Coupling Day Length Data and Genomic Prediction tools for Predicting Time-Related Traits under Complex Scenarios

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ABSTRACT

Genomic selection (GS) has proven to be an efficient tool for predicting crop rank performance of untested genotypes via their maker profiles; however, when the trait of interest have intermediate optima, as those traits related to crucial phenology stages of plants, this implementation might not be the most convenient when phenotypic information of the target environment is not available. In these cases, GS might deliver high rank correlations between predicted and observed values but incurring in serious bias. Days to heading (DTH) is a crucial development stage in rice for regional adaptability with a significant impact in yield potential. For this reason, the information about DTH is important for designing and planning rice cultivars experiments. We developed two methods that incorporate day length information (DL) in the prediction process: C method, which models DL and DTH aiding the prediction of tested genotypes in unobserved environments; CB method, which combines the C method plus genomic BLUPs for predicting untested genotypes in unobserved environments. The use of DL has clear advantages over the use of weather data since this information can be determined in advance just by knowing the location and planting date for establishing future cultivars. In this research, we considered three different prediction scenarios of interest for breeders: (*i*) CV2, predicting tested genotypes in observed environments; (*ii*) CV0, predicting tested genotypes in unobserved environments; and (*iii*) CV00, predicting untested genotypes in unobserved environments. Under CV00 scenario, the proposed CB method returned a square root of the mean square error (SMSE) of 7.3 days, a Pearson correlation of 0.93 and the environmental mean differences (EMD) ranged between -6.4 and 4.1 days. The conventional GS method produced a SMSE of 18.1 days for a correlation of 0.41 and EMD ranging between -31.5 and 28.7 days. For CV0, the proposed C method returned a SMSE of 3.9 days for a correlation of 0.98 and EMD ranging between -4.95 and 4.67 days. CV2's results were used for comparison purposes to assess the predictability levels that can be reached when complete information on genotypes and environments are available. Here, the conventional GS implementation produced a SMSE of 3.8 days for a correlation of 0.98 and an EMD ranging between -0.02 and 0.03 days. The results obtained with the proposed methods are promising and showed that the efficient use of DL information aids to improve significantly the predictive ability of time-related traits.

Key words: Time-related traits; days to heading; day length; genome-enabled prediction.

INTRODUCTION

To satisfy the daily demand of crop consumption for a growing population, it is necessary to improve the production systems to allow a sustainable increase of the yield potential and nutritional value of the new and up-coming varieties (FAO et al., 2018). In plant breeding there are different tools and methodologies to increase the rate of genetic gain (Bernardo 2002). Traditional plant breeding methods use phenotypic and/or pedigree information to perform selection of experimental lines for developing superior cultivars. However, phenotypic selection may not be permissible in many situations due to the high phenotyping costs; also, pedigree selection may not be accurate since recombination effects are not accounted for under this method.

Genomic selection (GS, Meuwissen et al., 2001) is an emerging tool that allows screening genotypes from a very large population without having to observe them in fields (Heffner et al., 2009; Jannink et al., 2010). This method only requires phenotypic and genomic information for calibrating models, then other genotyped candidates are selected based on the predicted values using their marker profiles (Crossa et al., 2010). In this context, the implementation of genomic tools and resources translates our understanding of the relationship between genotype and phenotype into predicted genetic merits for direct selection. This is highly desirable dealing with those traits that are controlled by a large number of genes with small individual effects, also known as complex traits (Jannink et al., 2010, Perez-de-Castro et al., 2012). GS has shown to be an effective tool for plant and animal breeding applications, especially when dealing with complex traits (de los Campos et al., 2013). The successfulness of GS predicting phenotypes from genotypic information has been reported in various crop species as reviewed by Voss-Fels et al. (2019).

In GS, the estimated breeding values (EBVs) of complex traits of untested genotypes are computed based on genome-wide marker information. The EBVs can be considered as deviations around a fixed mean (e.g., zero). Once the EBVs are obtained, the breeders may perform selections based on the ranked values by selecting those individuals in the tails. Depending on the trait, the individuals are selected either on the upper or lower tails of the ranked EBVs. For example, the upper side is selected for increasing genetic gains of traits like grain yield, stress resistance, or the content of essential nutrients. On the other hand, genotypes in the lower tail are selected for reducing lodging, shattering, the content of anti-nutrients, etc. In these cases, the EBVs provide sufficient information when the correlation between the EBVs and the direct genetic values (or phenotypic values) is high. However, the rank-based selection criteria might not be the most accurate when the target trait has an intermediate optima. This is the case of those traits related to phenological stages of crop plants. For example, days to heading (DTH) is one of the critical traits of ideotypes with an increased yield potential, and thus it is important to optimize DTH for high yield potential in different environments (Yin et al., 1997).

Usually multi environment trials (METs) are performed for developing stable cultivars across a wide range of environmental conditions, and the breeders may be interested in estimating DTH for a large set of environments instead of a single one. However, the EBVs may

contain a large bias if no phenotypic information from a targeted unobserved environment is available. The EBVs of one environment may not be valid for another environment due to variations in the environmental *stimuli*. These differences remain also for same genotypes observed in same locations but at different times (planting date, years). As consequence of the aforementioned issues, the problem of correctly estimating DTH for a set of untested genotypes in a wide range of unobserved environments becomes harder to solve than for non-time related traits. As pointed before, the advantage of GS techniques over conventional phenotypic methods is its ability for performing accurate rank predictions of untested genotypes based exclusively on their marker profiles without having to observe these in fields. Thus, a method capable of delivering highly accurate predictions of untested genotypes in unobserved environments without incurring in a large bias is desirable.

In this study, we propose a novel method to predict DTH in a precise and accurate way (i.e., small bias and high correlation between predicted and observed values). We demonstrated the potential of the method, analyzing a rice DTH dataset comprised of 112 genotypes evaluated in 51 environments. Because rice DTH is important for regional adaptability and has usually great impact on yield potential (Yin et al., 1997), genetic systems controlling DTH have been studied for a long time by integrating Mendelian genetics and genomics (Hori et al., 2016). The potential of GS in DTH has been evaluated in rice (Spindel et al., 2015 and Onogi et al., 2015) and sorghum (Li et al., 2018). Onogi et al. (2016) focused on the genomic prediction of DTH in multiple environments and demonstrated the potential of a novel method that integrates GS with a crop growth model. Li et al. (2018) also focused on the genomic prediction of DTH in multiple environments and demonstrated the potential of models combining GS with joint regression analysis on photothermal time. In this study, we also focus on the accurate prediction of DTH because its optimization is indispensable for high yield potential in different environments (Yin et al., 1997). The optimization will become more important in near future to attain high and stable yield under the changing climate conditions.

Firstly, we explored the usefulness of cumulative day length (CDL) information (planting date until heading) for predicting DTH. We found that CDL produced accurate predictions with small to null bias. However, for this method to work it is necessary know first the exact number of days that DL will accumulate from planting until heading and these values correspond to the response we want predict complicating the process.

An alternative way of using DL information is considering the DL values of the day when DTH are reached. In principle, DTH information of genotypes observed in different environments may be used for finding patterns that relate DTH and DL. Apparently, each genotype poses a unique fingerprint that relates the DL values at the occurrence day with DTH across environments. These patterns are characterized via a third degree polynomial equation that depends exclusively on the time covariate. This fingerprint provides a rough estimation of the DTH potential for a target candidate environment (location × planting-date combination). The ultimate goal of this research was to develop a method for predicting DTH of untested genotypes in unobserved environments. For this, a third degree polynomial equation was used for characterizing the DL at the DTH occurrence across environments of tested genotypes (one at

the time). Then, the DL values of a target unobserved environment were fitted via a third degree polynomial equation. An estimation of DTH of a tested genotype in an unobserved environment can be obtained by finding the intersection of these two equations.

This procedure was repeated for each genotype (one at a time) yet to be observed in the candidate environment. As was pointed before, one of the disadvantages of using GS methods for predicting DTH in unobserved environments is that a large bias between predicted and observed values may be generated if no phenotypic information of the candidate environment is available. In this case, the DTH environmental mean of the target environment is determined as the weighted mean of those environments in the training set. Thus, the genetic components become just random deviations around this weighted mean. If the real mean of this candidate environment is far from the weighted mean, a large bias may be expected predicting DTH, yet a high correlation can be obtained between the random deviations and the observed DTH values.

Thus, the problem of obtaining reliable predictions of untested genotypes in an unobserved environment may be solved just by finding the accurate DTH mean and then combine it with the genomic BLUPs obtained with GS methods. The BLUPs may be added just as deviations around this estimated DTH environmental mean. For assessing the proposed method, the square root of the mean square error (SMSE) and the Pearson correlation between predicted and observed values were computed within environments.

Three cases that are of interest for designing and planning experiments for addressing different problems were considered. CV2, predicting tested genotypes in observed environments (incomplete field trials); CV0, predicting tested genotypes in unobserved environments (targeting environments); and CV00, predicting untested genotypes in unobserved environments (targeting genotypes and environments).

Under the hardest cross-validation scheme (CV00), the results of the proposed method (CB) were compared with those obtained using the conventional GS model (E+G). For studying other applications for realistic scenarios, the two remaining cross validation schemes were also considered (CV2 and CV0). Results from CV2 scheme using the conventional GS model (E+G) were used to contrast the performance of the proposed method when complete information on genotypes and environments are available. Thus, these results may provide a better idea of the model performance of the proposed method when no information of genotypes and environments are available. Results under the CV0 scheme correspond to the proposed C method. Here the environments are considered “target” in a strict sense because based on theoretical DL values the best location and planting date can be determined before conducting field experiments.

Across environments, under the CV2 scheme the conventional GS model (E+G) returned a SMSE of 3.8 days and a Pearson correlation of 0.98. With CV00, the proposed BC method gave a SMSE of 7.2 days and a Pearson correlation of 0.93; also, the difference between the predicted and observed environmental means (DM) ranged between -4.1 and 6.4 days. On the other hand, the conventional GS model produced a SMSE of 18.1 days and a Pearson correlation of 0.41; the DM ranged between -28.73 and 31.53 days. Finally, the C method produced accurate results predicting tested genotypes in untested environments (CV0 scheme). It returned a SMSE

of 3.8 days and a Pearson correlation of 0.98; the DM ranged between -4.67 and 4.95 days. This method does not require genomic information at all neither phenotypic information from other genotypes.

MATERIAL AND METHODS*Phenotypic data*

The phenotypic data set comprises the seeding and heading date of 112 Japan rice cultivars tested in multiple locations from 2004 to 2017. This multi-environmental experiment was established to investigate heading related genes. During the first 2 years (2004 and 2005), the experiments were conducted in Tsukubamirai for 2 different cultivation periods. Then gradually more locations were considered for testing DTH, and these are distributed from North to South of Japan. Since 2012 at least 7 experiment per year have been conducted until 2018. Originally, the total number of trials/experiments (location-by-year-by-planting date combinations) is 79 (see Table A1); however, in this research we used information of only 51 of these (see Table 1).

The DTH was calculated as the difference between the heading date and the seeding date. The heading date was determined when more than 50% of the individuals reached the heading stage. However, DTH was measured in a different way in Kasai location. There, the heading date was initially determined as the event when 10% of the individuals started heading. Then, following the suggestions of an expert rice scientist, the final DTH for the genotypes tested in this location were obtained by adding 2 days to those cultivars from Hokkaido and 1 day to the cultivars originally developed in other locations. The replicated number of plants evaluated for each cultivar is different by experiments, and these ranged from 7 to 30. The cultivars that did not reach the heading stage at the end of the experiment were removed from the analysis.

Marker genotype data

For each variety, total DNA was extracted using the CTAB method (Murray et al., 1980). Next-generation sequencing data was obtained using the Illumina HiSeq 2000, HiSeq 4000 and HiseqX systems via paired-end sequencing (Illumina, Inc., San Diego, CA, USA). Data sets deposited in the DDBJ Sequence Read Archive (SRA106223, ERA358140, DRA000158, DRA000307, DRA000897, DRA000927, DRA007273, DRA007256) were reanalyzed. Adapters and low-quality bases were removed from paired reads using the Trimmomatic v0.36 program (Bolger et al., 2014). The preprocessed reads were aligned on the Os-Nipponbare-Reference-IRGSP-1.0 (Kawahara et al., 2013) by using the bwa-0.7.12 mem algorithm with the default options (Li, 2012).

SNP calling was based on alignment using the Genome Analysis Toolkit (GATK, 3.7-0-gcfedb67) (McKena et al., 2010) and Picard package V2.5.0 (http://broadinstitute.github.io/picard). The mapped reads were realigned using RealignerTargetCreator and indelRealigner of GATK software. SNPs and InDel were called at the population level using the UnifiedGenotyper of GATK with the -glm BOTH option. Heterozygous loci were treated as missing, owing to the assumption that almost all markers were well fixed in rice cultivars after several generations of self-pollination. We used only biallelic

non-missing sites over all cultivars with a minor allele frequency (MAF) ≥ 0.025. For further analysis, genotypes were represented as -1 (Homozygous Reference) and 1 (Homozygous Alternate).

Day length data

The original data set also consider environmental data (ED) and it was downloaded from the Agro-Meteorological Grid Squared Data (NIAES; https://amu.rd.naro.go.jp). However, in this study we only day length DL information was considered for developing the proposed model. Further developments will integrated both types of layers (DL & ED) in the analysis. The theoretical DL values were computed by the default method (Forsythe et al., 1995) included in the geosphere (v1.5-5) R package (Hijmans., 2017).

GBLUP model.

The GBLUP (Genomic BLUP) model is a main effects model used in GS applications (vanRaden et al., 2007, de los Campos et al., 2013). It attempts to explain the DTH value of the ith genotype (i=1, 2,…, I) observed in the jth environment (j=1, 2, …, J) as the sum of a common mean, random environmental and genomic effects, and an error term. It can be represented as follows:

$$Y_{ij} = \mu + E_j + g_i + \varepsilon_{ij} \qquad [1]$$

where μ is a common effect to all genotypes across all environments; E_j are IID (independent and identically distributed) outcomes from a $N(0, \sigma_E^2)$ representing the effect of the jth environment; $\boldsymbol{g} = \{g_i\} \sim N\left(\mathbf{0}, \mathbf{G}\sigma_g^2\right)$ is the vector of genomic effects, with $\mathbf{G} = \frac{\mathbf{XX}'}{p}$ as the genomic relationship matrix whose entries describe the genetic similarities between pairs of individuals, $\mathbf{X}$ is the standardized (by columns) matrix for p molecular markers; ε_{ij} are IID outcomes from a $N(0, \sigma_\varepsilon^2)$ representing measure errors; and σ_E^2, σ_g^2 and σ_ε^2 are the corresponding associated variance components of the described random effects.

Usually, when METs are analyzed the environmental component explains the larger proportion of the phenotypic variability across environments. For example, Crossa et al. 2016 and Jarquin et al., 2017 showed that this component captured between 65% and 95% of the variability in grain yield for different wheat populations (Mexican, Iranian and hard red winter). As consequence of this, from [1] we can conclude that the predicted value of the ith genotype in the jth environment strongly depends on an environmental mean, which is composed by a common mean across environments plus an environmental deviation that corresponds to the effect of jth environment.

Conceptually, the common mean effect can be viewed as the average performance of all genotypes across environments. The environmental deviation corresponds to the mean of the specific affectations of the current environmental *stimuli* in the set of the tested genotypes beyond the common mean. Thus, these environmental effects can be considered deviations around the common mean. Hence, by construction the contribution of the genomic component to the idealized phenotype is much smaller in comparison with the overall mean and the environmental effects.

If phenotypic information of the target environment is available, good approximations of the environmental effects can be computed. When this information is not available, the environmental effects are estimated using genotypes observed (phenotype) in other environments. In this case, the environmental component is estimated as the weighted average of the environmental effects in the training set. Since these random effects are conceptualized to be centered on zero, the weighted average will be close to zero. Hence, the predicted DTH value will be compose mainly of a common mean and a small genomic effect shrinking the predicted values towards the common mean. Dealing with traits where the selection process is based on rank performance (yield), the selected genotypes would correspond to those with the largest/smallest values (depending on the desired direction of the trait to improve) without considering the estimated values *per se*.

However, for those traits linked to important physiological crop growth stages (e.g., DTH) the selection based on rakings might not be the best strategy. Although this method usually delivers high correlations between predicted and observed values, it also might induce a large bias (MSE) when no information of the target environment is included. For solving this issue, we developed a method that incorporates DL information in the prediction process.

C method

The idea for using DL information in the prediction process was motivated examining the relationship between CDL and DTH of all genotypes across environments. A visual assessment of this relationship can be done with Figure A1. This plot contains 94% of the all possible combinations between 112 genotypes and 51 environments. Here, a simple linear regression returned an R-squared value of 0.99 exhibiting a strong relationship between DTH and CDL. It can be concluded that if CDL values are known then accurate estimations of DTH can be obtained for any genotype in any environment. However, a recursive issue is faced because in order to compute CDL the number of days between planting date and the heading occurrence should be known beforehand. Hence, DTH should be known first before compute CDL leading this to a recursive problem. However, analyzing the DL values of the day when a single genotype reaches the heading stage across environments could lead us to recognize clear patterns relating DTH and DL. Under this assumption, DTH becomes a function that depends on DL; however for a better understanding of the concept this relationship can be switched (i.e., DL depending on DTH).

For illustrating this concept, Figure 1 is used as example. It corresponds to genotype 56 observed in 50 environments out of 51 (1-46, and 48-51 but not in environment 47). The goal

here is to estimate DTH for this genotype in the remaining unobserved environment (environment 47: Tsukubamirai 2016 Late). For each environment, the planting date was considered as day 1 (*x-axis*). Then for these environments, the daily DL (*y-axis*) values were plotted (pink lines) from day 1 (planting) until genotype 56's DTH (blue point). Examining the cloud of blue points, a pattern can be identified where DL at the occurrence day depends at some grade on DTH. This gradient seems not to be strongly affected by the initial DL values at the different planting dates across environments. However, it appears to be linked with the initial DL's changing rate at the planting day. Those environments where DL's changing rates decreases at the planting time (late cultivars) showed shortest DTH values. On the other hand, the environments with increasing DL's rates (early cultivars) presented the largest occurrence times.

For predicting untested genotypes in unobserved environments (CV00) under the aforementioned concept (i.e., using DL information), the proposed method requires: (*i*) the estimation of DTH for a set of tested (training) genotypes (one at the time) yet to be observed in a target/unobserved environment (CV0); (*ii*) to compute the expected environmental mean of the unobserved environment using the estimated values from (*i*); (*iii*) to add the resulting blups of the untested genotypes derived from conventional GS methods to the estimated environmental mean. Further details of these steps are given next.

The DTH estimation of a tested genotype in an unobserved environment (CV0) requires the phenotypic information across several environments and their corresponding DL values at the occurrence time. The first step considers the characterization of DL at the heading occurrence time across environments. For this, a third degree polynomial equation is used for fitting DL as function of DTH (orange curve in Figure 1) as follows:
$dl_{ij} = b_0 + b_1 t + b_2 t^2 + b_3 t^3 + e_{ij}$, where dl_{ij} represents the observed DL of the ith genotype in the jth environment; t is the time independent variable; b_0, b_1, b_2 and b_3 are the model parameters; and e_{ij} is the error term and it is assumed to be IID $N(0, \sigma^2)$. For facility we will refer to this model as C_1 curve.

In a similar way, the daily DL information of the target environment (green curve) can be modeled using a third degree polynomial equation. The resulting fitted model is referred as C_2 curve. The solution to this system of two equations (C_1, C_2) will produce an estimate of DTH of the tested genotype yet to bet observed in the target environment. In order to find the intersection of these two curves, deterministic and numerical methods can be used. For facility in this study, the numerical evaluation method was considered as follows: (*i*) the fitted model C_1 (orange curve) was evaluated within the range of values of those phenotypes (DTH) of the same genotype tested in other environments. For example, in Figure 1 the smallest and the largest DTH values for genotype 56 were 67 and 137 days. Thus, the model was evaluated for the values in the range [67, 137] in steps of 1 day. Similarly, for the fitted model of the target environment it was evaluated in the same range in steps of 1 day. Then, the element to element differences between the two resulting vectors were computed and the label [67-137] of the position with the closets value to zero was considered as the intersection point (day 93 in Figure 1; dotted gray line).

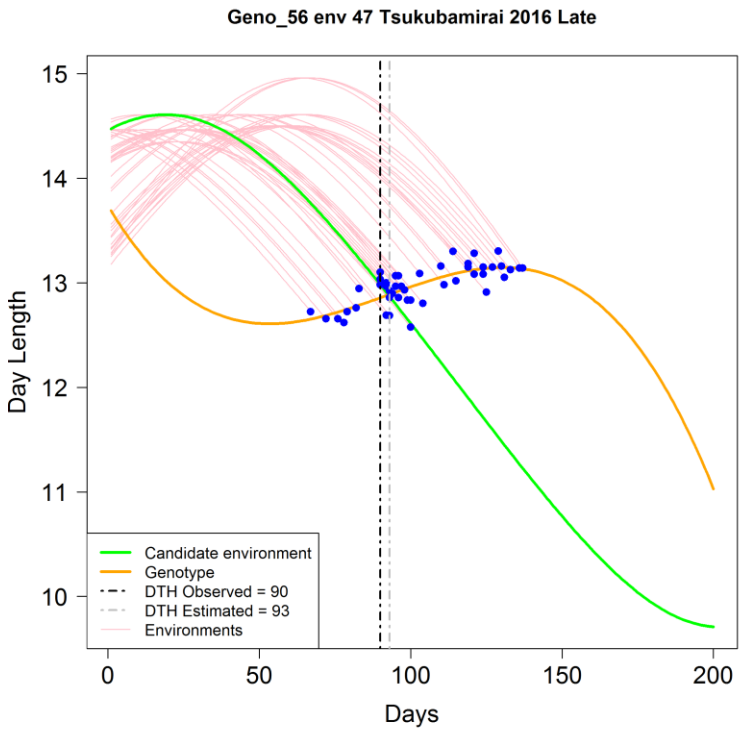

Figure 1. Graphical representation of the curve's C method for estimating days to heading (DTH) of genotype 56 in environment 47 (Tsukubamirai 2016 Late). Pink curves represent daily day length (DL) values from planting day (day 1) until heading time for genotype 56 in 50 environments (1-46, and 48-51). The blue points indicate the corresponding DL values when genotype 56 reached heading time. The green curve (C_2) represents the target environment (Environment 47) where no genotypes have been observed yet. The orange curve (C_1) depicts the fitted line for genotype 56 using a third degree polynomial equation relating DL at the occurrence time and DTH across environments. The vertical dotted gray line shows the intersection (93 days) between the green and the orange curves (C_1, C_2). This value is the estimated DTH for genotype 56 in the unobserved environment "Tsukubamirai 2016 Late". The vertical dotted black line corresponds to the observed DTH (90 days) in environment 47.

Empirical assessment

For testing the accuracy of this method for predicting DTH for a set of tested genotypes yet to be observed in a target environment, the C method was implemented considering one genotype at the time. To evaluate the performance of this method across environments, the previous procedure was implemented on each one of the 51 environment. The described method mimics the scenario of predict DTH for tested genotypes in unobserved environments. This scenario is similar to the cross-validation scheme CV0 (Jarquin et al. 2017) which attempts to predict already tested genotypes in unobserved/target environments by combining phenotypic and genomic information from the same genotype and from other genotypes observed in other environments. In this case, only the phenotypic information of the same genotype observed in other environments is used. Thus, the proposed C method does not considers genomic information, neither phenotypic information from other genotypes.

Since the ultimate goal is to predict DTH for untested genotypes in unobserved environments, the first step is to accurately estimate the expected DTH mean of the target

environment. Then, the final predicted value will be composed of the expected DTH mean of the target environment (computed ignoring any phenotypic record of the untested genotype) plus the genomic estimated breeding value ($\hat{g}_i$) of the untested genotype obtained via conventional GS methods (E+G model). For estimating the DTH mean, the C method was applied first to the genotypes in the training set to be observed in the target environment (one at the time). Then the environmental DTH mean was computed by averaging these predicted values. So far, we have not include any information (genomic and phenotype) from the untested genotype in the analysis.

Using a toy example consisting of four genotypes (G_1, G_2, G_3, and G_4) and three environments (E_1, E_2, and E_3), in Figure 2 we describe the procedure for finding the environmental mean of the target environment for untested genotypes. The top panel contains the observed values; the middle panel shows the predicted values presumably were obtained with the C method; and the bottom panel displays the conditional (given the tested genotypes) estimated DTH environmental means for each genotype at each environment. Once the DTH means are computed for each genotype in the candidate environment the prediction will be composed of these means plus the genomic effects obtained using model [1] under CV00 scheme. These genomic effects are estimated by deleting from the training set the information of the target environment and the information from the same genotype observed in other environments. Hence, within each environment the procedure of leaving out one genotype at the time was applied.

		G_1	G_2	G_3	G_4	Mean
Observed	E_1	65	69	71	66	67.75
	E_2	80	85	87	90	85.50
	E_3	100	105	108	110	105.75
Predicted	E_1	68	69	73	70	70.00
	E_2	77	83	85	88	83.25
	E_3	104	103	110	114	107.75
E-Mean	E_1	70.7	70.3	69	70	69.98
	E_2	85.3	83.3	82.7	81.7	83.24
	E_3	109	109	107	106	107.65

Figure 2. Representation of the procedure for estimating the conditional DTH environmental mean for untested genotypes in unobserved environments. The top panel contains the observed values for four genotypes (G_1, G_2, G_3 and G_4) observed in three environments (E_1, E_2, and E_3). The middle panel contains the predicted values presumably obtained with the C method assuming that these genotypes were not tested in a particular environment. This method attempts predicts DTH of tested genotypes in unobserved environments using the phenotypic information of these genotypes but observed in other environments (one genotype at the time). For example, the predicted value for G_2 in E_1 is 69 and it was computed using the information of G_2 tested in E_2 (85) and E_3 (105) via the C method. The bottom panel contains the estimated DTH means (E-Mean). These are particular to each genotype and were compute

as the mean of the predicted values of those genotypes in the training set for the current environment. For example, G_2 in E_1 (70.3) was computed as the mean of the predicted values of G_1 (68), G_3 (73) and G_4 (70) in E_1.

RESULTS

Descriptive statistics

Box-plot of DTH for each environment are depicted in Figure A2. The environments were ordered based on the environmental medians. In general, those environments with late planting date appeared first. The medians ranged between 59 (Fukuyama 2010 Late) and 116 (Akita 2015) days. The SDs of the environments varied between 5.07 and 16.83 days. Also, the smallest and the largest DTH values were 42 and 148 days and these were observed in "Tsukubamirai 2016 Late" and "Akita 2015" environments, respectively.

Evaluating the bias and accuracy of the methods

Table 1 contains the observed and the predicted environmental means (E-mean) for four different strategies (cross-validation scheme-method combination) as well as the corresponding square root of the mean squared error (SMSE) and the Pearson correlation between the predicted and observed values. These different strategies were used for different purposes. CB and GS methods were implemented under the CV00 scheme (CV00_CB, CV00_GS) for comparing the model performance of the proposed model with the conventional implementation (GS) predicting untested genotypes in unobserved environments. C method was implemented under the CV0 scheme (CV0_C) for assessing the predictive ability of the C method predicting tested genotypes in untested environments (also this is the intermediate step of the CB method used in the previous scheme). Lastly, the conventional method (GS) was implemented under the CV2 scheme (CV2_GS) for providing an idea of the levels of predictability that can be reached when complete information of genotypes and environments are available.

CV2 is the scenario that includes the most complete sets of information on genotypes and environments for calibrating models. Across environments, the CV2_GS strategy returned a SMSE of 3.8 days and a Pearson correlation of 0.98; within environments, the average SMSE and Pearson correlation values were of 3.7 days and 0.97, respectively. The difference of the environmental means (EMD) between predicted and observed DTH values ranged between -0.02 and 0.03 days. Since this scenario uses the most complete sets of information for calibrating models, these results can be used as reference for assessing the performance of the proposed methods under more complex prediction scenarios.

CV0_C results provide information of the ability for predicting tested genotypes in unobserved environment when considering only phenotypic and DL information of the same genotype using the C method. Across environments, this method respectively returned a SMSE of 3.9 days and a Pearson correlation of 0.98; within environments, the average SMSE and Pearson correlation were of 3.7 days and 0.98. The EMD ranged between -4.95 and 4.67 days.

CV00 corresponds to the scenario of predicting untested genotypes in unobserved environments and it is considered the hardest and most interesting prediction problem because no

phenotypic information is available for connecting untested genotypes in unobserved environments with calibration sets. Across environments, the conventional GS method (CV00_GS) respectively returned a SMSE and a Pearson correlation of 18.1 days and 0.41; within environments, the average SMSE and Pearson correlation were of 17.5 days and 0.69. The EMD ranged between -31.5 and 28.7 days. For the same scenario, across environments the proposed CB method (CV00_BC) respectively returned a SMSE and a Pearson correlation of 7.3 days and 0.93; within environments, the average SMSE and Pearson correlation were 7.2 days and 0.86. The EMD ranged between -6.4 and 4.1 days.

Table 1. Environmental means, Square root of the Mean Squared Error (SMSE) and the Pearson correlation between predicted and observed days to heading (DTH) for 112 rice genotypes tested in 51 environments. Three cross-validation schemes were implemented for mimicking realistic prediction scenarios. CV2 resembles incomplete field trails were some lines are tested in some environments but not in others. CV0 represents the scenario of predicting tested genotypes in unobserved environments. CV00 considers the prediction of untested genotypes in unobserved environments. CV2 was implemented using the conventional implementation (GS, E+G) leaving one phenotype out at the time. For CV0 the C method was used combining phenotypic and day length information (DL) of each genotype (one at the time) already tested in other environments. For CV00 two methods were considered: (*i*) the conventional implementation (GS, E+G) leaving one genotype out across environments and deleting any phenotypic information from the target environment; and (*ii*) the proposed CB method, which combines phenotypic and DL information from the tested genotypes (training set) with the genomic blup values ($\hat{\boldsymbol{g}}$) of the untested genotypes obtained in (*i*).

	Mean					Square root of the Mean Square Error				Correlation			
	Mean	CV00_CB	CV00_GS	CV0_C	CV2_GS	CV00_CB	CV00_GS	CV0_C	CV2_GS	CV00_CB	CV00_GS	CV0_C	CV2_GS
Tsukubamirai 2004 Late	79.4	76.7	85.9	76.3	79.4	7.0	12.6	4.6	2.5	0.86	0.70	0.99	0.98
Tsukubamirai 2004 Early	87.5	93.2	84.1	92.2	87.5	10.0	12.9	6.4	4.2	0.84	0.55	0.97	0.97
Tsukubamirai 2005 Early	98.5	102.1	87.1	101.0	98.5	6.9	14.0	3.5	2.0	0.87	0.75	0.98	0.99
Tsukubamirai 2005 Late	76.7	74.8	84.8	73.7	76.7	5.7	12.4	3.4	2.2	0.86	0.68	0.99	0.99
Kasai 2006	87.4	83.8	88.3	82.8	87.4	7.9	8.8	6.8	4.7	0.85	0.75	0.93	0.94
Fukuyama 2006	80.6	83.0	82.5	82.0	80.6	5.6	13.4	2.0	2.2	0.87	0.29	0.99	0.99
Fukuyama 2007	84.1	83.4	86.6	82.5	84.1	5.0	6.1	2.9	3.0	0.87	0.84	0.98	0.98
Tsukuba 2008	104.5	107.7	87.2	107.3	104.5	9.1	19.2	4.0	4.7	0.87	0.85	0.98	0.98
Kasai1st 2008	102.2	99.9	88.7	99.2	102.2	9.0	17.8	4.3	4.8	0.89	0.71	0.99	0.99
Fukuyama 2008	77.8	82.2	85.6	81.3	77.8	7.8	11.1	4.5	2.8	0.86	0.77	0.98	0.98
Tsukuba 2009	103.9	107.7	87.1	108.0	103.9	8.6	19.0	5.2	4.7	0.89	0.81	0.98	0.97
Kasai1st 2009	101.9	100.6	89.4	100.0	101.9	7.6	15.1	2.7	3.5	0.89	0.83	0.99	0.99
Fukuyama 2009	78.7	78.7	89.6	77.8	78.7	5.2	13.2	1.5	2.2	0.86	0.83	0.99	0.99
Tsukuba 2010 late	83.5	89.1	88.9	88.4	83.5	8.4	11.1	5.9	3.0	0.86	0.66	0.97	0.97
Kasai1st 2010	100.6	100.7	89.5	100.1	100.6	7.1	14.3	1.9	2.9	0.88	0.77	0.99	0.99
Fukuyama 2010	76.2	79.5	88.2	78.1	76.2	5.9	15.3	2.6	2.8	0.86	0.50	0.99	0.99
Fukuyama 2010 Late	59.0	65.4	87.7	62.5	59.0	9.2	29.7	4.2	7.7	0.84	0.82	0.96	0.90
Tsukuba 2010 Early	105.9	109.9	85.5	109.9	105.9	9.9	23.9	5.3	5.6	0.87	0.66	0.98	0.97
Tsukuba 2010 Late	82.8	80.8	90.0	80.4	82.8	5.9	11.9	4.1	3.6	0.86	0.78	0.95	0.96
Tsukuba 2010 Middle	94.7	95.7	90.9	95.4	94.7	6.5	11.1	3.0	3.1	0.88	0.67	0.98	0.98
Kasai 2011	101.4	100.6	89.3	100.0	101.3	7.8	15.7	2.4	3.6	0.89	0.76	0.99	0.99
Fukuyama 2011	75.7	79.4	91.1	78.6	75.7	6.4	16.9	3.1	2.0	0.87	0.83	0.99	0.99
Fukuyama 2011 Late	66.4	72.7	97.9	66.2	66.4	8.9	32.4	2.9	5.7	0.68	0.58	0.95	0.86
Tsukuba 2012 Early	97.1	96.0	86.9	101.1	97.1	5.7	12.4	5.8	3.2	0.81	0.74	0.97	0.94
Tsukuba 2012 Late	84.3	81.6	88.2	81.0	84.3	6.1	7.5	4.3	3.5	0.86	0.80	0.96	0.97
Tsukuba 2012 Middle	92.6	95.4	88.4	94.8	92.6	6.3	8.3	3.7	2.9	0.87	0.82	0.97	0.97
Kasai 2012	103.8	102.7	86.4	102.1	103.8	7.9	21.3	2.7	3.8	0.89	0.60	0.99	0.99
Fukuyama 2012	75.2	78.8	89.9	78.0	75.2	6.2	16.3	3.1	2.0	0.87	0.83	0.99	0.99
Fukuyama 2012 Late	71.5	77.0	103.1	68.6	71.6	7.5	32.4	3.4	4.6	0.70	0.66	0.98	0.92
Fukuoka 2012	79.6	76.0	89.1	75.4	79.6	6.7	13.0	5.2	4.2	0.84	0.69	0.96	0.96
Akita 2012	113.1	115.9	90.1	114.5	113.1	8.3	26.1	2.8	4.4	0.86	0.54	0.98	0.97
Kasai 2013	104.8	104.1	87.3	103.5	104.8	8.0	20.1	3.2	4.7	0.90	0.80	0.98	0.98
Fukuyama 2013	74.9	80.1	89.4	79.3	74.9	7.4	16.3	4.6	1.8	0.87	0.82	0.99	0.99
Fukuyama 2013 Late	62.1	64.8	85.7	64.1	62.1	6.3	27.1	3.6	4.9	0.83	0.36	0.93	0.95
Fukuoka 2013	77.4	77.3	93.3	76.7	77.4	5.7	17.5	1.9	2.6	0.86	0.77	0.99	0.98
Akita 2013	116.6	115.8	91.1	115.1	116.5	8.5	28.1	2.4	4.9	0.88	0.66	0.99	0.98
Kasai 2014	102.4	105.1	88.9	104.4	102.3	8.4	18.0	2.8	4.4	0.90	0.66	0.99	0.99
Fukuyama 2014	79.6	82.1	87.9	81.3	79.6	5.9	12.1	2.1	1.3	0.88	0.68	0.99	0.99
Fukuyama 2014 Late	67.6	64.5	88.1	62.9	67.6	6.7	21.4	5.1	6.0	0.83	0.79	0.98	0.94
Fukuoka 2014	81.8	77.7	80.4	77.1	81.8	6.7	11.5	5.4	3.1	0.87	0.45	0.97	0.97
Akita 2014	115.2	116.2	89.6	115.6	115.2	9.2	28.5	2.2	5.8	0.88	0.68	0.99	0.98
Fukuyama 2015	76.3	72.6	88.1	71.9	76.3	6.5	14.4	4.6	3.3	0.86	0.63	0.99	0.98
Akita 2015	118.1	119.8	89.4	117.6	118.0	7.7	29.9	2.5	3.6	0.81	0.76	0.99	0.98
Kasai 2016	102.3	101.2	85.6	100.7	102.2	7.2	20.4	3.1	3.9	0.90	0.61	0.98	0.98
Fukuyama 2016	79.1	81.1	85.2	80.8	79.1	6.0	8.9	2.4	2.9	0.86	0.82	0.99	0.98
Fukuyama 2016 Late	67.5	65.2	89.6	64.6	67.5	6.8	26.1	4.5	6.3	0.79	0.63	0.95	0.91
Tsukubamirai 2016 Late	73.6	77.9	84.6	77.2	73.6	7.0	13.6	4.9	1.9	0.90	0.80	0.98	0.99
Kasai 2017	108.6	106.2	85.7	105.5	108.6	8.3	26.3	4.3	4.4	0.89	0.56	0.98	0.98
Fukuyama 2017	77.4	80.2	87.7	79.6	77.4	6.3	16.3	2.7	2.0	0.88	0.42	0.99	0.99
Fukuyama 2017 Late	68.4	67.7	88.8	67.0	68.4	6.0	22.9	3.0	4.9	0.82	0.69	0.97	0.95
Tsukubamirai 2017 Late	69.5	73.1	86.9	72.2	69.5	6.8	19.8	4.3	1.6	0.87	0.65	0.98	0.99
Mean						**7.2**	**17.5**	**3.7**	**3.7**	**0.86**	**0.69**	**0.98**	**0.97**

Only the results of CB and GS methods used under CV00 scheme are directly comparable between them. Figure 3 depicts the scatter plot between predicted and observed DTH values for all genotypes across environments. The top panel contains the results obtained with the proposed CB method, while the bottom panel shows the results derived from the conventional implementation (GS, E+G). The blue and black lines correspond to the fitted and the on target predictions with respect to the observed DTH values. In general, across and within environments (Table 1) the results obtained with CB method showed a better fit than those derived from the GS model.

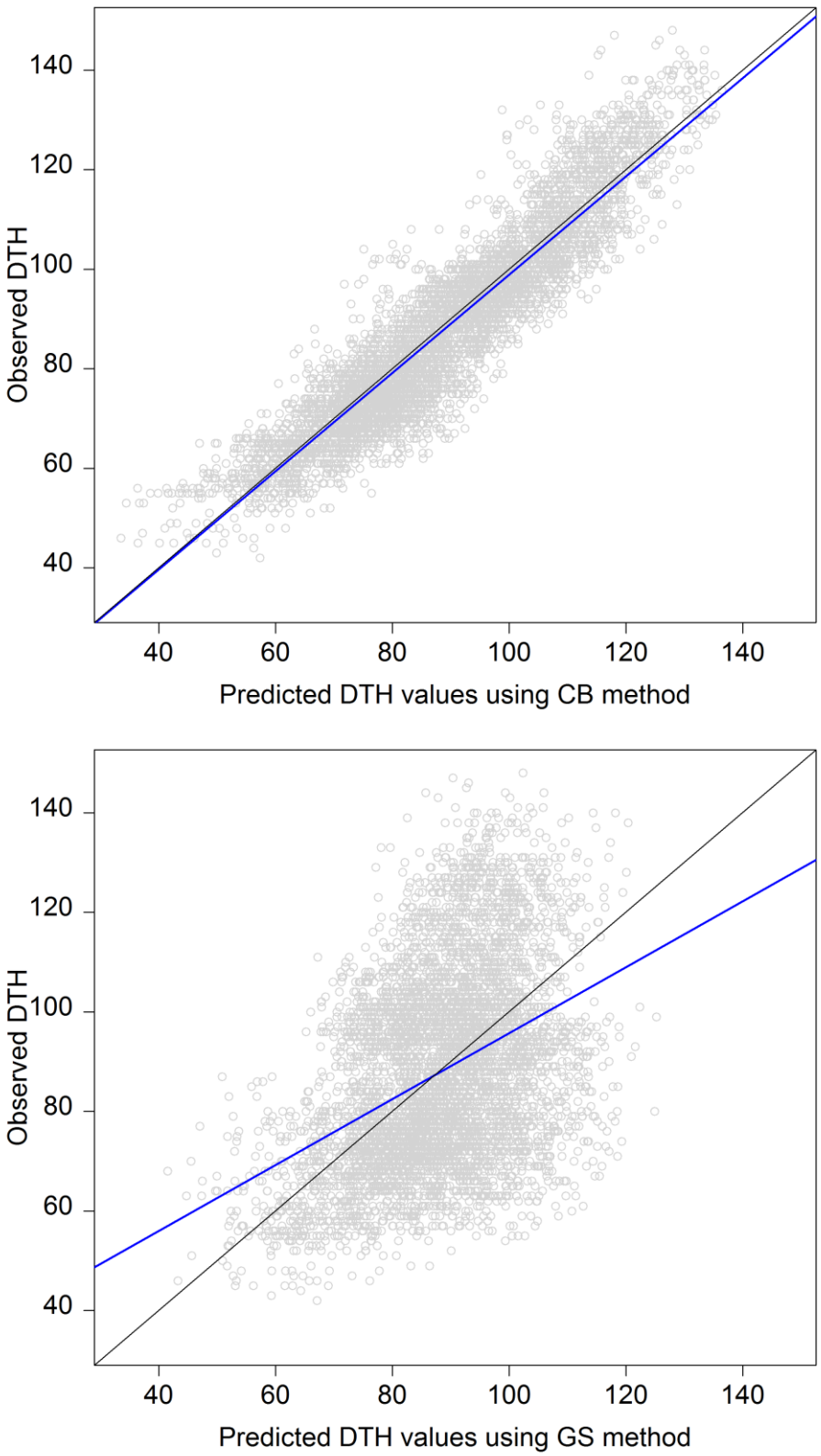

Figure 3. Scatter plot of predicted (*x-axis*) and observed (*y-axis*) DTH values using two different methods (CB a and GS) for predicting untested genotypes in unobserved environments (CV00 scheme) for a rice data set comprising 112 genotypes tested in 51 environment in Japan between 2005 and 2017. The top panel presents the results

obtained with the proposed CB method. It combines the predicted environmental means (E-mean) obtained using the proposed C-method with the genomic blups ($\hat{\boldsymbol{g}}$) derived from the conventional implementation (GS, E+E) under the CV00 scheme. The bottom panel displays the results obtained with the conventional implementation (GS, E+G GS). The blue line represents the fitted line between predicted and observed DTH values while the black line show the exact match (on target) of the predicted values on the observed values.

The advantages of considering the proposed CB method for predicting DTH under the CV00 scheme are more clearly appreciated analyzing the predicted environmental means (E-means). Figure 4 displays the predicted (*x-axis*) and observed (*y-axis*) E-means. The predicted E-means obtained with the CB and GS methods are indicated in orange and blue colors, respectively. As expected, when no information from the target environment was available for calibrating models the conventional implementation (GS, E+G) regresses the predicted values towards the overall mean (~87.74) from the training sets. On the other hand, the CB method consistently returned closer values to the diagonal black line, which represents the exact match.

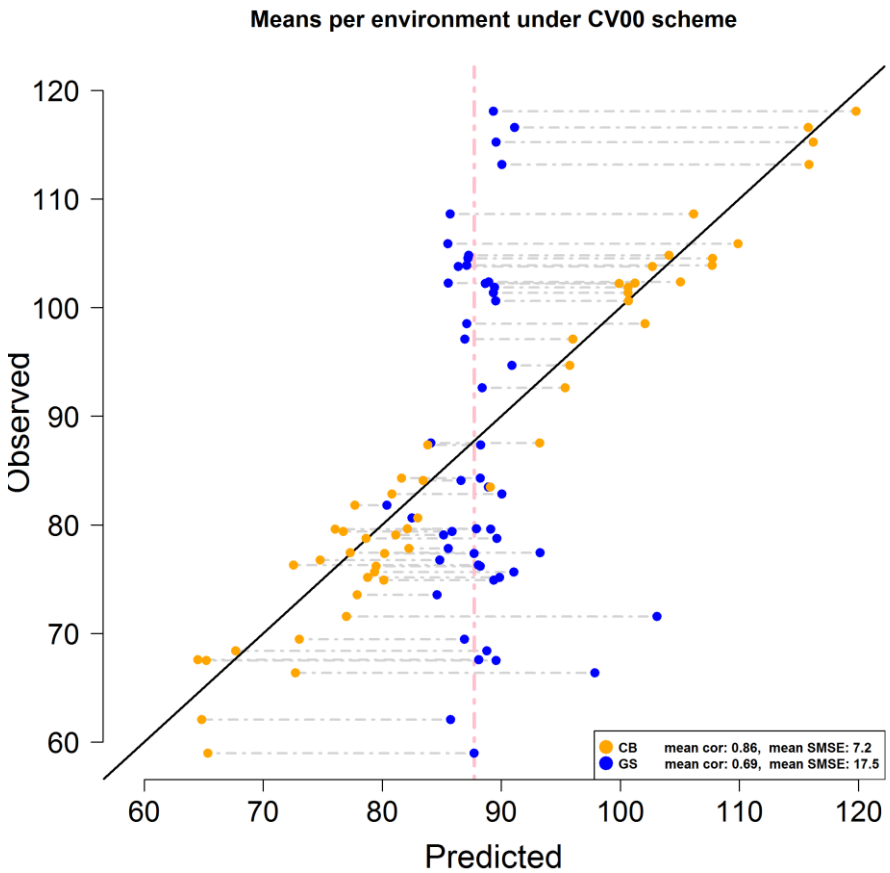

Figure 4. Scatter plot of predicted and observed environmental means (E-means) using the proposed CB method (orange) and the conventional implementation (GS, E+G) (blue) under the CV00 scheme (predicting untested genotypes in unobserved environments). The vertical pink line indicates the position of the overall phenotypic mean across environments (87.74 days). The diagonal black line shows the exact match between predicted and observed means. SMSE and cor stand for square root of the mean square error and the Person correlation between predicted and observed DTH values. These were computed within environments; and the mean SMSE and mean cor correspond to the across environments means.

Since the objective of this study is to develop a method for predicting DTH for untested genotypes in unobserved environments, the predicted values obtained with the CB method were used for fitting the DTH curves for each one of the 112 genotypes across environments. Figure A3 shows the fitted curves for the predicted (orange) and observed (gray) DTH values across the 51 environments. In principle, these fitted curves can be used for predicting DTH for other environments not included in this study. The information needed for defining a target environment are the location and planting date allowing the computation of the theoretical DL values using different methods (Forsythe et al., 1995). Once these theoretical values are obtained the C method is implemented to find the estimated DTH E-means. In general, the fitted curves (orange) derived from the predicted DTH values were very similar to those derived from the observed DTH values (blue).

DISCUSION

The boxplot from Figure A2 shows a high heterogeneity among the environments in the study with those with "late" planting date showing smaller phenotypic variability and shorter occurrence time for DTH in comparison with the "early" planting environments. Conventional GS models assume homogeneity of variances among environments and the violation of this assumption may induce serious inconsistencies in the results. Especially when no genotype-by-environment parameters are include in models.

The results obtained with the CV2_GS strategy can be viewed as the threshold of the predictive ability levels that can be reached with the conventional implementation when complete information about genotypes and environments is available. On the other hand, the results from the CV00_GS strategy provide information of the model performance of the conventional implementation when no phenotypic information is available for connecting genotypes in target environments. This cross-validation scheme is among the three cases (CV2: tested genotypes in observed environments; CV0: tested genotypes in unobserved environments; and CV00: untested genotypes in unobserved environments) the most interesting, useful and challenging for breeders. Dealing with traits where the selection process is based in ranks rather than in the estimated *per se* values, the conventional implementation (GS) usually provides good results (moderate to high correlations) under this scheme (CV00).

However, in traits related with important growth stages like DTH where the estimated *per se* values are crucial for designing, planning and establishing experiments the implementation of the conventional implementations (GS) might not be best choice. When no phenotypic information of the target environment is available, the E-mean of the target environment is determined as a weighted average of those environments in the training set forcing it towards a general mean. Hence, the final prediction is composed of this general mean plus deviations due to genomic effects. Since these deviations are conceptualized to be centered in zero, under CV0 and CV00 schemes the GS methods would produce similar environmental means (i.e., centered on an overall mean determined by the environments in the calibration set).

The obtained results showed that the C method provides good estimations of DTH for the CV0 scheme. Across environments, the SMSE was of 3.9 days and a Pearson correlation of 0.98 was obtained. These results were very similar to those obtained using the conventional GS models when complete information of the genotypes and environments were available (i.e., CV2 scheme). Under CV2, the SMSE and Pearson correlation across environment were of 3.8 days and 0.98, respectively.

As mentioned before, it is expected to obtain similar environmental means using either CV0 or CV00 schemes under the conventional GS implementations. The, CV00_GS strategy returned, across environments, a SMSE of 18.1 and a Pearson correlation of 0.41. The EMD between predicted and observed values ranged between -31.5 and 28.7 days while for the CV0_C strategy these ranged between -4.95 and 4.67 days. In this case, the CV0_C strategy used only the phenotypic information of the genotype to be predicted but observed in other environments. Since the CV0 and CV00 schemes produce similar E-means when no information of the target environment is available using the conventional implementations (GS) it is also expected that the proposed C method will produce good results with respect to the CV0_GS strategy not considered in this study.

As was pointed before, the ultimate goal of this research is to develop a method able to deliver small SMSE when predicting unobserved genotypes in unobserved environments (CV00 scheme). The BC method seems to produce good results under this scenario. The SMSE across environments was 7.3 days with a Pearson correlation of 0.86. These results are very encouraging compared with those obtained using the conventional implementation (GS) under this scheme (SMSE: 18.1 and cor: 0.41). In this case, the BC method doubled the accuracy of the conventional GS method and reduced the SMSE by 60%. However, the advantages of this method were more evident when comparing the range of the differences between the predicted and observed environmental means. Under the conventional implementation (CV00_GS) these differences ranged between -31.5 and 28.7 days while with the proposed CB method (CV00_BC) these ranged between -6.4 and 4.1 days. The proposed CB method reduced the extremes of this range between 80 and 85%, respectively. These improvements can be observed in Figure 4 were the environmental means of the proposed BC method (orange dots) were always closer to the diagonal line, representing the exact match with true means, than those means obtained using the conventional GS implementation (blue dots).

At beginning, the implementation of the proposed methods (C and BC) requires enough information for an accurate characterization of the genotype's DL curves. For this, sets of genotypes are tested in several environments. Perhaps it might sound expensive conduct these experiments but this will allow the accurate estimation of the environmental means of the unobserved environments. Once this information is available, potentially any untested genotypes could be accurately predicted in any unobserved environment. Thus, the obtained results should hold not only for the set of analyzed genotypes and environments of this study but also for others.

CONSLUSIONS

GS is an emergent tool successfully implemented for screening candidate genotypes based on their rank performance via the genomic estimated breeding values (GEBV). However, dealing with traits linked to important growth development stages like DTH, the punctual prediction of these traits (*per se* time wise values) becomes more important than the rank accuracy. In scenarios where partial information of the target environment is available (CV2 and CV1 schemes), good estimations of the environmental means can be obtained. However, when no information of the target environment is available (CV0 and CV00 schemes) a large bias between the predicted and estimated environmental means can be expected; even when the rank correlations may exhibit high values.

The developed methods here presented (C and BC) showed that the use of DL information significantly improves the predictive ability of the conventional implementations (GS) also reducing the bias between the predicted and observed DTH values. An advantage of the C method is that if phenotypic information of the same genotype is available for a set of environments, it is easy estimate the expected DTH in an unobserved environment without need molecular marker information or phenotypic information from other genotypes. The BC method improved the prediction of DHT of untested genotypes in unobserved environments by coupling the marker profiles of the untested genotypes with the phenotypic and DL information from other genotypes.

Both of the proposed methods require the day length (DL) information of the environments (training and testing) in their construction. The convenience of the proposed method resides in the fact that the theoretical DL information can be obtained in advance even before establishing the experiments and thus it can be exploited during the planning and designing stages. The proposed methods capture the genotype-by-environment interaction (G×E) component via the fitted curves thus the explicitly inclusion of this term is not needed. The conventional implementation (GS) returns the same estimated genomic effect across environments for a particular genotype when the G×E component is absent. The curves precisely capture the sensitivity experienced by genotypes under different environments driving specific responses to each environment. Thus, although the proposed BC method is composed of the genomic component computed without explicitly considering the G×E term the final prediction accounts for this interaction term via the curves. In addition, the formal modeling of heterogeneity of variances is not necessary because these patterns are also accounted with the current method via the different curves.

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APPENDIX

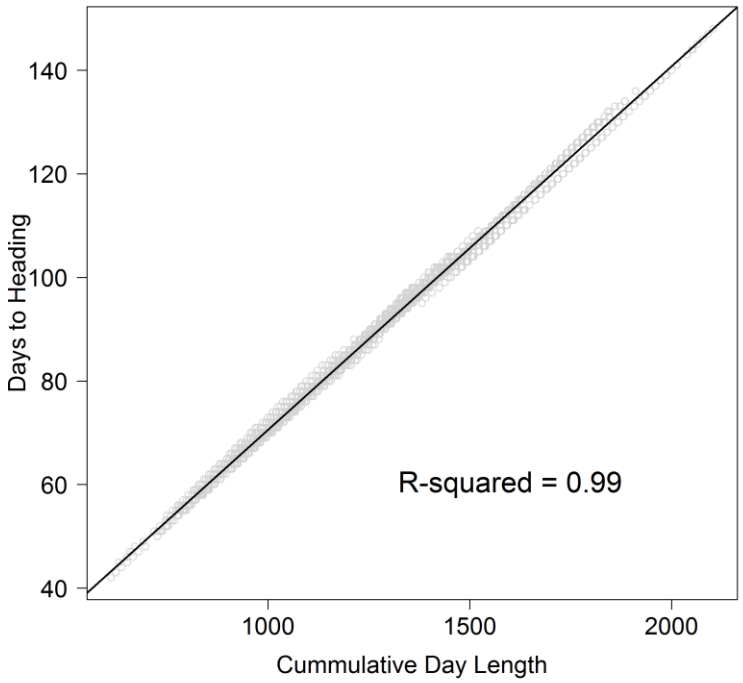

Figure A1. Scatter plot of days to heading (DTH) as function of the cumulative day length (CDL) from planting date until the occurrence event for a rice data set comprising 112 genotypes observed in 51 environments (in Japan between 2005 and 2017). From the total of all possible genotype-environment combinations (112×51=5712), 94% (5365) of these were observed. The black line represents the regression line between DTH and CDL (R-squared of 0.99).

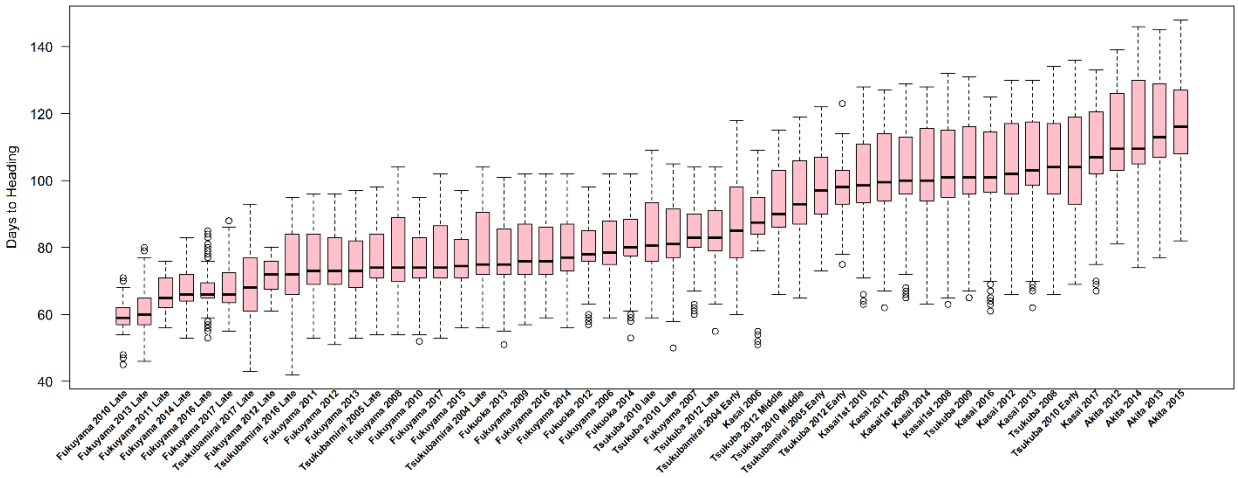

Figure A2. Boxplot for days to heading (DTH) for a rice data set comprising 112 genotypes tested in 51 environments (in Japan between 2005 and 2017). From the total of all possible genotype-environment combinations (112×51=5712), 94% (5365) of these were observed. Environments were ordered based on the environmental medians.

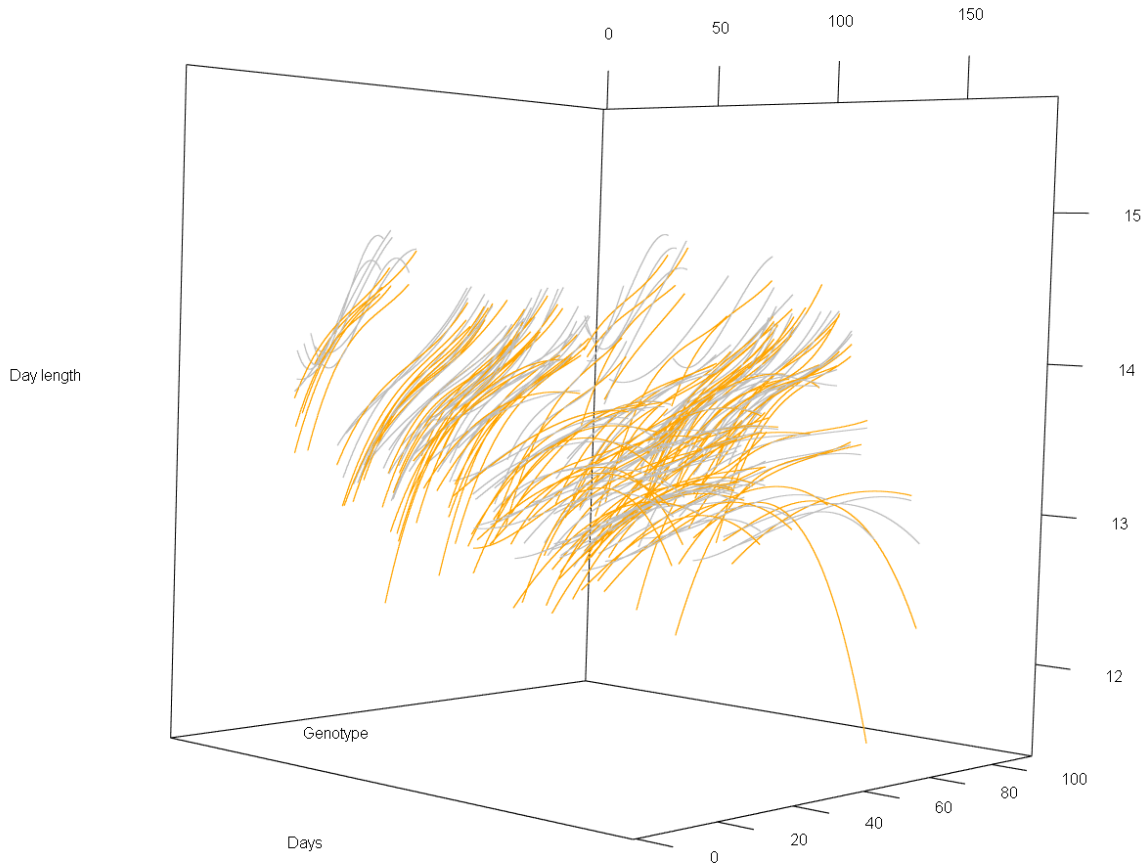

Figure A3. Fitted curves for observed (gray) and predicted (orange) DTH (Day length-*axis*) values for a rice data set comprising 112 genotypes (Genotype-*axis*) tested in 51 environments between days 1 (planting date) and 180 (Days-*axis*). The orange color curves were fitted using the predicted values obtained with the CB method under the CV00 scheme (predicting untested genotypes in unobserved environments) while the gray color curves considered the observed data.

Table A1. Rice trials for studying days to heading for 112 genotypes tested in 79 environments (site-by-year-by-planting date combinations) in multiple locations in Japan. In locations with more than 1 experiment per year different seeding times were tested.

Location	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Latitude	Altitude
Fukuoka									1	1	1	1	1	1	33.21	130.49
Fukuyama			1	1	1	1	2	2	2	2	2	2	2	2	34.5	133.39
Kasai			1	1	2	2	2	1	1	1	1	1	1	1	34.88	134.86
Tsukubamirai	2	2										1	2	2	36.01	140.02
Tsukuba					1	1	2	3	3	1	1				36.02	140.11
Toyama												1	1	1	36.62	137.23
Hokuriku												1	1	1	37.11	138.27
Akita									1	1	1	1	1	1	39.48	140.49
Sapporo										1	1	1	1	1	43.01	141.41