotypic selection).

Results

Phenotypic analysis

Plant height records of genotypes in 2014 season were in average greater than in 2013, in which Hoh was the location with the shortest plant stand while Tul had the tallest one and in the across environment analysis plant height was, on average approximately 81.02 cm (Table 2). For the sake of the subsequent analyses we decided to exclude six of the extremely tallest genotypes categorized as outliers based on their across

environments' BLUEs. These "outgroup" lines included several genetic resources with exotic genetic background from the US, Canada, Bulgaria and Ukraine: DGE-1, Agathe, AC Navigator, Cirpan 13, No3026 and Nursith. On average, the heading date was 155 days after January with a significant later heading behavior in 2013. High entry-mean heritabilities are reported here for both covariates analyzed and, moreover, their genetic variances were always higher than both genotype \times environment and residual variances. In addition, a significant and direct association between both covariates were detected in the environments Hoh13 and Tul14 (Table 2).

The artificial inoculation of *Fusarium* was successful in all environments, and a broad FHBms variation could be found within the individual environments as well as in the across trail environments analysis ranging from resistant to highly susceptible genotypes. Tul14 and Oli14 were the most and least affected environments, respectively, and the later was the environment with the largest FHBms variation and heritability (Table 2). The updated top resistant lines included the Italian variety "Belfuggito" released in the 1960's, the Russian cultivar "Amazonka", the cultivar "Soldur" and several breeding lines from breeding program of the University of Hohenheim. FHBms had a moderate to high heritability, even though its genotypic variance component which was just slightly higher than both the residual and the $G \times E$ components (Table 2), thus confirming the good quality of the scoring for *Fusarium* in this multi-environment study.

Both covariates plant height (PH) and heading date (HD), exhibited significantly negative correlations with FHBms of $r = -0.25$ and $r = -0.63$

Table 2 Summary statistics of the phenotypically estimated values of 178 durum wheat genotypes for the traits: heading date (HD), plant height (PH) and Fusarium head blight mean severity (FHBms) as well as their variance components,repeatability and entry-mean heritabilities (H²) and correlations between traits (r) for each of the five environments (Env) and across them

Trait	Env	Min	Mean	Max	σ_G^2	σ_{GE}^2	σ_e^2	Rep/H ²	r(~ FHBms)	r(~ PH)
HD (d)	Hoh13	151.07	163.19 ^b	171.62	8.17		2.82	0.90	− 0.65***	0.31***
	Oli13	169.27	176.59 ^a	187.98	16.01		2.37	0.95	− 0.73***	0.12
	Hoh14	126.49	138.72 ^e	148.54	9.77		2.33	0.94	− 0.75***	0.07
	Oli14	149.23	155.54 ^c	162.14	3.71		0.78	0.93	− 0.60***	− 0.12
	Tul14	140.50	144.49 ^d	150.00	1.80		0.87	0.86	0.11	0.15*
	MET	148.10	155.51	163.45	5.93	2.00	2.36	0.91	− 0.63***	0.14
PH (cm.)	Hoh13	58.29	70.16 ^e	83.65	19.2		16.12	0.78	− 0.33***	−
	Oli13	58.02	74.29 ^d	90.05	30.29		9.97	0.90	− 0.06	−
	Hoh14	63.98	82.49 ^c	103.16	27.92		9.57	0.90	− 0.13	−
	Oli14	69.57	87.03 ^b	108.19	31.94		8.54	0.92	− 0.05	−
	Tul14	71.54	91.11 ^a	113.33	35.27		14.25	0.88	− 0.08	−
	MET	64.65	81.02	99.04	22.77	5.14	14.69	0.92	− 0.25***	−
FHBms (1–9)	Hoh13	2.21	4.29 ^d	6.24	0.55		0.28	0.85	−	−
	Oli13	2.13	4.94 ^c	7.50	0.86		0.26	0.91	−	−
	Hoh14	3.35	5.53 ^b	7.52	0.63		0.18	0.91	−	−
	Oli14	1.36	4.04 ^e	7.58	0.83		0.21	0.92	−	−
	Tul14	3.97	6.08 ^a	8.64	0.92		0.43	0.86	−	−
	MET	2.85	4.98	6.66	0.38	0.35	0.37	0.80	−	−

Trait values presented in days for heading date, cm for plant height and in 1–9 scale for FHBms with 1 for no visible symptoms and 9 for more than 95% of all spikelets per plot affected. Correlations with *, *** superscripts stand for significance level at α level 0.05 and 0.001 respectively. Mean values for each trait between environments were compared with Tukey's HSD test with $\alpha = 0.05$. Env (Environment). Genotypic variance σ_G^2 , genotype \times environment variance $\sigma_{G \times E}^2$, and residual variance σ_e^2 .

Field trials: Hoh= Heidfeldhof (DE), Oli= Oberer Lindenhof (DE), Tul=Tulln (AT)

($p < 0.001$), respectively. Despite of dropping six outlier genotypes for plant height PH, its correlation with FHBms was still highly significant also for the single environment Hoh13. FHBms was in all environments but Tul14 as well as in the multi environment (MET) analysis highly associated with HD as seen by coefficients higher than $r = -0.60$.

Correction effects

The variability of FHBms BLUEs decreased when they were corrected by either HD or HD plus PH in terms of their coefficient of variation under any of the methods and the same effect was detected for their heritabilities. A comparison between their distributions revealed how their frequencies increased around the mean value being the more pronounced case the FHBms^{ADJ.C} BLUEs (Supplemental Figure 1).

Amongst the correction methods considering only HD as covariate, ADJ.A and ADJ.C performed nearly similar, both adjusting completely for the unfavorable FHBms-HD correlation while ADJ.B reduced it until non-significance only for the environment Oli13. The former methods though led the coefficient of the FHBms-PH trade-off to significance ($p < 0.05$). Almost the same first mentioned pattern was found when FHBms' BLUEs were adjusted exclusively for PH under the former methods however, ADJ.B significantly increased the correlation towards PH except for the environments Hoh14 and Oli14 (Table 3).

The adjustment of the FHBms' BLUEs considering simultaneously both covariates was evaluated only when worth it i.e. the environment Hoh13 and the MET case. ADJ.A was the only capable to effectively decrease the magnitude of correlation coefficients

Table 3 Pearson coefficients of correlation between both covariates HD and PH and the adjusted Fusarium head blight mean severity (FHBms^{ADJ}) values corrected under three methods (A, B, C shortly described in Table 1) singled out by ~ sign for the covariate(s) adjusted

	Heading date—HD				Plant height—PH					
	Hoh13	Oli13	Hoh14	Oli14	MET	Hoh13	Oli13	Hoh14	Oli14	MET
FHBms	− 0.65***	− 0.73***	− 0.75***	− 0.60***	− 0.63***	− 0.33***	− 0.06	− 0.13	− 0.05	− 0.25***
A ~ HD	0.00	0.00	0.00	0.00	0.00	− 0.17*	0.03	− 0.12	− 0.16*	− 0.21**
A ~ PH	− 0.58***	− 0.56***	− 0.37***	− 0.43***	− 0.53***	0.00	0.00	0.00	0.00	− 0.11
A ~ HD + PH	0.00	−	−	−	0.00	0.00	−	−	−	− 0.09
B ~ HD	− 0.29***	− 0.12	− 0.34***	− 0.23**	− 0.26***	− 0.25***	0.02	− 0.14	− 0.12	− 0.24**
B ~ PH	− 0.66***	− 0.62***	− 0.50***	− 0.54***	− 0.63***	− 0.44***	− 0.37***	− 0.07	− 0.04	− 0.36***
B ~ HD + PH	− 0.33***	−	−	−	− 0.30***	− 0.39***	−	−	−	− 0.33***
C ~ HD	0.00	0.00	0.00	0.00	0.00	− 0.17*	0.03	− 0.12	− 0.15*	− 0.20**
C ~ PH	− 0.58***	− 0.56***	− 0.37***	− 0.43***	− 0.53***	0.00	0.00	0.00	0.00	− 0.11
C ~ HD + PH	− 0.26***	−	−	−	0.07	− 0.38***	−	−	−	− 0.16*

Coefficients with *, **, *** superscripts stand for significance level at α level 0.05, 0.01 and 0.001 respectively

towards both covariates and turn them non-significant. In the other hand, ADJ.B and ADJ.C merely reduced the ~ HD coefficients' magnitudes and regarding PH both methods raised the coefficients in the environment Hoh13 and the former method did so in the MET case (Table 3).

Genomic predictions

Prediction abilities

Prediction abilities of FHBms, across environments ranged from PA = 0.61 in Oli14 to PA = 0.69 in Hoh14 (Supplemental Table 1). However, when predictions accounted for the $G \times E$ interaction term, the prediction ability reached a significant higher value of PA = 0.75. The above values corresponded to the FHB mean severity estimators predicted without any correction or parameter tuning in the GBLUP model. It must be mentioned that both prediction abilities and expected selection gain G_e rates for all the models were calculated taking as reference the non-adjusted estimators of FHBms. This consideration turned out to be a remarkable point since, specifically for the correction methods, it allowed us to compare PAs from any type of approach.

Significant lower mean prediction abilities were obtained when the corrected FHBms estimators were used for genomic prediction as expected since these estimators deviated from the non-adjusted FHBms. In fact, this occurred irrespective of the applied adjustment method, except when PH was the covariate included (Fig. 1 left panel). Analogously, if the ~ PH models are ignored since their predictabilities are comparable to the reference model, prediction abilities under the ADJ.B method were less diminished and they, in turn, outperformed their equivalents from both ADJ.A and ADJ.C methods. In the other hand, what stands out in Fig. 1 (right panel) is the significant improvement in the prediction abilities of the trait-assisted TA.GP approach, reaching PA = 0.80, when HD was included as covariate. There was no predictability upsurge for any of the multi-trait MT.GP models and even for such version involving both covariates a slight diminution in mean predictability was found. Our so-called G + HD and G + HD + PH alternatives had poorer predictabilities than the multi-trait GP models though greater than their respective ADJ.A and ADJ.C correction models versions.

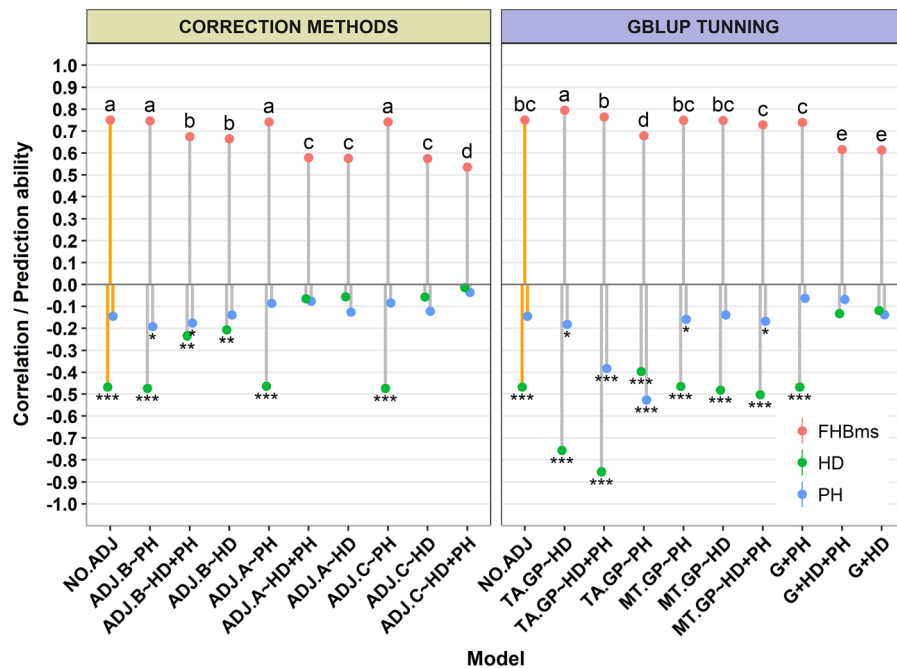


Fig. 1 Stem plots for the mean values of prediction ability PA (above the zero line) and correlation with the covariates (below the zero line) obtained from 40 cycles of five-fold cross validation of genomic prediction for Fusarium head blight mean severity (FHBms) and the covariates heading date (HD) and plant height (PH). Prediction abilities for three FHBms correction methods (left panel) and three GBLUP model parameter tuning (right panel) including multi-trait versions, are illustrated as the correlation of phenotypically estimated BLUEs of both the target trait and covariates with the GEBVs

In addition, relative low PA = 0.60 resulted when GEBVs obtained from either MT.GP~HD or MT.GP~HD+PH were weighed by restricted selection indexes (Fig. 2 right panel), however the lowermost predictabilities amongst all the methods were obtained when the restricted indexes were derived from ST.GP' GEBVs involving both HD and HD plus PH versions (Fig. 2 left panel). Lastly, only for the TA.GP approach a slightly predictability increase was detected when the three traits at issue were fit taking the NO.ADJ GP model as reference and the tri-variate versions of both ADJ.C and IDX.ST models were the only ones for which a significant lower prediction ability was observed in a within version's model comparison.

for the target trait. Mean PAs and correlation scores for the non-adjusted values are highlighted in orange bars and the rest of the models/methods are gray colored and decreasingly sorted by the within method average PA. Mean PAs were compared with Tukey's HSD test with $\alpha = 0.05$ and displayed in compact letters display fashion. Correlations with *, **, *** superscripts stand for significance level at $\alpha = 0.05$, 0.01 and 0.001 respectively. Every model is denoted by its abbreviation (see Table 2) followed by "~" symbol and the covariate(s) incorporated

Correlation with covariates

Genome-based estimators for non-adjustment FHBms were negligibly associated ($r = -0.14$, $p > 0.05$) with PH though still highly correlated ($r = -0.47$, $p < 0.001$) with HD. Genomic predictions under the correction method ADJ.B presented still significant ($p < 0.01$) correlations towards HD, nevertheless the genomic predictions using ADJ.A and ADJ.C methods -omitting their ~PH versions- were able to decline the correlation with HD to marginal non-significant levels. Regarding the trade-offs with PH, FHBms' predictions became significant ($p < 0.05$) when both PH and HD + PH were corrected for in method ADJ.B (Fig. 1 left panel).

All versions of MT.GP and TA.GP models' predictions were highly associated ($p < 0.001$) with HD, and for the latter model correlation coefficients up to $r = -0.85$ and $r = -0.38$ were detected for HD and

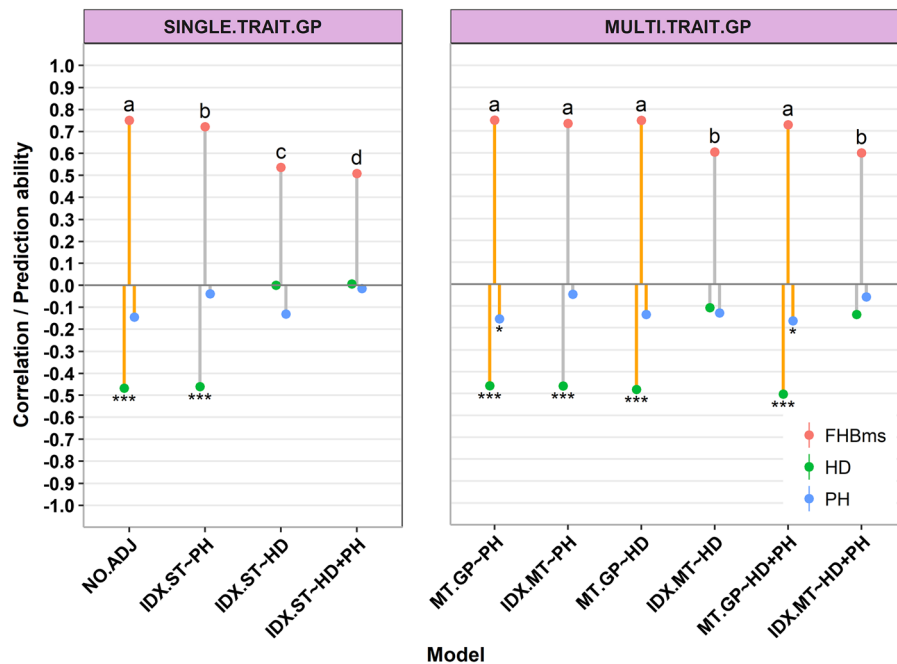


Fig. 2 Stem plots for the mean values of prediction ability PA (above the zero line) and correlation with the covariates (below the zero line) obtained from 40 cycles of five-fold cross validation of genomic prediction (GP) for Fusarium head blight mean severity (FHBms) and the covariates heading date (HD) and plant height (PH). In each panel with gray bars are shown the scores for different selection indexes applied over the single and multi-trait GP models' outputs. In the right panel, the scores for the GP model of the non-adjusted FHBms are presented with

orange bars. In the left panel, the multi-trait genomic GP models including the covariates both together and separately are presented with orange bars. Mean PAs were compared with Tukey's HSD test with $\alpha = 0.05$ and displayed in compact letters display fashion. Correlation coefficients with *, **, *** superscripts stand for significance level at $\alpha = 0.05, 0.01$ and 0.001 respectively. Every model is denoted by its abbreviation (see Table 2) followed by "~" symbol and the covariate(s) incorporated

PH respectively (Fig. 1 right panel). The only MT approach for which a non-significant trade-off between FHBms and PH was found was MT.GP~HD. On the other hand, the method G + COV presented no relevant correlation coefficients between their predictions and both covariates, excepting when PH was the incorporated trait. Non-significant correlations with both PH and HD were observed when predictions obtained either from ST or MT models were weighted by restricted indexes (Fig. 2).

Expected selection gain

A single round of genomic-based selection was performed targeting the 5, 10, 15 and 20% of the most resistant genotypes, and their standardized expected selection gain values are presented in Fig. 3 ticked by approach type and both traits FHBms

and HD. Selection based on the most accurate model (TA.GP) showed a larger indirect response towards HD than the conventional GP model for uncorrected FHBms values and such response was even comparable to the one coming by means of phenotypic selection when more than 5% of the lines were selected (Fig. 3 central panel). In contrast, if the GEBVs from ST.GP models were scaled by restricted indexes, their gain differentials in heading time was almost reduced to zero (Fig. 3 right panel). ADJ.A and ADJ.C methods followed IDX.ST model being the most effective alternatives to counteract the gains in heading date (Fig. 3 left panel). G + COV and IDX.MT methods performed similar decreasing by about a half standard deviation the unfavorable expected selection gain towards a later average heading date. Expected selection gains for FHBms amongst approaches kept the same prediction

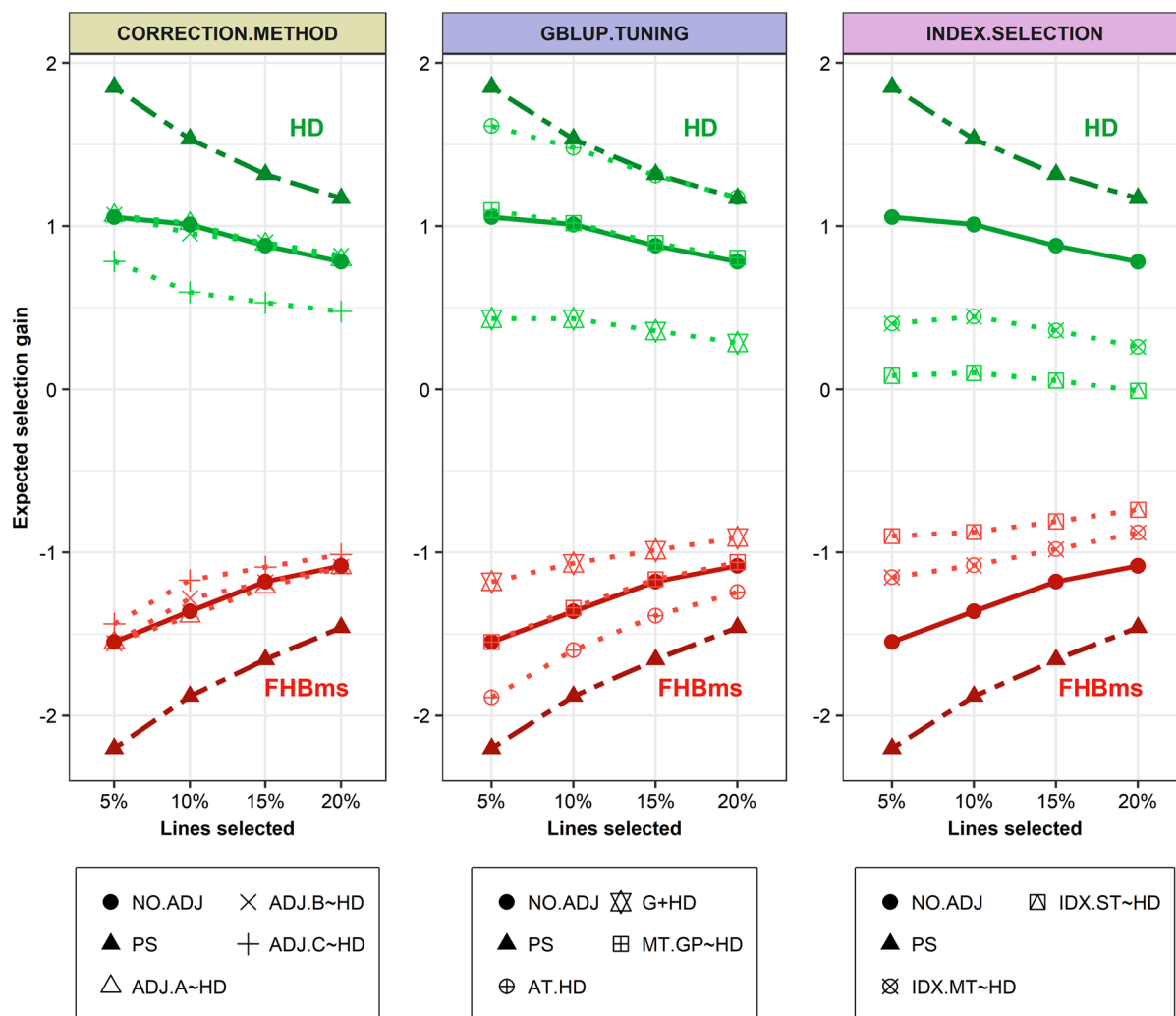


Fig. 3 Models performance in terms of expected selection gain (G_e). Models that predicted FHBms corrected values (left panel), GBLUP model tuning (central panel) and application of restricted selection indexes (right panel) when predicting FHBms –dotted lines–. G_e values are displayed for FHBms and heading date HD across several selection intensities. In each

of the facets are included also the G_e scores for the non-adjusted FHBms predictions –solid lines– and for the selection based exclusively on the phenotypic BLUE values PS –two dashed lines. Every model is denoted by its abbreviation (see Table 2) followed the covariate(s) incorporated

abilities' ranking with the TA.GP model notably increasing the disease resistance gains. Moreover, confirmed after the simulation of the genomic selection of the best 20% genotypes proportion, the latter was the only method which significantly decreased the FHBms from the GP model with no modifications (Fig. 4). Contrarily, the rest of methods increased in average the susceptibility with exception of the multi-trait MT.GP model for which no meaningful change

was detected. Additionally, from Fig. 4 it can be advised that all methods, excluding MT.GP and TA.GP, had the capability to choose more genotypes having both susceptibility and heading date estimators under the overall mean than the reference non-adjusted model.

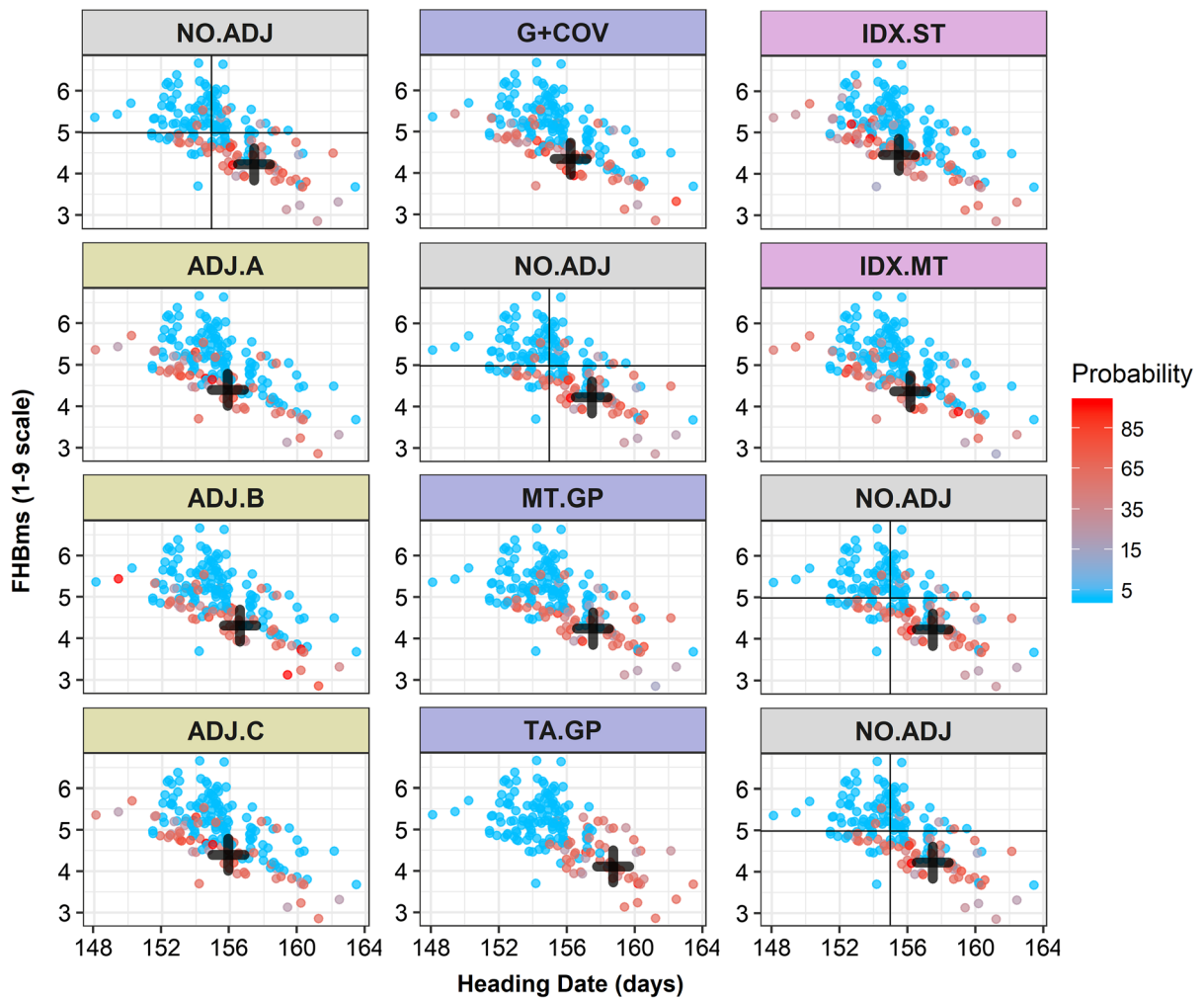


Fig. 4 Layout of 178 durum wheat genotypes according to their FHBms and HD BLUE values. After a single round of simulated genomic-based selection aiming the 20% most resistant lines, the probability for a genotype to be selected based on 40 cross-validation cycles is highlighted in a blue-red scale. The mean

values of the selected genotypes in each panel are cross-pointed and the overall mean values are marked in the non-adjusted panels. Every panel is labeled with the model abbreviation (see Table 1)

Discussion

Simultaneous selection for multiple traits is an exigent task in plant breeding, hence some considerations have to be made in order to exploit favorable or to adjust unfavorable trade-offs between traits. The present research focused on the prediction of FHB severity in the framework of its correlation with plant height and heading date, using a diverse durum wheat panel as a case study. The investigated alternatives for addressing these relationships ranged from corrections applied prior and post genomic prediction either

through adjustments of FHBms estimations or usage of restriction indexes, respectively, as well as the tuning of GP model parameters including multi-trait GP alternatives.

The highly quantitative nature of FHB resistance regarding this case study panel was unveiled by Miedaner et al., (2017) via genome-wide association mapping, reporting nine loci explaining between 1% to 14% of the genetic variance. Combining minor FHB_r loci detected in durum varieties with major QTL from bread wheat has been showed certain efficiency in decrease the levels of susceptibility e.g. Prat et al.

(2017) reported that concurrently introgressing the major locus *Fhb1* with either loci on chromosome 2BL or 5AL could even overcome the negative effects on FHB of the *Rht-B1b* allele and, Zhao et al. (2018) combined the introgressed QTL *Qfhb.ndwp-7A* from the Chinese line PI 277012 with both minor loci on chromosomes 2A and 7A to significantly increase FHB resistance. Such allele combination and deployment in durum populations has been typically achieved by phenotypic selection or MAS in the past, although genomic selection might nowadays be pre-figured as a recommended strategy to capture the genetic variation generated by many small-effect QTL and might assist breeders by shortening the breeding cycle or improve the selection gain in early stages where typically more resistant lines could in this way be selected. Moreover, among the studies demonstrating the higher efficiency of GS over traditional FHB breeding strategies, Mirdita et al. (2015) evidenced a GS predictability three times superior than MAS in the absence of major QTL using a large set of 2325 European winter wheat genotypes evaluated in 11 environments and, Arruda et al. (2016a, b) and Steiner et al. (2018) also showed the same accuracy boost that was even increased when significant FHB loci were included as fixed effects in the GP models. Predictability of MAS in the present dataset reached 0.65, reported by Miedaner et al. (2017), being even higher than some of our investigated approaches (Figs. 1, 2). However, the latter MAS' feature could be an overestimation due to the relatedness between lines which in turn is a drawback that GS could overcome as revealed for instance by independent versus cross validation sampling comparisons, that makes GP models' performance more stable in cases like European wheat breeding programs with slow allele frequency dynamics Jiang et al. (2017).

Correction of covariates tradeoffs as an alternative in GP

Roughly eight QTL for plant height explaining minor proportions of phenotypic variation and not being localized nearby any FHB locus were revealed for the panel in study. Apart from those loci, the *Rht-B1* (*Rht1*) locus explained more than half of the phenotypic variation in plant stature. It has been verified, for other durum germplasms as well, that *Rht1* can increase or even duplicate measurements of FHB

severity (Buerstmayr et al. 2012; Prat et al. 2017; Talas et al. 2011). We partially mitigated this trade-off by leaving out six out of nine of the tallest lines carrying the *Rht-B1a* wild type allele which met the outlier exclusion criteria, by doing so the range and variance of FHB severity ratings did not dramatically change and the correlation coefficient was reduced from -0.37 to -0.25 ($p < 0.001$). The latter exclusion could be taken as a step in order to focus on genomic predictions for elite durum wheat rather than genetic resources. Unlike plant height, for the tandem FHB severity-days to heading/flowering, both positive (Clark et al. 2016; Prat et al. 2017; Steiner et al. 2004; Zhao et al. 2018) and negative (Buerstmayr et al. 2012; Gervais et al. 2003; He et al. 2016; McCartney et al. 2016; Paillard et al. 2004; Schmolke et al. 2005; Somers et al. 2003; Yi et al. 2018) type of correlations have been reported in bread and durum wheat. Co-localization of these traits' loci have been found in durum on chromosomes 4AL and 6AS according to Prat et al., 2017 and on chromosome 7B to Buerstmayr et al. (2012). In all environments analyzed in this study as well as in the across environments analysis strong negative FHBms-HD trade-offs were detected with the exception of Tul14 (Table 2), a circumstance that may be attributable to the moist-keeping conditions during flowering in this location and both its lower HD repeatability and range (e.g. just 9.5 days compared with 20.5 days in Hoh13). In addition, HD loci detected for this panel were not in LD respect to FHB resistance QTL yet when FHBms BLUES were adjusted for this covariate (Miedaner et al. 2017), therefore the great influence of specific differences in weather factors or ripening and possible pleiotropy events might be plausible explanations for this trade-off.

Some alternatives have been investigated to assess true genotypic effects of FHB resistance and dissect it from a passive mechanism of resistance such as plant height or development stage i.e. flowering time. For instance Klahr et al. (2007) used a covariance analysis and detected the only stable QTL *QFhb.hs-5B* non-affected by plant height and heading date in a RIL population of European bread wheat. Likewise, Miedaner et al. (2006) adjusted the FHB ratings to the effect of heading date and evaluated the effect of QTL introgressed from resistant donors in European elite spring wheat lines. Other covariance considerations highlight additional advantages over traditional

mapping methods when trying to identify QTL, i.e. He et al. (2016) and Lu and Lillemo (2014) included HD and PH as covariates into the QTL mapping algorithms, while Yi et al. (2018) suggested a conditional QTL mapping on either HD or PH. In the context of genome-wide association mapping and genomic prediction studies for FHB resistance in hexa- and tetraploid wheat, several studies (Arruda et al. 2015, 2016a, b; Miedaner et al. 2017) have used the strategy first proposed by Emrich et al. (2008) and included the trait HD as a quantitative covariate in the mixed linear model from where the adjusted BLUE/BLUP values were obtained. The former covariance analysis was also examined in this study under the method ADJ.B (4) for which the unexpected higher FHBms-PH correlations obtained after correction for this covariate in addition to the unsatisfactory association levels of the FHBms^{ADJ.B} predictions discourage considering this method for either low or moderately correlated traits. Furthermore, as proposed by Song et al. (2017), our so-called G + COV approach evaluated the inclusion of the covariates as fixed effects in the GP model stated in [8] and it turned out to be the most efficient method to accomplish both higher predictabilities and negligible trade-offs ($p > 0.05$) with the covariates regardless whether HD alone or together with PH were involved.

Regression-based correction methods are frequently employed in a plant breeding perspective to assess negative trade-offs between major agronomic traits e.g. disease resistance and both maturity and plant height (Bormann et al. 2004; Bradshaw et al. 2004) or grain yield and protein content (Monaghan et al. 2001). In fact, one of the most extended applications of regression-based adjustments addressing the latter traits in wheat are the so-called grain protein deviations GPD, which are defined as the residuals of regressing protein content on grain yield (Rapp et al. 2018; Thorwarth et al. 2018). The ADJ.A and ADJ.C methods took advantage of this procedure as did the correction of FHBms based on regression coefficients derived from either single or multi-variate mixed models on a plot basis, respectively. The fact that the predictions for the FHBms values corrected by the latter methods were among the poorest in terms of predictability can be explained since these adjustments deviated the most from the original FHBms; although, this attribute led, in turn, to a highly

effective reduction of the respective trade-offs both at BLUEs and GEBVs levels.

Single-trait predictions comparison with multi-trait models

Multi-trait genomic prediction MT.GP models tested in this study provided no predictability' advantage over the reference non-adjusted model. These observation is supported by other empirical studies, where the usage of multi-trait GP models did not necessarily result in an increase in prediction abilities (Fernandes et al. 2018; Schulthess et al. 2017; Guo et al. 2014), but is in disagreement with reports that showed important performance' improvements when either i) higher heritabilities for the indicator traits and/or ii) significant correlations between traits were evidenced (Calus and Veerkamp 2011; Guo et al. 2014; Jia and Jannink 2012). In a simulation study Calus and Veerkamp (2011) showed that only the inclusion of traits with genetic correlations stronger than 0.5 and higher heritabilities can lead to greater predictabilities and Schulthess et al. (2017) suggested that the benefits of MT.GP models could be limited since achieving last mentioned requirements might be somewhat unrealistic, as was evinced for our case where no pronounced differences in heritabilities were found. Plant height having moderately high phenotypic associations with FHBms in this study, did not seem to play a major role in multi-trait GP models as expected if compared to, for instance, the evidence showed in Lado et al. (2018) who have demonstrated that the inclusion of a third mildly correlated trait into MT.GP models was not suitable to increase predictabilities in comparison to the inclusion of a single highly correlated indicator trait.

The multi-trait GP approach type that improved the prediction ability was the one for which phenotypic records of the correlated covariates were already available in the validation population when predicting the target trait i.e. the trait-assisted selection model TA.GP Fernandes et al. (2018) also known as phenotypic imputation Jia and Jannink (2012). This predictability' upsurge was evinced for both the TA.GP model fitting HD alone and HD+PH simultaneously, which was significant in the former case. A similar effect has been noticed in several studies with different crops (Fernandes et al. 2018; Jia and Jannink 2012; Rutkoski et al. 2012, 2016; Schulthess et al. 2017;

Wang et al. 2017), although the degree of association between the target trait' predictions and the covariates has not been extensively assessed e.g. in Fernandes et al. (2018) since the trade-offs between yield biomass and either moisture or plant height in sorghum were not of concern. While predictions based on both approaches MT.GP and TA.GP exhibited the higher predictabilities among the alternatives studied here, the former had similar correlation levels with the covariates than the NO.ADJ model but the TA.GP models exhibited coefficients up to 44% higher respect to HD and regarding to PH it was even more than tripled in the TA.GP ~ PH model. Moreover, it was demonstrated after selection simulation that under TA.GP model none of the moderately resistant and early flowering lines would have been selected. Using this phenotypic imputation for multi-trait GP, Steiner et al. (2018) revealed an important increase in the trade-off FHB severity-PH when both PH and PH+FD (flowering date) were fit in the GP models since in their analyzed panel the only moderately correlated trait with FHB severity was plant height. The actual usage of such previous generated records, that are routinely assessed in earlier generations during variety development for GP models should therefore be examined in the light of the indirect responses which rise for such scenarios.

Weighting predictions by genomic restriction indexes

Final products delivered by breeders in form of new cultivars must satisfy a certain number of requirements. In this context, a durum cultivar is desirable if it displays moderate resistance levels to *Fusarium*, high yields, short stature and early flowering. Breeders have been traditionally conducted multi-trait selection by methods like tandem selection, independent culling levels and index selection (Dudley 1997), while usage of selection indexes is generally more efficient than the former ones (Wang et al. 2018). Since the net merit index proposed by Smith (1936) and Hazel (1943) many modifications like restriction index have appeared, where the aim is to improve a given trait while keeping the response to others at zero (Kempthorne and Nordskog 1959). Similarly, Pešek and Baker (1969) presented an approach to achieve a certain rate of desired gains for a set of traits. In our case, weighting the GEBVs of FHBms, HD and PH

obtained in ST.GP models by employing this desired gain index as restriction indexes had the best performance in annulling any response towards the respective indicator traits HD and PH though with the subsequent largest predictability penalization for FHBms. However, the utter goal in a multi-trait GP framework may not necessarily be the cancellation of the unfavorable correlations towards the indicator traits but rather their reduction till negligible levels of either magnitude or significance, as it was accomplished here using the restriction indexes ponderating the GEBVs derived from MT.GP. In the same regard, the use of this genomic indexes deflated the enlarged FHB severity-PH trade-off detected after either ST or MT genomic-based predictions in a durum wheat panel (Steiner et al. 2018). The authors of the mentioned study did not detect significant differences between the index usage in either single or multiple trait models in terms of predictability although on average they were significantly lower than their unweighted counterparts. In this regard, it must be said that the most plausible models through which both the less drastic predictability penalization and non-significant ~ HD correlation levels were attained appeared to be $G + HD$ and $IDX.MT \sim HD$, although some features would give the former certain advantage: (1) its slightly higher predictability, (2) due to it is less computationally demanding and, (3) since it was more prone to avoid the selection of the earliest flowering-moderately susceptible genotypes after the genomic selection simulation round (Fig. 4).

Conclusion

Highly significant associations of FHB severity and the indicator traits PH and HD estimations were detected, although the trade-off with HD turned out to be the only one relevant and persisted after genomic predictions. Including PH as the only covariate in genomic prediction models resulted in imperceptible changes in the GP performances, except when PH was fit in the trait-assisted GP model for which a significant decline in predictability was detected. Incorporating simultaneously both covariates did not improve the predictability and for the models dealing with multi-trait frameworks such configuration led to worse predictabilities. The approaches that corrected FHBms phenotypic estimations by regression-based methods

and weighting the FHBms' genomic breeding values by restriction indexes derived from single trait GP models were the most effective alternatives controlling the indirect responses towards the covariates and even decreased by up to two standard deviations the most important trade-off. Multi-trait genomic prediction approaches significantly outperformed in predictability the non-adjusted reference model only when HD records for the validation population were already available, which led in turn to the largest rise in the correlation between HD and FHBms. Fitting HD as fixed effect in the GP model (G + COV) and correcting the FHBms' genomic estimations using restriction indexes derived from multi-trait GP models achieved smaller predictabilities but were on the other hand capable to reduce HD trade-offs, and therefore represent alternative models with the highest relevance in this study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Publication 2

Dissecting the Contribution of Environmental Influences, Plant Phenology, and Disease Resistance to Improving Genomic Predictions for Fusarium Head Blight Resistance in Wheat

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Article

Dissecting the Contribution of Environmental Influences, Plant Phenology, and Disease Resistance to Improving Genomic Predictions for Fusarium Head Blight Resistance in Wheat

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Abstract: Environmental factors like temperature and humidity are presumed to greatly influence Fusarium head blight FHB infections in wheat. Anther retention AR, on the other hand, is a morphologically neutral trait that shares a common genetic basis with FHB resistance. In this study, our aims were to: (i) Evaluate two types of corrections of FHB severity scores, namely method-1 via linear regression on flowering time (FT), and method-2 via a best-subset multiple linear regression analysis comprising FT plus accumulated thermal time variables; and (ii) assess the performance of multi-trait genomic selection (MT.GS) models for FHB severity assisted by AR. The forward prediction scenarios where GS models were trained with data from the previous years revealed average prediction accuracies (PA) of 0.28, 0.33, and 0.36 for FHB severity scores that were uncorrected or corrected by method-1 and method-2, respectively. FHB severity scores free from the influences of both environment and phenology seemed to be the most efficient trait to be predicted across different seasons. Average PA increments up to 1.9-fold were furthermore obtained for the MT.GS models, evidencing the feasibility of using AR as an assisting trait to improve the genomic selection of FHB resistance breeding lines.

Keywords: Fusarium head blight (FHB); wheat; genomic selection; environment; anther retention

1. Introduction

Fusarium head blight FHB has become a major threat for wheat production, particularly in warm and humid regions [1]. FHB is caused by several members of the *Fusarium* genus yet evidence supports that resistance to FHB is neither *Fusarium*-species- nor isolate-specific [2–4]. The economic impact of this fungal disease is caused by either subtle to severe grain yield and quality losses, or mycotoxin accumulation [5]. According to recent studies, the combination of tolerant varieties, fungicides, and specific management practices might be used to decrease FHB losses [6,7] such as the \$1.18 billion reached in United States in 2015–2016 [8].

Genetic improvement of host resistance is considered the most sustainable and suitable approach to manage this disease [9]. Conventional breeding, however, is limited mainly by both the lack of highly resistant germplasm and the quantitative nature of the resistance to FHB [10]. More than 500 quantitative trait loci (QTLs) for FHB resistance have been mapped into 44 chromosomal regions

spreading across all 21 wheat chromosomes [10–14]. To date, ten QTL [10] have been either validated or employed in Marker-Assisted Selection (MAS) breeding: 2B-2, 2D-2, 3A-1, 3B-1 (*Fhb1*), 3B-2, 4B-1 (*Fhb4*), 5A-1 (*Fhb5*), 6A-2, 6B-1 (*Fhb2*), and 7B-1. From these loci, *Fhb1* is the most intensively studied, and it has been deployed, through MAS, in a few registered cultivars in the United States [15–17], Canada [18], Australia [19], and Europe [20]. Genomic selection (GS) appears to be ideal to target the complex genetic architecture of FHB resistance under its assumption that at least some of the markers are in linkage disequilibrium (LD) with loci associated with the trait of interest [21]. Several studies [10,11] have assessed the effectiveness of GS models in FHB resistance improvement in wheat and some report higher accuracies and selection gains than MAS. The performances of GS models varied in whether a major QTL was included, the traits representing FHB resistance, the size and composition of the training and validation populations, and the types of prediction models. Multivariate GS models generally improve prediction accuracies employing strongly correlated and highly heritable traits as covariates [22,23], and even more when the records of the indicator traits are also available for the tested genotypes [24–27].

Morpho-agronomical and phenological traits plus the environment directly affect FHB infection [1]. Plant height, heading/flowering date, and floral morphological traits like anther retention (AR) are traits that have been extensively studied in the context of FHB disease [11]. Concerning earliness, it has been shown that there is no systematic type of its association with field FHB resistance, which frequently results in ambiguous associations with reports of negative [28,29] and positive [5,30–33] correlations. It has been postulated that season-specific weather conditions at flowering and inoculation time rather than a shared genetic control might better explain this apparent tradeoff [34–36].

Variations in environmental temperature and humidity in the atmosphere are major factors modulating FHB infection, and there is a general consensus that warm and wet conditions at anthesis favor FHB disease severity [4,37–40]. Wetness periods of at least 24 h and temperatures above 15 °C are required for successful infections by most of the FHB causing agents [4,39], although some epidemics have occurred in seasons with lower temperatures and above-average precipitation at anthesis [41,42]. Temperature also plays an important role from inoculum production and dispersal to its infection of wheat heads [43]. Optimum temperature conditions for disease development depend on the *Fusarium* species, inoculum type and virulence, and the affected tissue [39]. The two most prevalent *Fusarium* species that produce the mycotoxin deoxynivalenol (DON) are *Fusarium culmorum* and *Fusarium graminearum*. Their growth temperature optima under in vitro growth are 24–28 °C and 20–25 °C for *F. culmorum* and *F. graminearum*, respectively [39,43,44]. This is in concordance with the fact that *F. graminearum* is predominant in the United States, Canada, Australia, and parts of continental Europe with hotter summers than North Western Europe, where *F. culmorum* and *F. avenaceum* are among the more predominant species [45]. Ideal weather conditions at anthesis not only promote infection but also encourage the vegetative spread of mycelium to neighboring florets by favoring disease cycle components like perithecia maturation and ascospore formation [40,46–48]. Earliness and environmental variables have been studied together in another pathosystem on wheat via a step-wise multiple linear regression and resulted in temperature and rainfall measurements preferred over heading date [49].

The extent of retained anthers after flowering is, on the other hand, a trait phenotypically associated with FHB resistance [50] specifically with resistance to initial infection [51]. Anther retention (AR) possesses a quantitative genetic nature and a shared genetic correlation with FHB traits as twelve QTL have been reported to be associated with both FHB resistance and AR [51–58]. In the field, AR usually shows a positive correlation with FHB severity, i.e., partially or not extruded anthers are indicative of higher FHB infection and fully extruded anthers are associated with reduced FHB infection [56]. When anthers are either partially extruded or stuck between palea and lemma, they become the media to facilitate the FHB infection into the floret cavity. However, if the anthers are fully extruded, it is more difficult for the FHB pathogen to colonize the spikelet tissue [52]. It has been postulated that the selection of wheat lines with low AR could be a good strategy for breeders when breeding against FHB

susceptibility [56,57,59]. Successful cases have been reported in breeding programs in China [60] and promising results in Europe [61].

In this study, we aim to compare the performance of FHB severity scores from a best-subset multiple linear regression analysis involving flowering time and thermal variables within a genomic prediction framework. Additionally, we are motivated to evaluate multi-trait GS models for FHB severity having anther retention as an indicator trait measured in both the training and validation sets.

2. Materials and Methods

2.1. Plant Material and Field Experiments

Training Sets (TS) for GS models were tested in three trials seasonally evaluated between the years 2015 and 2017 totaling 853 genotypes, being either F_{4:6}, F_{5:7} or double haploid breeding lines. On the other hand, the 143 overlapping lines each evaluated in two consecutive years in the time period 2015 to 2018 were used as Validation Sets (VS). The lines belonged to 429 bi-parental families with sizes varying from 1 to 22 individuals derived from 305 parents. For the purposes of this study, the latter will be referred to as 16-OV1 (2015–2016), 17-OV2 (2016–2017), and 18-OV3 (2017–2018). All the trials were phenotyped for Fusarium head blight severity (FHBs) in an artificially inoculated disease nursery at the experimental station of the Department of Agrobiotechnology in Tulln (16°04, 16' E, 48°19, 08' N, and 177 m above sea level). Within each trial, two replicates per genotype were sown in double-rows of 1 m length with 17 cm spacing. A DON-producing *Fusarium culmorum* isolate (Fc91015) was applied at a conidial concentration of 2.5×10^4 spores mL⁻¹ several times at anthesis with an automatic backpack sprayer. The anthesis date itself was recorded as flowering time (FT) observed as days after 1 May. Constant humidity/moisture conditions were kept through a mist irrigation system during 20 h after each inoculation. Anther retention (AR) was measured at five days post-anthesis as the proportion of 20 florets per plot: Four basal florets of five heads were manually opened and inspected on whether anthers remained within the floret or between lemma and palea. FHBs symptoms were scored as percentage of infected spikes for each plot on 10, 14, 18, and 22 days after inoculation (dai). The area under the disease progress curve (AUDPC) scores were standardized to a 0–100% scale as stated by [62].

$$\text{AUDPC} = \left[\sum_{t=1}^{r-1} \left(\left(\frac{y_t + y_{t+1}}{2} \right) \times (d_{t+1} - d_t) \right) \right] \div (d_r - d_0) \quad (1)$$

where y_t is the observed data at time t and d_t is the t th day of measurement, going from $t = 10$ to 22, and r is the total number of observations $r = 4$.

Accumulated thermal time (ATT) at each of the disease evaluation's scoring dates plus 1, 2, 4, and 7 dai were determined as the sum of degree-days per every single 24 h period, calculated in a modified form as suggested by [63]:

$$\text{ATT} = (T_{\max} - T_{\min}) \div 2, \quad (2)$$

where minimal (T_{\min}) and maximal (T_{\max}) temperatures are measured in °C. Base temperature, defined as the one below which growth in the system ceases, was ignored.

2.2. FHB Severity Correction Methods

The following correction methods were applied over the raw data in a plot observations basis for each trial separately. In addition, every application was done every time a given TS or VS was sampled.

2.2.1. Method-1: Residual Method

AUDPC scores were first corrected as the residuals from the regression on the FT scores x_{FT} , as stated in [64]:

$$AUDPC^1 = AUDPC - \beta_0 - x_{FT}\beta_{FT}, \quad (3)$$

where β_0 and β_{FT} are the intercept and the regression coefficient, respectively.

2.2.2. Method-2: Feature Selection

Alternatively, a preceding filtering step of ATT variables was conducted targeting nonlinear temporal trends. Basically, only the ATT variables showing a high correlation of $r = \pm \sqrt[3]{0.60}$ with respect to flowering time were considered in the next analysis stages. A lasso regression [65] model was employed for selecting relevant predictors amongst the input variables of flowering time, as well as the thermal variables. Let y be the dependent variable, i.e., FHB_r, and p be the total number of predictors x_i ; the original linear regression model can be written as follows:

$$y = \beta_0 + \sum_{i=1}^p \beta_i x_i + \varepsilon, \quad (4)$$

The lasso algorithm estimates linear regression coefficients (β) through L1-constrained least squares, minimizing the residual sum of squares subject to the sum of the absolute value of the coefficients being less than a constant (s). Specifically, for model (4), the constrained L1 norm can be given by the following inequality:

$$\sum_{i=1}^p |\beta_i| < s, \quad (5)$$

The lasso parameter estimates' calculation is a problem equivalent to minimizing the following loss function in a typical Lagrangian form for model (4):

$$\beta_{lasso} = \frac{1}{2} \sum_{l=1}^N \left(y - \beta_0 - \sum_{i=1}^p \beta_i x_{i,l} \right)^2 + \lambda \sum_{i=1}^p |\beta_i|, \quad (6)$$

where N is the sample size and $\lambda \geq 0$ is a complexity tuning parameter controlling the degree of shrinkage. It was obtained through cross-validation as implemented in the *cv.glmnet* function of the *glmnet* R package [66]. Similar to (3), the new subset of features (p') were the new predictors used to correct AUDPC scores:

$$AUDPC^2 = AUDPC - \beta_0 - \sum_{i=1}^{p'} \beta_i x_i, \quad (7)$$

2.3. Field Trials Analysis

Best linear unbiased estimates (BLUEs) were derived from each trial and trait from a linear mixed model of the form:

$$y_{i,j} = \mu + g_i + r_j + e_{ij}, \quad (8)$$

where μ is the overall mean; $y_{i,j}$ are the plot-basis observations of the traits AR, FT, AUDPC, AUDPC-1, and AUDPC-2; g_i is the fixed effect of the i th genotype; r_j is the random effect of the j th replication; and e_{ij} is the random residual effect. Model (8) was expanded to account for the effect of the k th trial t_k as follows:

$$y_{i,j,k} = \mu + g_i + t_k + (g \cdot t)_{ik} + (t \cdot r)_{kj} + e_{ijk}, \quad (9)$$

where μ is the overall mean; y_{ijk} are the plot-basis observations of the traits AR, FT, AUDPC, AUDPC-1, and AUDPC-2; g_i is the fixed effect of the i th genotype; t_k is the effect of the k th trial; $(g \cdot t)_{ik}$ and

$(t \cdot r)_{kj}$ are the interaction terms of “genotype \times trial” and “trial \times replication”, respectively; and e_{ijk} is the random residual effect. Entry-mean heritabilities (h^2) for both training and validation sets were calculated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \frac{1}{2} \text{MVD})$, where σ_g^2 corresponds to the genetic variance and MVD to the mean variance of a difference of the BLUEs [67]. Additionally, plot-basis heritabilities (H^2) were calculated as $H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{nr}}$ for the training sets and as $H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gt}^2}{nt} + \frac{\sigma_e^2}{nt \times nr}}$ for the validation sets where nr is the number of replications, σ_e^2 is the residual variance, nt is the number of trials, and σ_{gt}^2 is the “genotype \times trial” variance component.

2.4. Genomic Predictions

2.4.1. Genotypic Data

DNA from all the lines was extracted with a modified protocol by [68], and each one was genotyped with the genotyping-by-sequencing (GBS) approach (Diversity Array technologies P/L). Quality control filtered out markers with more than 10% of missing data or a minor allele frequency smaller than 5%, which resulted in a set of 5700 single nucleotide polymorphism (SNP) markers.

2.4.2. Validation Schemes

Every validation step consisted of randomly sub-setting 25 and 200 lines as validation (VS) and training (TS) sets, respectively, from their larger sets. The latter sampling was repeated 300 times. Only forward prediction scenarios were considered, resulting in each of the three OV being predicted using their previous trial as TS (Figure 1a). In addition, 17-OV2 and 18-OV3 were predicted with enlarged training sets of 400–600 lines composed of the sum of 200 sampled lines from each of the previous two or three trials (Figure 1b).

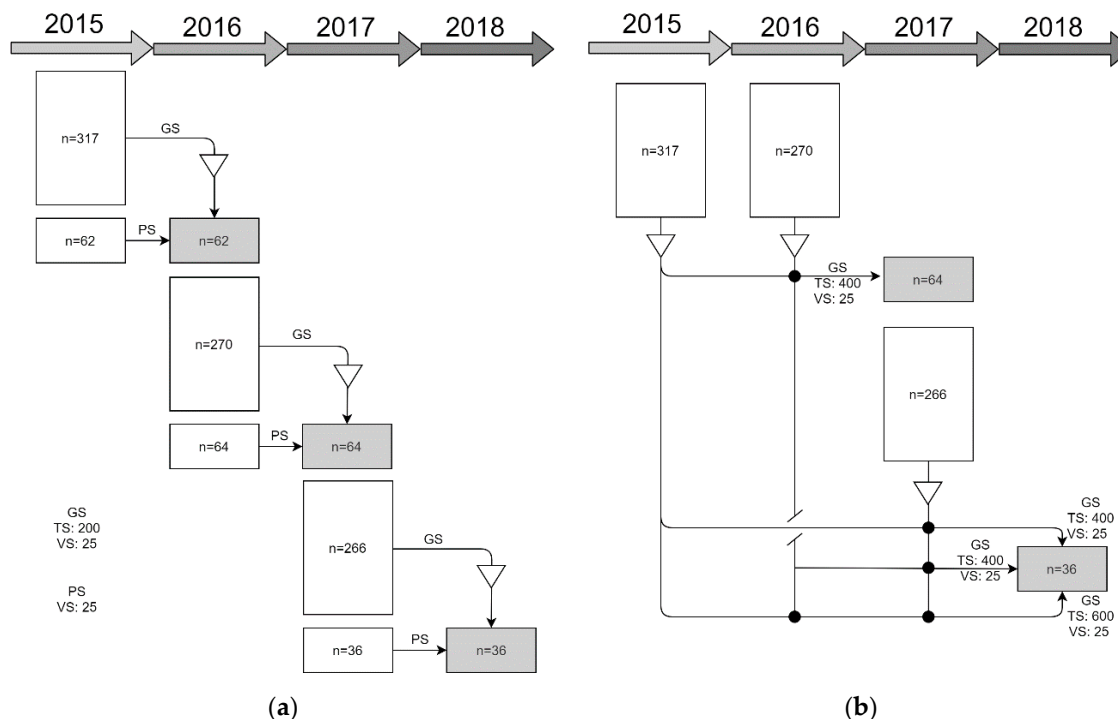


Figure 1. Validation schemes followed in this study. Single trial validation (a) and combined trials validation (b). Triangles point to sampling steps at each validation run. Filled circles reflect the merge of sampled lines from different trials. GS stands for Genomic Selection model, PS for Phenotypic Selection, TS for Training Set, and VS for Validation Set.

2.4.3. GS Models

Genomic best-linear unbiased predictions were obtained from the following model:

$$y = X\beta + Zg + e, \quad (10)$$

where y contains the calculated AUDPC estimates either obtained by (1), (3), or (7); β is the vector of the fixed effects containing the overall mean; g is the vector of genomic estimated breeding values (GEBVs) [$g \sim N(0, G\sigma_g^2)$], with σ_g^2 being the genotypic variance estimated by the restricted maximum likelihood (REML) approach. X and Z are the design matrices for fixed and random effects, respectively; e is a vector containing the residuals $e \sim N(0, \sigma_e^2)$; and G accounts for the genomic relationship matrix as [69] $G = ZZ^T / 2 \sum p_j(1 - p_j)$, where Z is the matrix of m markers and n individuals with elements $z_{ij} = x_{ij} - 2p_j + 1$, x_{ij} is the value of a given allele for the i th genotype at the j th locus, and p_j the allele frequency of the j th marker. Model (10) includes a fixed effect for the k th trial when multiple trials was combined as a training set.

Additionally, a so-called trait-assisted genomic prediction [25,26] was evaluated, in which for each validation run, the assistance trait (AR) estimators of the validation set were taken into account and represented pre-existing information about the genotype performance corresponding to the AR scores measured in the trial preceding the validation year.

Multi-trait genomic best linear unbiased prediction (GBLUP) models followed this general equation:

$$y_t = X_t\beta_t + M_tg_t + e_t, \quad (11)$$

where y_t is the array for a given number of t traits, i.e., AUDPC and AR, containing the BLUE from the phenotypic analysis; and g_t is the array containing the GEBVs [$g \sim MVN(0, \Sigma_g \otimes G)$] with Σ_g as the complete unstructured variance-covariance matrix, $\begin{pmatrix} \sigma_{g1}^2 & \sigma_{g1}\sigma_{g2} \\ \sigma_{g2}\sigma_{g1} & \sigma_{g2}^2 \end{pmatrix}$, and M_t as its design matrix. The terms σ_{g1}^2 , σ_{g2}^2 are the genetic variances of AUDPC and AR, respectively. The residual array follows a distribution: [$e \sim MVN(0, \Sigma_e \otimes I_n)$], where I_n stands for an $n \times n$ identity matrix and Σ_e for the completely unstructured variance-covariance matrix accounting for the residual variance and correlations between both traits.

Prediction accuracy (PA) was estimated as the Pearson correlation coefficient between the BLUE estimates for AUDPC-corrected scores and their respective genomic-estimated breeding values GEBVs accordingly divided by the square root of the entry-mean heritability h^2 .

3. Results

3.1. Phenotypic Values

Disease severity was considerably higher in 2016 than in the other years while the lowest range and less variable scores were observed in the 2017 trial. The 2018 trial was the most variable in terms of flowering time contrarily to the 2017 trial that had the smallest range in FT. The lines evaluated in the 2017 trial were more prone to trap anthers across the largest training sets (Figure S1).

Flowering time was the trait with the highest heritabilities' estimates concerning the across-trials analysis. Heritabilities for AUDPC were moderately high and slightly lower in magnitude than both corrected AUDPC scores, suggesting that the latter were able to capture a higher proportion of genotypic variability.

However, across training sets, heritabilities of the corrected AUDPC scores were lower (Table 1) and a closer look at the repeatability at plot level within each trial showed that they were not improved by the applied corrections (Table 2). Interestingly, the genetic variances (σ_g^2) were higher for the validation sets than for the training sets.

Table 1. Descriptive statistics of the traits studied specified in the training and validation sets.

	Trait ¹	h ²	H ²	σ^2_g ²	σ^2_{gt} ³	σ^2_e ⁴	Min	Mean	Max
Training Sets	AU-R	0.837	0.675	88.63		30.66	0.17	16.35	83.36
	AU-1	0.824	0.624	80.18		32.31	0.17	19.83	83.36
	AU-2	0.816	0.606	76.27		32.33	0.17	20.22	83.36
	FT	0.823	0.602	5.01		1.10	12.00	29.58	40.00
	AR	0.772	0.746	322.99		187.98	0.00	51.82	100.00
Validation Sets	AU-R	0.735	0.751	111.62	122.66	50.23	0.67	16.49	86.67
	AU-1	0.769	0.765	119.56	121.82	49.89	0.67	18.18	86.67
	AU-2	0.757	0.759	116.62	122.46	51.11	0.67	18.08	86.67
	FT	0.914	0.939	4.52	0.202	0.81	12.00	29.11	39.00
	AR	0.862	0.869	331.12	20.75	162.90	0.00	50.66	100.00

¹ AU—followed by either R (raw scores) or the respective numbers standing for correction method-1 and method-2, FT: flowering time, AR: anther retention. ² Genetic variance. ³ Genotype-by-trial variance. ⁴ Residual variance.

Table 2. Performance of the models for Fusarium head blight (FHB) severity under two different correction methods.

Training Sets	<i>n</i>	Method-1		Method-2		Repeatability			
		R ²	AIC	Features Selected	R ²	AIC	AU-R ¹	AU-1 ²	AU-2 ³
2015	317	0.26	−34.56	FT, ATT02, ATT14	0.32	−59.66	0.84	0.83	0.82
2016	270	0.07	148.20	-	-	-	0.87	0.86	0.86
2017	266	0.20	−35.40	ATT04, ATT07, ATT22	0.21	−39.20	0.79	0.75	0.75

¹ AUDPC raw scores. ² AUDPC scores corrected by method-1. ³ AUDPC scores corrected by method-2.

3.2. Trait Correlations and Variable Selection

Disease severity scores in the 2017 trial were atypically correlated toward FT as they showed a moderate negative coefficient ($r = -0.46$), while in both the 2015 and 2016 trials, those traits were strongly and moderately positively correlated with coefficients of $r = 0.51$ and 0.29 , respectively. The smaller validation set in the 2018 trials also exhibited a stronger positive tradeoff $r = 0.73$ (Figure S2).

The correlations between accumulated thermal time (ATT) and AUDPC scores were specific to each trial and differed in magnitude and sense. ATT features in the 2015 trial showed a two-clustered profile correlation with AUDPC being positive from one to four dai and negative from 14 to 22 dai. In the 2016 trial, none of the ATT variables seemed to play a major role concerning disease severity nor flowering time, and this led to method-1 and method-2 to be equivalent in this specific TS. ATT variables at one, two, and eighteen dai were discarded from the feature selection process in the 2017 TS and the rest of the ATT features were significantly positively correlated with disease severity except ATT at 22 dai. In the 2018 trial, all ATT features were considered relevant, and conversely to the 2017 trial, disease severity and earliness were directly related, meaning that the most affected and late-flowering genotypes accumulated a higher degree-day rating (Figure S2). The latter led to the observation that, typically, the most affected and earlier genotypes in this trial were also the ones that accumulated much more degree-days.

Concerning the training sets of both 2015 and 2017 trials, a maximum of three predictors were selected under method-2 and none of those were recurrent. Flowering time was not present on the best-features set in the 2017 trial presumably replaced by ATT at 22 dai as both were negatively correlated with disease severity. The models derived through method-2 significantly improved their counterparts with FT as the unique predictor in terms of R² and Akaike information criterion (AIC) (Table 2). FT was consistently selected as the best explanatory variable in 2017 OV lines, and ATT04 was the unique variable producing the best fit in the 2018 trial (Table 3).

Table 3. Performance of models for FHB severity regarding the overlapping lines evaluated in consecutive years.

Validation Sets	n	Method-1		Method-2		Phenotypic Selection				
		R ²	AIC	Features Selected	R ²	AIC	AU-R	AU-1	AU-2	
15-OV1	62	0.34	−22.13	ATT04, ATT14	0.36	−23.40	0.57 ^B	0.59 ^A	0.59 ^A	DS ¹
16-OV1	62	0.09	46.34	-	-	-	0.55 ^A	0.51 ^B	0.51 ^B	IS ²
16-OV2	64	0.09	45.17	-	-	-	0.74 ^B	0.77 ^A	0.77 ^A	DS
17-OV2	64	0.01	19.86	FT	-	-	0.38 ^B	0.39 ^B	0.40 ^A	IS
17-OV3	36	0.01	−30.98	FT	-	-	0.18 ^C	0.51 ^B	0.55 ^A	DS
18-OV3	36	0.52	−32.24	ATT04	0.59	−38.50	0.58 ^A	0.31 ^B	0.29 ^C	IS

¹ Direct phenotypic selection. Uppercase letters reflect significant differences between correlation scores collected through 300 rounds of selection and compared by Tukey's HSD test with alpha of 5%. ² Indirect phenotypic selection based on anther retention.

3.3. Phenotypic Selection

The highest phenotypic correlation of AUDPC raw scores between overlapping lines was detected between the 2016 and 2017 trials ($r = 0.74$) and the lowest between the 2017 and 2018 trials ($r = 0.18$). Nonetheless, phenotypic selection based on corrected AUDPC scores was superior to the AUDPC raw-based selection across every validation set (Table 3). Significant increments of 4% were on average obtained when selecting lines from 16-OV1 and 17-OV2 regardless of the correction method and, remarkably, selection of the 18-OV3 lines based on corrected scores led to up to more-than-threelfold increments where method-2 outperformed method-1 (Table 3).

Given that anther retention AR scores were used to select for disease severity (indirect selection IS), the correlation coefficients were lower than the direct selection described above except for the selection of AUDPC raw scores of 18-OV3 lines. This indirect selection based on AR was most favorable in the selection of 16-OV1 lines because of both the high correlations AR-AR and AR-AUDPC on the implied trials (Table 3, Figure S3).

3.4. Genomic Selection

3.4.1. Single Trials

Across the three validation scenarios with TS composed of single trials (schemes detailed in Figure 1a), prediction accuracies PA averaged 0.28, 0.33, and 0.36 for the GS models based on AUDPC raw scores (AU-R), and corrected under method-1 (AU-1) and method-2 (AU-2), respectively (Figure 2a). Hence, genomic predictions based on corrected AUDPC scores under method-2 showed a slightly superior PA among all three scenarios, being most notorious for the prediction of both 16-OV1 and 18-OV3 sets. This advantage of method-2 over method-1 reflects the better model fit in the phenotypic analysis of FHB severity in both TS of the 2015 and 2017 trials, as well as in the 18-OV3 VS by selecting additional and/or different features than flowering time (Table 2, Table 3).

Increments from 8% up to more than sixfold were achieved when anther retention scores from their previous year served as the assisting trait in multi-trait genomic selection (MT.GS) models predicting 16-OV1 and 18-OV3 lines, respectively (Figure 2a). Single-trait ST.GS models predicting 18-OV3 lines performed poorly in terms of PA; however, their MT versions produced the largest increments when modeling AUDPC-corrected scores (Figure 2a). On the other hand, the prediction of the 17-OV2 lines was the scenario where the most accurate ST genomic predictions were obtained, and it was precisely the one that did not represent an opportunity for their MT versions with poorer PA performances, which was 3% lower regardless of the type of AUDPC scores (Figure 2a). The latter observation for such a case might be partly explained by the fact that this was the only validation scenario where, on average, prediction accuracy is sensibly higher than the correlation of AR-AUDPC seen in the indirect selection based on AR scores from the previous year.

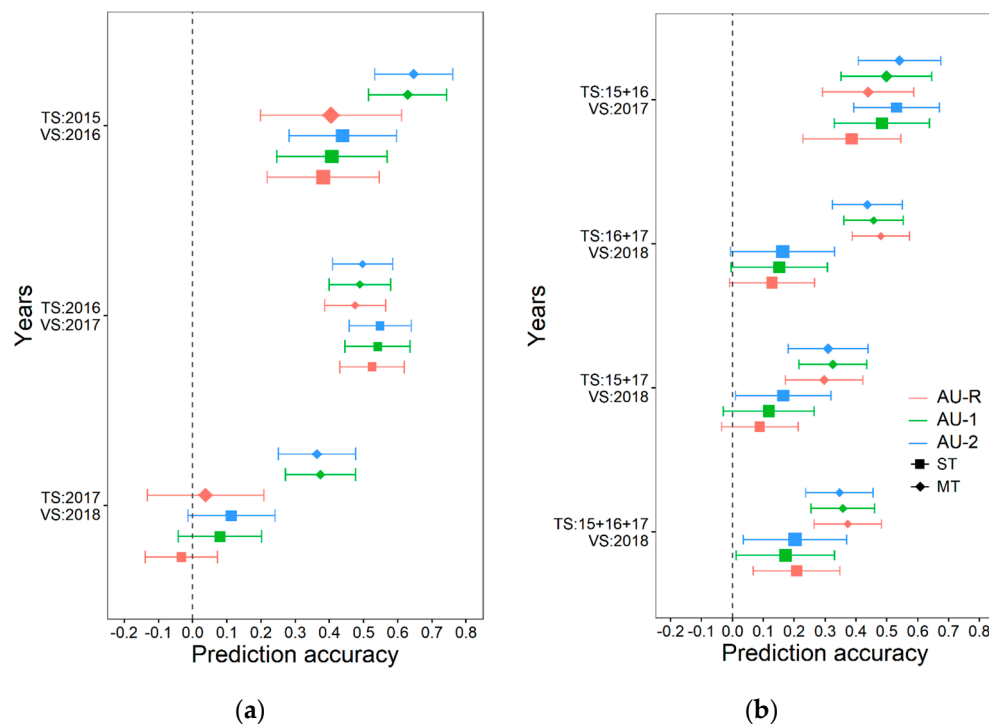


Figure 2. Prediction accuracies PA for the proposed genomic selection schemes: (a) For GS models with training sets composed of lines from single trials and (b) with training sets composed of lines from combined trials. TS and VS stand for training and validation sets, respectively. AU-R, AU-1, and AU-2 stand for area under the disease progress curve (AUDPC) scores either uncorrected or corrected under method-1 or method-2, respectively. ST and MT explain whether the GS model included disease severity alone or another retention was included as an assisting trait, respectively. Standard deviations are presented as the length of each segment, and the width of either the squares or diamonds correspond to the 95% confidence interval.

3.4.2. Combined Trials

The GS models aiming to predict 17-OV2 lines trained with the combination of lines from the 2015 and 2016 trials led to worse PA performances for both traits AU-1 and AU-R scores (PA = 0.48 and 0.41) compared with the model trained only with the 2016 trial (PA = 0.52 and 0.54). In the latter case, the prediction of the AUDPC scores corrected by method-2 (AU-2) outperformed the other traits and was equivalent to its respective single-trial counterpart (Figure 2b).

The following outcomes might be highlighted from the three scenarios of the genomic selection of the 18-OV3 lines employing TS composed from various trials (Figure 2b):

The poor performance of models exclusively trained with lines from the 2017 trial was improved and, more specifically, genomic predictions based on AU-2 scores resulted in performances averaging PA = 0.18 and 0.36 for the ST and MT models' versions, respectively, versus models based on AU-1 scores PA = 0.15 and 0.38, and models based on AUDPC-raw scores PA = 0.13 and 0.38.

The only scenario where the type of FHB severity score represented significant differences in PA was when 2015 and 2017 trials were combined to train the single trait GS model, leading to the following order according to their PA: AU-2 > AU-1, AU-R.

The highest PA rates for the ST.GS models were obtained when the TS was combined with lines from the three previous trials regardless of the type of severity score, while for their MT.GS model versions, the most successful cases were models trained with the merge of lines from 2016 and 2017 trials. Across all scenarios in consideration, the relative increments in PA of MT.GS models averaged 3.1, 2.6, and 2.1 folds for the AU-R, AU-1, and AU-2, respectively, from their single trait model versions.

4. Discussion

This study evaluated a procedure consisting of a best-subset multiple linear regression analysis accounting for earliness and temperature variables to produce corrected FHB severity scores that were subsequently compared with the original trait under various genomic prediction scenarios. The convenience of including anther retention as an indicator trait in phenotypic and genomic selection was also investigated, and by doing so, this becomes the first report, at least to the best of our knowledge, of such an attempt in winter wheat.

QTL mapping studies have found twelve overlapping loci between HD/FT and FHB resistance traits, with the most frequently detected QTL being close to the *Vrn* and *Ppd* genes that control, among others, the vernalization requirement and photoperiodic sensitivity in wheat [11]. However, when it comes to multi-environment trials, the nonsystematic type of FT-FHBr associations have been detected [36] and several studies follow a pre-correction of the FHBs scores to dissect it from passive mechanisms of resistance [34,70,71]. In order to tackle this trade-off, genome-wide association mapping and prediction studies [27,70–72] oftentimes follow the approach described in [34], which is basically the same as that referred to in this study as method-1. Our findings constitute in this way an enhancement from the latter approach that exclusively considered earliness traits and increased the explainability of the genetic variability of FHB resistance of wheat lines evaluated in consecutive years. According to our results, up to three thermal variables were preferably selected over FT in 2017 and 2018 trials as best predictors. Noticeably, stepwise linear regression has often been used to identify the best predictors when modeling epidemiologic data including the FHB pathosystem [73–75]. However, the usage of the forward and backward elimination of variables has been discouraged, with all subset selections being a more statistically grounded option [74], whereas lasso regression often outperforms the latter methods due to a higher coefficient stability, especially in cases of multicollinearity between predictor variables [76].

Wheat heads are most susceptible to FHB infection at anthesis [77] but infection can occur up to the soft dough stage [78]. ATT at the day of anthesis/inoculation was significant and directly correlated with FHBs, except in the 2016 trial, and it became the best predictor in the TS of the 2015 trial and the 18-OV3 lines. ATT variables closer to the date of anthesis were likewise chosen in 2015 (two dai) and in 2017 (both four and seven dai). On the other hand, ATTs at 14 and 22 dai were additionally selected in 2015 and 2017 trials, respectively. Models employed to predict the risk of FHB epidemics [73,79] often consider among their predictors temperature measurements at up to 15 days post-anthesis and interactions with either rainfall or humidity variables. Considering the latter weather measurements, as well as alternative nonlinear responses of FHBs to environmental variables [63], might improve the proposed method-2. Concerning relative humidity, it was not accounted for in the presented approach because of the assumption that mist irrigation conditions kept this factor at high and constant levels in trials conducted for the study at hand.

The accumulation of temperature seemed to favor the development of disease at early stages of disease development (one to seven dai) across all trials. Contrarily, less visible symptoms were correlated with ATT variables at later evaluation stages in 2017 (at 22 dai) and especially in 2015 with the difference that in the former trial, earliness was associated with more susceptible genotypes. A shift in the daily temperature might explain those changes between 43 and 51 days after May 1st in 2015 where a decrease of almost 11 °C in the daily mean temperature was detected. Seemingly in 2017, there was a sustained increase between 48 and 53 days after 1 May 2017 of 8.5 °C (Figure S4).

The composition and size of the training sets are of utmost importance in GS as evidenced by simulated and empirical data [80–82], and in that regard, this study proposed validation scenarios using complete trials as folds to validate genotypes in unobserved years. Poor performances of GS models predicting 18-OV3 lines were found in absolute terms of prediction accuracies that were improved on average 2.9- and 1.9-fold for 2–3 times larger training set sizes, respectively, by adding both lines and additional trials. Nevertheless, the contrary effect was observed for 17-OV2 lines especially for uncorrected AUDPC scores, where the prediction accuracy was 20% lower when the

TS consisted of lines from both 2015 and 2016 trials. As both the 2015 and 2018 trials were heavily dependent on earliness and temperature, adjusting their AUDPC scores from these strong phenological influences putatively allowed the GS models to exploit genomic relatedness more efficiently. Prediction accuracy is supposed to be directly related to the degree of genetic relationship between training and validation sets. The performance of GS models in terms of prediction accuracy for FHB resistance in wheat has been validated in independent samples [83] and found to be comparable to cross-validated schemas conditioned to the relatedness level TS-VS. Additionally, based on empirical results, it has been proposed that less biased predictions across breeding cycles/years can be expected for highly heritable traits like FHB or plant height, as shown in barley [84] compared to quality traits in wheat [85] and sugar beet [86].

The correspondence between a high proportion of retained anthers and an increased FHB severity has been systematically found across wheat populations [58]. AR is considered a morphological marker for FHBr, especially for its Type I or resistance to initial infection [52,59]. Here, indirect selection based on AR measurements led to correlations between 0.30 and 0.51, and they were on average 34% lower than the actual direct selection based on AUDPC scores. Anther retention keeps the advantages of traits like plant height or flowering time as a low-cost phenotype indirectly associated with FHBr, but unlike the latter two, it is agronomically more neutral in the sense that further breeding implications/considerations like the selection of unfavorable tall genotypes might be largely avoided. Although AR was also correlated with plant height in the germplasm studied here, this correlation was low and mostly attributed to the presence/absence of the dwarfing alleles at the Rht-B1 and Rht-D1 loci (data not shown). Multi-trait GS models in the FHB resistance context in wheat have been studied, most of them accounting for flowering time and plant height [27,87,88] as covariates or related traits like FHB incidence, DON concentration, or Fusarium damage kernel index (FDK) [89,90]. An upsurge in prediction accuracy of MT.GS models was confirmed here with AR as the indicator trait for most of the validation scenarios. For instance, increments in prediction accuracy averaged 46% across the three scenarios consisting of multiple trial-training sets predicting 18-OV3 lines; however, the MT.GS models performed slightly worse (−3%) when aiming to predict the set of 17-OV2 lines with lines from the previous year. From simulation and empirical proofs, it has been noted that both higher heritabilities and correlations are needed for the indicator trait in order to yield higher accuracies [23,88,91,92]. As both FHBr and AR had a similar heritability in the study at hand, the degree of their correlation in each GS scenario seemed to be the major constraint for improving the prediction accuracy. Hence, in cases when this correlation was low, the prediction accuracy could not be improved beyond the respective single-trait model, while an advantage of using AR as an assisting trait in MT.GS models was generally seen with an increase in the correlation of AR-FHBr. Finally, MT.GS models assisted by AR also represented an advantage over their indirect phenotypic selection counterparts.

5. Conclusions

The approach presented here to account for temperature variations within and among seasons for correcting FHB severity scores, although not being the most comprehensive, turned out to be a simple and fast way to produce consistent predictions for observed and unobserved winter wheat genotypes. Furthermore, multi-trait genomic selection models evidenced the feasibility of the usage of AR as an assisting trait even in cases without FHB records available (e.g., early breeding stages), which could be scored and used for indirect FHBr selection and will not require additional considerations to compensate for undesirable indirect effects during selection.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/12/2008/s1>, Figure S1: Boxplots on the distribution of the traits analyzed distinguished by both training and validation sets. (a) Flowering time measured in days after May, (b) AR in percentage, and (c) AUDPC in a 0–100 scale. Figure S2: Correlation plots of the traits analyzed in the years (a) 2015, (b) 2016, (c) 2017, and (d) 2018. The abbreviations are as follows, AR: Anther retention, FT: Flowering time, ATT: Accumulated thermal time at different days post-infection. AU-R: AUDPC scores, AU-1: AUDPC scores corrected under method-1, AU-2: AUDPC scores corrected under method-2. Lower diagonals represent the training set and upper diagonals the validation set.

Figure S3: Correlation plots between the overlapping lines in two consecutive years: (a) 2015–2016, (b) 2016–2017, and (c) 2017–2018. Abbreviations of each trait are followed by the last two digits of the year of the respective trial. AR: Anther retention, FT: Flowering time, AU-Raw: AUDPC scores, AU-1: AUDPC scores corrected under method-1, AU-2: AUDPC scores corrected under method-2. Figure S4: Temperature profiles of each of the seasons (a) 2015, (b) 2016, (c) 2017, and (d) 2018 measured at the experimental station of the Department of Agrobiotechnology in Tulln (16°04, 16' E, 48°19, 08' N, and 177 m above sea level).

Author Contributions: J.M.-A. wrote the manuscript and analyzed the data. J.M.-A. and S.M. conceived the analysis. F.L. and H.B. initiated and guided through the study. All authors have read and agreed to the published version of the manuscript.

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Discussion

The correction methods accounting for earliness applied in publication #1 revealed a lower across-environments genetic variance for FHB resistance, which was detrimental to the cross-validated prediction abilities. On the other hand, it was also revealed the effectiveness of these methods including the usage of restriction indexes to control the indirect response to selection for the correlated traits earliness and plant height. Taking together, the tighter the relationship of the correlated traits with the trait of interest i.e. FHB resistance, the higher were the losses in prediction abilities. This finding was in concordance with a follow up study in durum wheat [40]. However taking a closer look on seasons' contrasts, publication #2 proposed thermal measurements at several days *post* infection as additional covariates related to FHB severity. Such correlations were at least of moderate strength and mostly positive in all the evaluated years except in 2016, which was the year with the least variable temperatures in the inoculation plus evaluation time window (Appendices – Figure S4). It also suggests that these measurements were more explanatory of the variability on both training and validation sets than flowering time alone for FHB severity across seasons and at early stages of disease development. Even more, thermal patterns across years during flowering and disease evaluation presented drastic changes that were in correspondence with the thermal variables chosen as relevant explanatory features. Therefore, adjusting for this source of extra variability of FHB severity was the pivotal step to get further accuracy improvements in both phenotypic and genomic selection.

Aside from the genetics, cultural practices or management strategies are also involved as key players to prevent FHB pandemics. For instance “ultra-early” planting date is an important management strategy for durum wheat in Canada to achieve higher attainable grain yield and quality and is also a matchable strategy with early maturing cultivars to diminish the probabilities of coincidence with the optimal conditions of FHB infection and other diseases [4, 111]. However West et al [112] suggested that such adaptation might have the unexpected consequence towards FHB. Based on simulation studies Madgwick et al [113] predicted higher epidemic FHB risk in the southern UK and mycotoxins levels exceeding the EU limits by 2050. On the other hand, [114] discussed how early flowering time winter wheat is a desirable feature given seasonal fluctuating conditions, plus the potential to mitigate drought stress, improve some logistics and crop management, and evade pest and pathogen damage specifically the ergot fungus, *Claviceps purpurea*, and orange wheat blossom midge (OWBM; *Sitodiplosis mosellana*). Most likely climate change will directly impact the prevalence and severity of FHB and mycotoxins concentrations in wheat crops since weather is one of the main factors affecting the severity of epidemics and also the proportions of the different FHB causing agents [112, 115]. The European Food Safety Authority (EFSA) has for instance suggested

that in the next 20-50 years extreme changes in rainfall/drought, elevated temperatures and CO₂ will result in a much earlier ripening in cereals in the Mediterranean basin and central Europe [116]. Temperature and relative humidity are the most relevant ecophysiological factors influencing both fungal colonization and mycotoxin production [117]. Precisely, one of the manifestations of climate change is the raise in temperature [118] and this can inhibit, stimulate or not have any effect over fungi growth [117]. Warmer and more dry conditions for instance might provoke different reactions to mycotoxigenic fungi that are believed to adapt to changes due to their plasticity [119]. Biotic constraints like diseases affect wheat productivity and they must be included in the assessments of climate change studies. Several model simulations have been conducted [120–122], however they must be interpreted carefully and more studies are needed specially restricted to more locations [115]. One example are the prediction tools combining weather cropping practices and mycotoxins concentrations [DONCAST, TOOLBOX]. In general, mycotoxins increases with rainy days and days with relative humidity > 75% but decreases with temperatures <12 or > 32C [123]. In this light, Fernandes et al [124] showed a higher FHB risk factor under climate change scenarios, rendering the development of resistant cultivars one of the most effective measurement to control FHB.

The scale on which FHB severity was assessed differed across publication 1 and publication 2 since in the former it was presented as the average percentage taking four evaluations and in the latter with the area under the disease progress curve (AUDPC). This aspect turn out to be of great importance and there might be some indications that disease progress as AUDPC might be able to better represent drastic time course variations than individual scores and the correlations with other traits like DON concentration [125, 126]. In practical scenarios with large populations, FHB evaluations are done at fewer time points. Hence, special attention must be placed to handle those measurements as well as to come up with proper standards for their use and share.

In both publications, earliness was analysed either as heading date or flowering time. Both heading date and flowering time are generally presented as the amount of days required to reach that respective stages with temperature being one of the major determinants [127]. Several authors agree on the switch of the quantification, report, and share of the such phenological records towards thermal units such as degree days (Cd) after sowing [128] or grown degree days GDD. Thermal time is considered a most reliable, steady and robust and widely accepted manner to represent HD/FT as relies on the collection of daily mean temperatures instead of a the number of days [129] [130]. In fact GDD has been used to predict anthesis date in perennial ryegrass [131] and other development stages in barley, corn and wheat [127, 132]. Bogard et al [133], Hyles et al [134] suggested that it is the most suitable

manner when conducting across-environments studies like the ones conducted by Rose and Kage [135] or Kahiluoto et al [136].

Exotic taller lines considered as outliers were excluded from the analysis in the durum wheat panel of publication 1, which led to lessen the negative trade-off towards disease severity that was significant in only one of the environments. To some extent, PH played a less important role than heading date in that panel just in contrast to a couple of studies in hybrid [137] and durum wheat [40]. In those analyses PH was evaluated in trait- assisted genomic selection for FHB, and better predictabilities were found with authors recognizing that further considerations like culling or selection indexes should be adopted to counteract the above-mentioned unfavourable trade-off. When selecting for FHB resistance the usage of selection indexes to mitigated the selection towards higher genotypes have been investigated in an elite durum wheat panel [40]. A drawback of selection indexes might though in general be that the eventual exclusion of the best individuals for single traits and their poor ability to control for inbreeding [138]. A multi-objective optimized breeding strategy that assessed the unfavourably trade-off of multi correlated traits in the simulated selection of parent combinations. Another alternative is the application of culling levels as studied in [139], who applied the average of the predicted plant height resulted in winter wheat genotypes smaller than 97 cm for improving FHB and Septoria leaf blotch resistance in Western European breeding material. Steiner et al 2018 additionally culled the earliest and latest genotypes in a durum wheat panel based on a phenotypic pre-selection followed by a genomic assisted selection strategy.

In a recent published study [140] in spelt (*Triticum aestivum* subsp. *spelta* (L.) Thell.), significant negative correlations were found between plant height and FHB severity while positive coefficients were reported for anther retention and FHB severity. From a previous study [141] it can be assumed that in traditional spelt wheat genes other than the Rht-B1 and Rht-D1 are responsible for the variation in plant height. Plant height has been hypothesized to play a 'disease escape' role from natural FHB infections via morphological and structural changes that led to differences in canopy microclimate [142]. It was also found a protective effect of tough glumes, which accumulated higher DON concentrations than those in the grains, towards FHB resistance in agreement with other studies in such crop [143, 144]. The mentioned study [140] was conducted in three European locations seasonally evaluated between 2016 and 2018 and evaluated a diversity panel of 80 genotypes included spelt landraces and modern and old varieties. It was point out in addition that modern spelt varieties tended to fall into the susceptible to highly FHB susceptible group, possibly due to introgressed Rht-alleles from bread wheat.

Regarding the study of correlated characters, multi-trait analyses are an interesting approach to better understand genetic correlations based on pleiotropy or linkage. An example is the general approximation of BayesC π presented by [145] in which the genetic architecture of the traits can be stated via assumption of a mixture of priors, avoiding the -always violated- assumption that a given locus has an effect either on all traits or none of them and by allowing cases where a given marker has a null effect on a trait but not on the others. It was highlighted that the latter method can favour the prediction of highly heritable disease traits with low-heritable traits correlated in loblolly pine [146]. A more recently study [147] tested the same dataset with a multi-trait Bayesian Lasso model that applies a differential shrinkage of the marker effects and it outperformed both the BayesC π and MT.GBLUP models. It is expected that if there is a sort of homogeneity on the multi-trait vectors of the effects most methods will perform seemingly. On the other hand, if there are indications of certain level of sparsity, methods like the ones recently mentioned most likely outperformed counterparts like GBLUP. Moreover, it has been suggested from animal breeding studies that the accuracy of MT.GBLUP might be improved if marker effects are weighted by their heterogeneous covariances resulting from Bayesian methods [148, 149]. Genomic prediction problems are unique depending on the species, the trait as well as its underlying data structure it seems meaningless to compare the power of different prediction machines across datasets. Several studies have come to the common place of there is no universally best prediction machine [89, 102, 103, 150–153] and in some cases they can also lead to simplistic solutions if truly complex quantitative traits like diseases are studied.

GS accuracies in the experiment proposed in publication 2 were in most of the cases beneficiated when MT.GBLUP. This approach has been called trait-assisted or CV-2 [100, 154, 155] when the additional secondary trait' records of the tested lines are included. This GS approach led to marginal accuracy increments compared to phenotypic selection only in one of the scenarios (TP15 \rightarrow VP16, PS=.59 GS=.62), however that increments were usually more important in comparison to indirect selection based on the assisting trait (IPS=.31 vs GS=.4, IPS=.59, GS=.62). Selection gains were assessed in publication 1 where the most accurate GP model selected genotypes that were on average around 2% more resistant in comparison to the base model. The most efficient model to restrain the gains in the covariates (IDX.ST) allowed the selection of lines almost 2 days earlier flowering than genomic selection without considering the trade-off HD and FHB (Figure 3). Tessema et al [156], Gaynor et al [157] conducted simulated long-term wheat breeding programs to compare the genetic gains of PS vs GS and found increments up to 1.12 and 3 times higher respectively, mainly via increments in selection accuracy. Herter et al [139] reported an advantage of realized genetic

gains of 10.6 percentage for FHB resistance in a panel of 2500 winter wheat lines when training model with 1120 lines. Despite the former and further evidences from simulation and cross validated studies reporting higher accuracies for GS over conventional selection until this date, there is no breeding practical proof that GS increase long-term genetic gain [91, 158]. Increasing the amount of selection cycles per time unit is crucial and it might now be attainable throughout methods like speed breeding [159]. Recently, Jighly et al [160], Sekine et al [161] showed improvements in accuracies of simulated combinations of shortening breeding cycles and genomic selection for several traits in tall fescue and onion, although the former study also assessed the consequent side effect of a higher inbreeding rate.

The validation scheme in the publication 2 was an attempt to mimic more realistic scenarios of a forward prediction compared to typical cross validated schemes. The accuracies ranged overall from very poor to moderate even with larger training populations, although beneficial effects of increasing training set sizes on prediction accuracies are well known [162–164]. Despite correction by season specificities, difficulties to predict FHB severity of genotypes in 2018 by models trained with data from the previous year can be partially explained by the larger genetic distance as shown in a population structure analysis [Appendices – Figure S5]. Most precisely [165] reported prediction accuracies of new genotypes in new environments were lower than those obtained by cross validation, with the latter being the later overestimated on average by 12% for FHB resistance in European winter wheat. They echoed other references recommending sampling both genotypes and environments to cross-validate either in MAS or GS approaches. Forward validation -or prediction of genotypes' performance in untested environments- schemes resulted in lower prediction accuracies compared to cross validated within years for grain yield in oats and barley [166]. Conversely, larger and closer-related training sets led to better prediction accuracies for yield and quality traits in strawberry [167].

In publication 2, the accuracies when predicting 2018 lines trained with sets of lines composed from different trials fluctuated less when corrected either by earliness or by thermal variables. The degree of genetic relatedness between training set and selection candidates is an important factor affecting accuracy [168, 169]. Despite demonstrations that enlargement of training sets with closely related individuals might optimize its composition, quality of the phenotypic records and genetic diversity of the training sets are also important criteria [170]. In addition, multi-objective optimizations have been envisioned in the design of training populations that would accordingly balance genetic diversity, relatedness and other criteria [138].

Conclusion

The correlation of FHB severity towards earliness confirmed the ambiguity of this particular trade-off across the datasets analysed in this thesis. While in the durum wheat dataset the usage of corrected scores led to poorer cross-validated predictabilities, a similar approach was the most effective under independent validation scenarios across different seasons in winter wheat. Plant height and/or days to heading were used in the former while in the latter the adjustment targeted earliness and thermal variables. Therefore, an appropriate assessment of the genetic architecture of the traits involved in genomic prediction as well as the associations between them is crucial to avoid undesired side effects because of selection and also to get better prediction accuracies.

Considering that anther retention has already been suggested as a trait to be selected for when FHB resistance is desired, here was presented one of the first demonstrations of the usage of anther retention as an assisting trait in genomic predictions for FHB resistance in wheat. Hence and more specifically, the multi-trait and across cycle genomic selection assessed might be seen as an appealing alternative to be further studied in wheat and hybrid wheat breeding programs.

Evidence of shifting from single to multi-trait approaches in genomic prediction studies has been previously demonstrated and corroborated here. In the studied pathosystem, the accuracy of the genomic selections of lines based on the resistance to FHB must be revised in the light of the incorporation of additional correlated traits. This thesis has thus envisaged starting point alternatives when forth-coming numerous traits generated from the latest technologies need to be jointly analysed.

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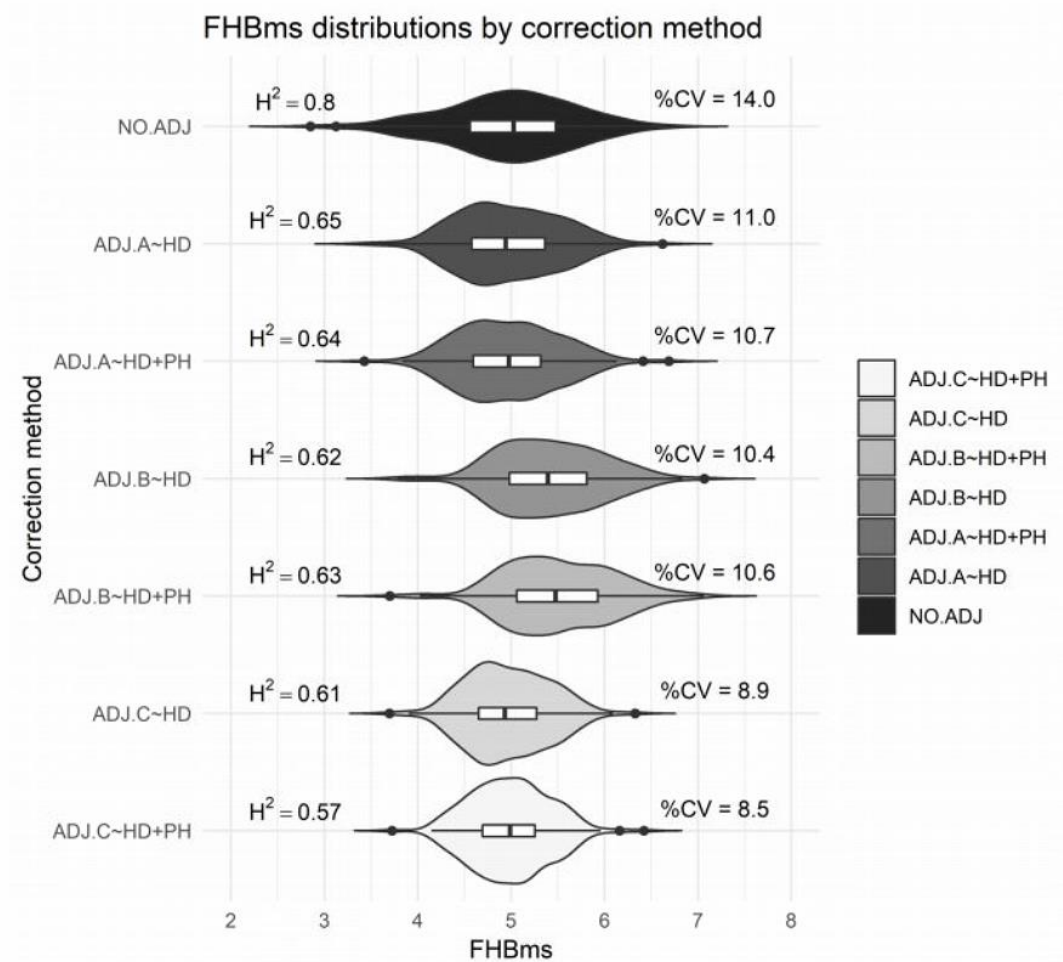
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Appendices

Supplementary material of publication 1



Supplemental Figure 1: FHB mean severity distributions by several correction methods.

Supplemental Table 1: Mean prediction ability for Fusarium head blight severity and standard deviation for each environment

Environment	Prediction ability	Standard deviation
Hoh13	0.63 ^c	0.09
Oli13	0.63 ^c	0.09
Hoh14	0.69 ^a	0.07
Oli14	0.61 ^c	0.09
Tul14	0.67 ^b	0.08

Mean prediction ability values were compared with Tukey's HSD test with $\alpha=0.05$. Field trials: Hoh= Heidfeldhof (DE), Oli= Oberer Lindenhof (DE), Tul= Tulln (AT); evaluated in 2013 and 2014.

Supplementary material of publication 2

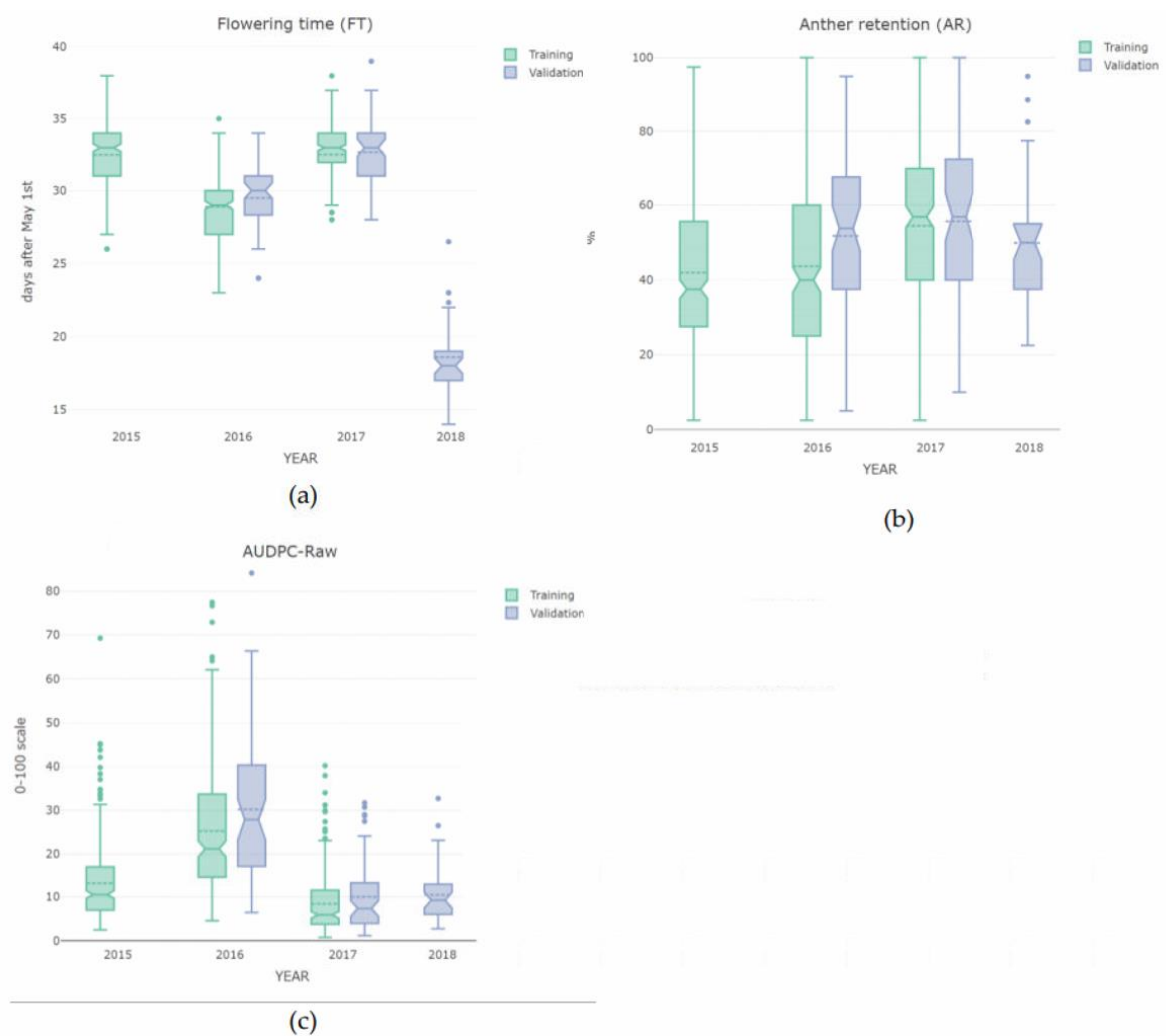


Figure S1. Boxplots on the distribution of the traits analyzed distinguished by both training and validation sets. (a) Flowering time measured in days after May, (b) AR in percentage and (c) AUDPC in a 0-100 scale.

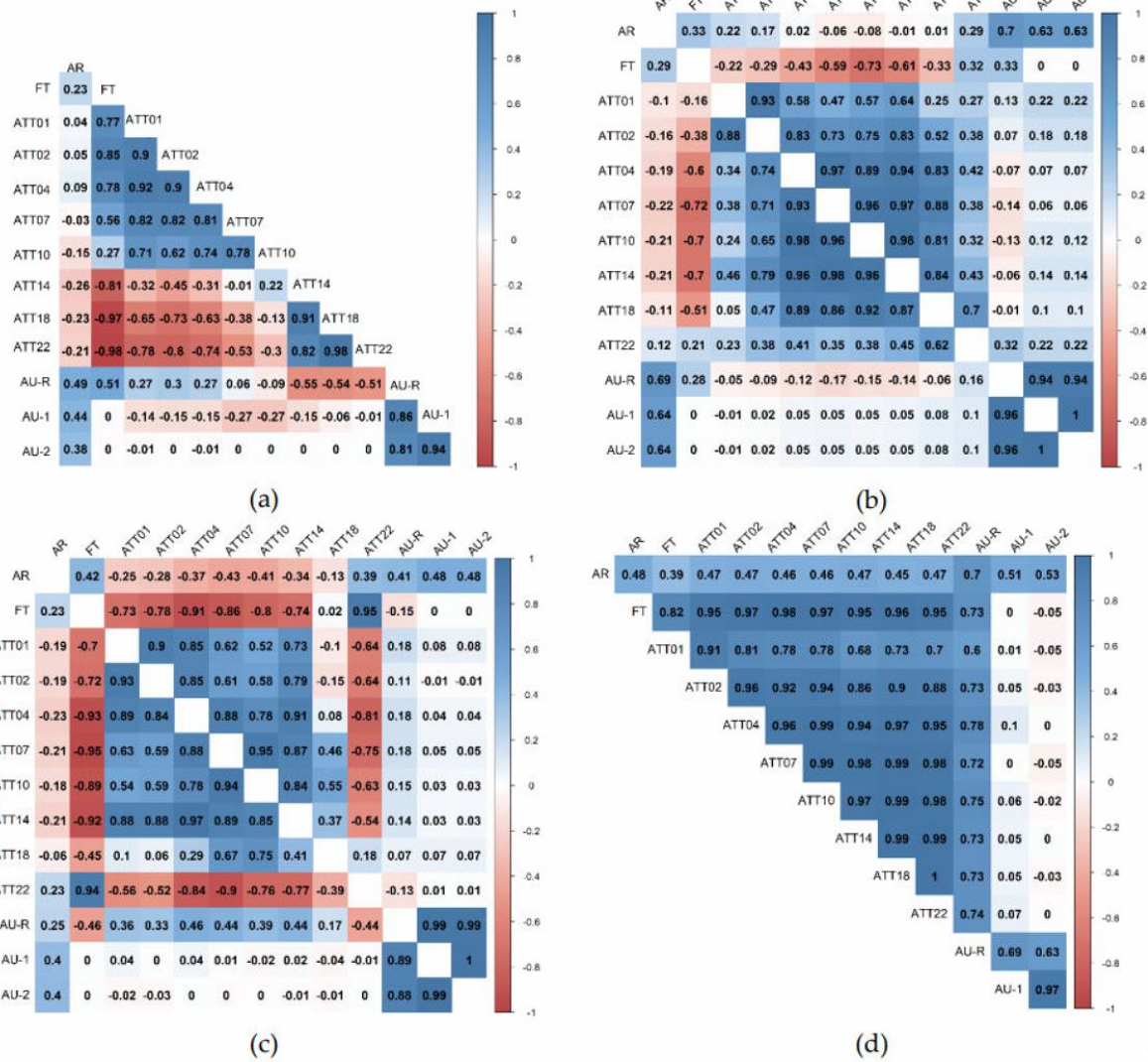


Figure S2. Correlation plots of the traits analyzed in the years (a) 2015, (b) 2016, (c) 2017 and (d) 2018. The abbreviations stand as follows AR: anther retention, FT: flowering time, ATT accumulated thermal time at different days post infection. AU-R: AUDPC scores, AU-1: AUDPC scores corrected under method-1, AU-2: AUDPC scores corrected under method-2. Lower diagonals represent the training set and upper diagonals the validation set.

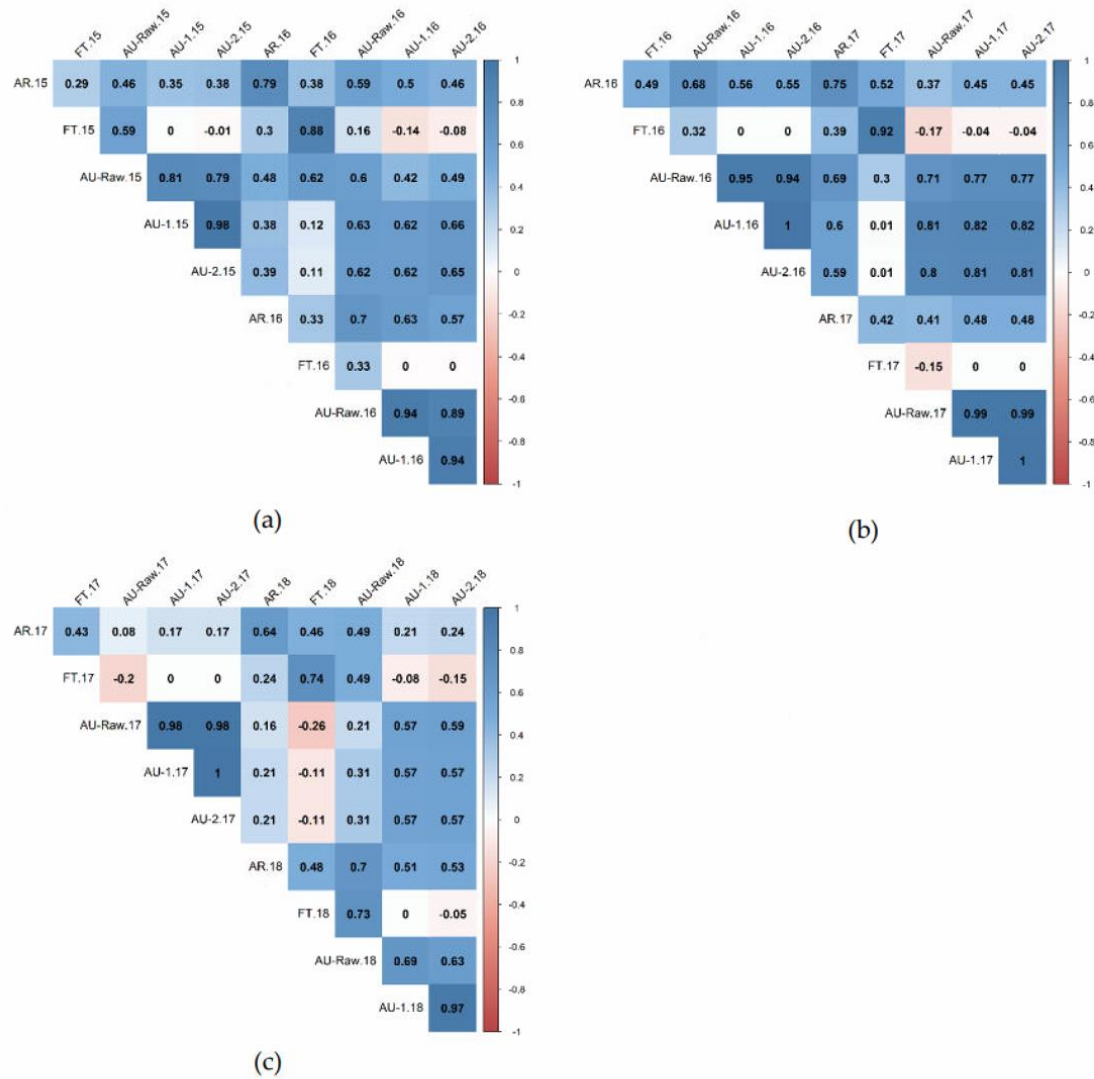


Figure S3. Correlation plots between the overlapping lines in two consecutive years (a) 2015-2016 (b) 2016-2017 and (c) 2017-2018. Abbreviations of each trait are followed by the last two digits of year of the respective trial. AR: anther retention, FT: flowering time, AU-Raw: AUDPC scores, AU-1: AUDPC scores corrected under method-1, AU-2: AUDPC scores corrected under method-2.

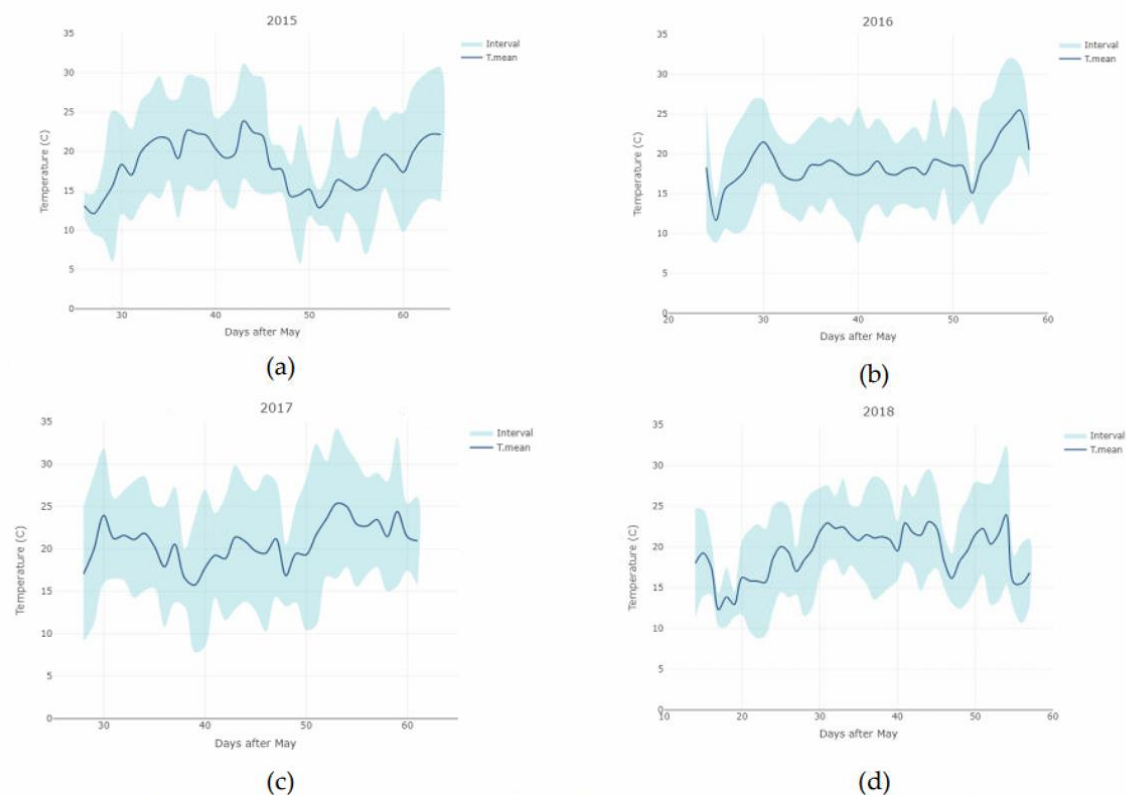


Figure S4. Temperature profiles of each of the seasons (a) 2015, (b) 2016, (c) 2017 and (d) 2018 measured at the experimental station of the Department of Agrobiotechnology in Tulln (16°04, 16'E, 48°19, 08'N, and 177m above sea level).

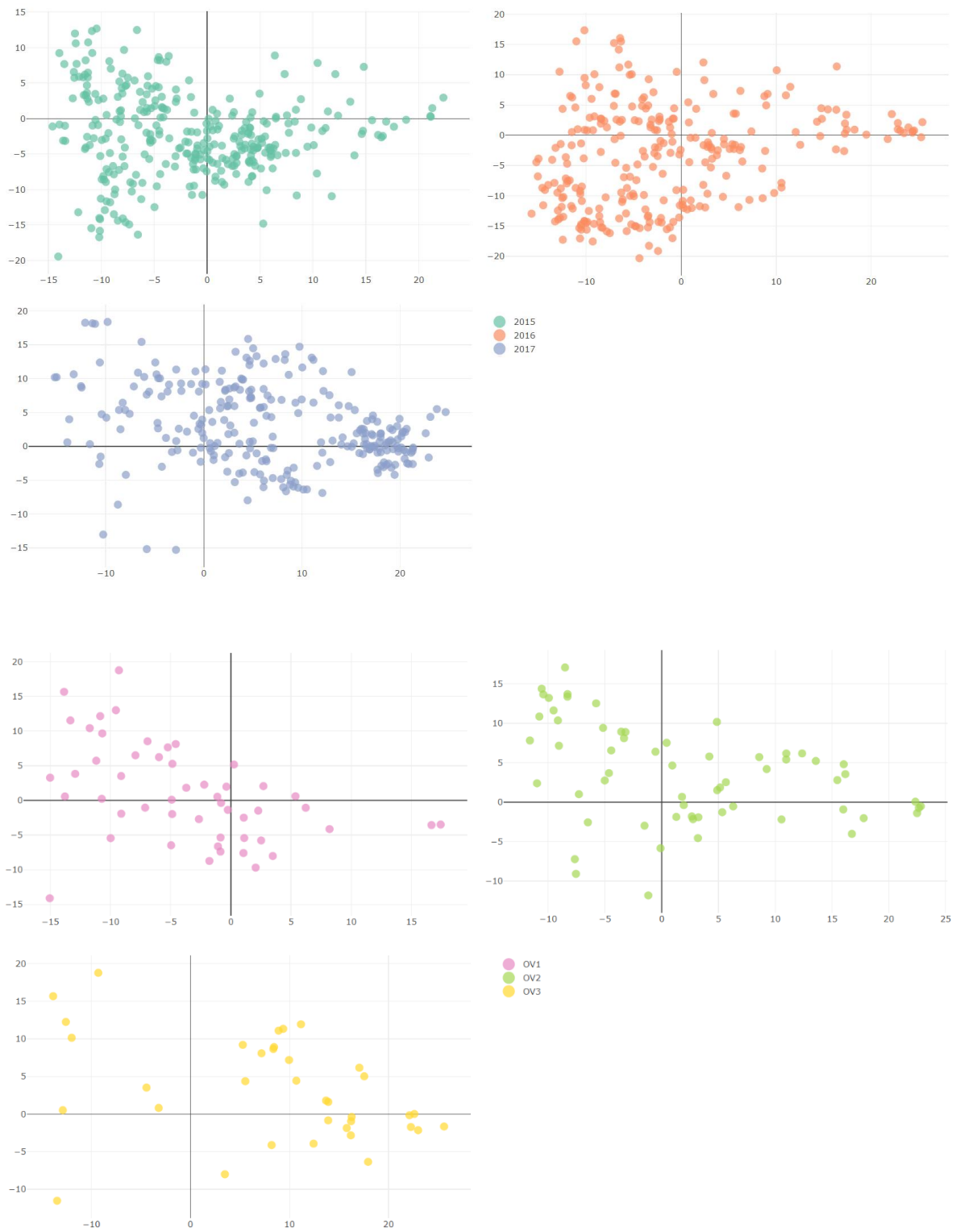


Figure S5. Population structure of each of the training sets (above panel) and validation sets (below panel).