Field Crops Research 214 (2017) 331–340

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Nitrogen nutrition index predicted by a crop model improves the genomic prediction of grain number for a bread wheat core collection

Delphine Ly[a], Karine Chenu[b], Arnaud Gauffreteau[c], Renaud Rincent[a], Sylvie Huet[d], David Gouache[e], Pierre Martre[f], Jacques Bordes[a], Gilles Charmet[a,*]

[a] UMR GDEC, INRA, UCA, 5 chemin de Beaulieu, 63000 Clermont-Ferrand, France
[b] The University of Queensland, QAAFI, 203 Tor Street, Toowoomba, QLD 4350, Australia
[c] Institut National de la Recherche Agronomique, UMR Agronomy, Thiverval Grignon, France
[d] Institut National de la Recherche Agronomique, MAIAGE – Applied Mathematics for Computer Sciences, Jouy-en-Josas, France
[e] Terres Inovia – Institut technique des oléagineux, des protéagineux et du chanvre, 75378 Paris, France
[f] UMR LEPSE, INRA, Montpellier Supagro, 34060 Montpellier, France

ARTICLE INFO

Keywords:
Crop model
Environment characterization
Nitrogen stress
Wheat
Genomic prediction
Genotype-by-environment interactions

ABSTRACT

In plant breeding, one of the major challenges of genomic selection is to account for genotype-by-environment (G × E) interactions, and more specifically how varieties are adapted to various environments. Crop growth models (CGM) were developed to model the response of plants to environmental conditions. They can be used to characterize eco-physiological stresses in relation to crop growth and developmental stages, and thereby help to dissect G × E interactions. Our study aims at demonstrating how environment characterization using crop models can be integrated to improve both the understanding and the genomic predictions of G × E interactions. We evaluated the usefulness of using CGM to characterize environments by comparing basic and CGM-based stress indicators, to assess how much of the G × E interaction can be explained and whether gains in prediction accuracy can be made. We carried out a case study in wheat (*Triticum aestivum*) to model nitrogen stress in a CGM in 12 environments defined by year × location × nitrogen treatment. Interactions between 194 varieties of a core collection and these 12 different nitrogen conditions were examined by analyzing grain number. We showed that (i) CGM based indicators captured the G × E interactions better than basic indicators and that (ii) genomic predictions were slightly improved by modeling the genomic interaction with the crop model based characterization of nitrogen stress. A framework was proposed to integrate crop model environment characterization into genomic predictions. We describe how this characterization promises to improve the prediction accuracy of adaptation to environmental stresses.

1. Introduction

Wheat yields in Europe have been stagnating since 1990 despite constant progress in the genetic improvement of cultivars. This is partly because genetic gains from improved cultivars are being counteracted by negative effects of climate change, which is causing more frequent heat and drought stresses (Brisson et al., 2010). The introduction of fertilizer reduction policies aimed at reducing nitrate pollution are also impeding the expression of wheat yield potential. Plant breeding is therefore increasingly challenged to account for how the genetic makeup of a chosen cultivar determines its responses to such environmental factors (Cormier et al., 2013; Ortiz et al., 2008).

In both wheat breeding and varietal recommendation, it is necessary to predict the differential responses of genotypes to their environments, and more specifically to the types of environments that are encountered in real farming systems. Characterizing relevant environmental stresses, and in particular the severity and timing of those stresses during crop development, is essential (Chenu, 2015). For example, the impact of abiotic stress, like drought, will depend on the developmental stage of the crop when the stress occurs (Denmead and Shaw, 1960). In early studies of this kind, environments were characterized using climatic, geographical and/or pedoclimatic variables (Balfourier and Charmet, 1991; Berger and Turner, 2007; Pollak and Corbett, 1993), but more recently new stress indices have been considered to account for crop phenology including the timing of stress during the crop growth cycle (Chapman et al., 2000b; Chenu et al., 2011; Lecomte, 2008).

Crop models provide a way to refine environment characterization as they can account for simultaneous impacts from various

* Corresponding author: INRA UMR GDEC, 5 chemin de Beaulieu, 63 000 Clermont-Ferrand, France.
E-mail address: gilles.charmet@inra.fr (G. Charmet).

http://dx.doi.org/10.1016/j.fcr.2017.09.024
Received 15 December 2016; Received in revised form 15 September 2017; Accepted 19 September 2017
Available online 28 September 2017
0378-4290/

environmental factors affecting crop growth and development. Chapman et al. (2000a) used a sorghum crop model (Hammer and Muchow, 1994) to characterize different environments based on a drought stress index, which integrated climatic, soil, management, and crop development factors and also accounted for the timing and severity of the stress. This index was used to distinguish three patterns of drought stress in sorghum and showed that in severe terminal stress conditions, the performance of cultivars ranked differently than in mid-season or mid-terminal stress conditions. The approach has been extended to wheat to characterize stress patterns of growing conditions representative of the Australian wheat belt (Chenu et al., 2013) and used to unravel genotype × environment (G × E) interactions in breeding trials (Chenu et al., 2011). Environment characterization for drought has now been carried out for several crops in different regions of the world (Chapman et al., 2000a; Chauhan et al., 2013; Chauhan and Rachaputi, 2014; Chenu et al., 2011; Harrison et al., 2014). Crop modeling has also been used in the USA to define maize target populations of environments in terms of photoperiod, maximum temperature and average radiation (Löffler et al., 2005), and in Australia to look at the impact of frost or heat on wheat (Lobell et al., 2015; Zheng et al., 2015, 2012). Indices related to water deficit and nitrogen (N) availability between booting and heading, two factors impacting grain yield and protein concentration, were also computed with a crop simulation model for durum wheat to characterize agro-climatic conditions in France (Lacaze and Roumet, 2004).

To characterize N stress, so-called N satisfaction indicators have been defined to account for the balance between supply and demand (Lecomte, 2008), such as the ratio between plant N at maturity and the number of grains (Meynard and Limaux, 1987). The N nutrition index (NNI) is another example. NNI is the ratio between the concentration of N in the plant (*CropN*, kg N kg^{-1} DM) in the total above-ground biomass (*CropDM*, t DM ha^{-1}) and the critical N concentration (*CropN*$_c$, kg N kg^{-1} DM) that would be required for optimal crop growth (Lemaire and Gastal, 1997). However the evaluation of N satisfaction indicators requires laborious destructive sampling of plants and the resources to do so. It could thus be advantageous to use crop modeling to simulate patterns of N deficiency over time or to estimate indices such as NNI without expensive and time-consuming sampling.

Quantitative trait loci (QTL) influencing grain yield and grain number in response to either low or high N levels have been identified (Laperche et al., 2007). QTL for traits relative to N use have also been reported in wheat (Bordes et al., 2013; Habash et al., 2007; Hirel et al., 2007). Such QTL can be used to adapt the genetics of N responses through marker assisted selection (MAS, Lande and Thompson, 1990). An extension of MAS is genomic selection (GS), where the QTL detection step is omitted but instead markers covering the whole genome are used as predictors of the overall breeding value (genomic estimate of breeding value, GEBV) of the individual genome (Meuwissen et al., 2001). GS is a suitable method for predicting highly polygenic yield-related traits and is currently being used to improve N use efficiency (Cormier et al., 2016).

Recently researchers have started to couple crop modeling with GS. A wheat crop model has been used to predict phenology and thus derive stress indicators fitted to phenological stages. For example, the sum of daily temperatures between meiosis and flowering or the number of days when the temperature is > 25 °C during grain filling can be considered as genotype-adapted environmental covariates. Such covariates were then used in GS models (Heslot et al., 2014). Another approach is to use GS to estimate the genetic parameters of crop growth models (CGM), then run the CGM with parameters tailored to a variety to predict its performance (yield, grain composition, etc.) in observed or simulated environments (e.g. in a climate change scenario). This approach has been demonstrated using approximate Bayesian computation to estimate genetic parameters of CGM Technow et al. (2015). Recently, Ly et al. (in press) developed a genomic factorial regression prediction model (FR-gBLUP) to focus on how a genotype responds to particular environmental covariates, such as drought and heat stress. The FR-gBLUP model was shown to improve grain number prediction compared to the classical additive genomic model when modeling the genomic reaction norms to a major stress influencing G × E.

The objective of this study was to assess whether crop modeling could define biomass-based environmental covariates which could be used to explain G × E interactions. Specifically, we evaluated the explanatory and predictive ability of GS models in capturing the G × E interactions due to N stress of a core collection of wheat varieties without measuring N indices during the experiments. Crop modeling was used to estimate indicators of N stress, first in a set of experiments where N indices were measured to validate the model, and then in multi-environment trials (MET) of the core collection. Different stress indices were computed and compared to investigate the G × E interactions in the MET. In these trials, N treatments were applied to induce stresses before anthesis, so that grain number had a high heritability and was the main driver of yield. Genomic predictions of grain number were made considering the response to N stress, with or without the N indices derived from the crop model.

2. Materials and methods

2.1. Core collection experimental data

The French National Institute for Agricultural Research (INRA) curates a bread wheat (*Triticum aestivum*) core collection of 372 accessions (Balfourier et al., 2007). From this core collection 194 bread wheat accessions were selected to encompass a wide diversity in geographic origins and registration dates, while limiting the likelihood that plants would be susceptible to lodging. The material was evaluated for yield-related traits during the 2006–2007 (hereafter referred to as 07) and 2007–2008 (hereafter referred to as 07) cropping cycles in three locations in France, namely Estrées-Mons (EM; 49°53′N, 3°00′E), Le Moulon (LM; 48°40′N, 2°10′E) and Joze (JO; 45°86′N, 3°30′E). In each trial, genotypes were grown under high N (HN) or low N (LN) fertilizer input. Crop management also included different sowing densities, disease treatments and growth regulator application (Table 1). The year × location × management combinations resulted in a total of 12 environments. Environments were given six-character code names where the first two characters represent the location (EM, LM, or JO), followed by two characters for the year of the trial (07 or 08), and two characters indicating the level of N in crop management (LN or HN).

For each year × location × management combination, the 194 accessions were sown in 6 incomplete blocks, according to their expected heights in order to minimize plant competition. Six check varieties were planted in all 6 blocks. Phenotypic data were corrected for block effects, which were estimated with reference to the phenotypes of the 6 check varieties, because the other varieties had not been randomly assigned to blocks.

N deficiencies affect grain number according to both the intensity and duration of N stress (Abbate et al., 1995; Jeufrroy et al., 1999). Of the possible yield-related phenotypic measurements, we focused on grain number as most of the differences in N application occurred before anthesis. More information on the trials and genetic materials can be found in Bordes et al. (2013).

2.2. Genotypic data

Varieties were genotyped with the 420 K Axiom array, containing markers for 105 000 insertion-site based SNPs (Paux et al., 2010), 140 000 intergenic SNPs, 150 000 genic SNPs, including 10 000 candidate gene-derived polymorphisms, and roughly 30 000 additional SNPs described in the literature or by the BreedWheat project (http://www.breedwheat.fr/). After filtering out missing data and markers giving a minor allele frequency greater than 0.025, 53 615 SNP markers were selected. Missing data were imputed by the observed allele frequency of

Table 1
Summary of experiments conducted at Estrées-Mons (EM), Le Moulon (LM) and Joze (JO) for the 2006–2007 (2007) and 2007–2008 (2008) growing seasons on bread wheats from a core collection. Growth regulators were applied to prevent plants from lodging. Adapted from (Bordes et al., 2013).

	Estrées-Mons (49°53′N, 3°00′E)				Le Moulon (45°86′N, 3°30′E)				Joze (45°86′N, 3°30′E)			
	2007		2008		2007		2008		2007		2008	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
Environment code	EM07HN	EM07LN	EM08HN	EM08LN	LM07HN	LM07LN	LM08HN	LM08LN	JO07HN	JO07LN	JO08HN	JO08LN
Sowing date	17 Oct		17 Oct		26 Oct		23 Oct		29 Oct		25 Oct	
N application (kg ha^{-1}):												
1 - Tillering	33		40		33		40		35		50	
2 - Jointing	60	40	80	40	60	40	80	40		40		50
3 - Heading	60	40	40		60	40	40		40		40	
4 - Anthesis									50	50	40	40
Seeding density (plant m^{-2})	220	220	220	220	220	220	220	220	280	170	280	170
Disease treatment	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Growth regulators	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes

the corresponding SNP over all accessions (Rutkoski et al., 2013).

2.3. Calibration and testing of the APSIM-Wheat crop model

The APSIM-Wheat version 7.6 crop model (Wang et al., 2002; Holtzworth et al., 2014) was used to simulate the growth of the winter cultivar Soissons, for which enough data from multi-environment trials were available. First, the model for this cultivar was parameterized with two sets of field experiments. The first set of experiments was conducted in five years (1992, 1995, 1996, 1998, 1999) in Grignon (GR), France (48°51′07″N, 1°55′05″E) with N management systems differing in how many times (6, 10, 6, 5 or 5), when and how much N was applied (Jeuffroy and Bouchard, 1999). The second set of experiments was conducted at Villiers-le-Bâcle (VB), France (48°43′32″N, 2°07′32″E) during the 2011–2012 and 2012–2013 winter cropping cycles each with two types of N management. In all trials, information about the soil (maximum rooting depth, soil texture, and organic and inorganic N content), N management and anthesis date were recorded. Leaf area index, total above-ground biomass, and N at different stages from emergence to final harvest, and grain dry matter and grain number at harvest were measured. NNI was calculated as:

$$NNI = \frac{CropN}{CropN_c} \quad (1)$$

*CropN*c was calculated using the N dilution curves established by Justes et al. (1994) for wheat:

$$CropN_c = \begin{cases} 4.4, & CropDM \leq 1.5\text{t DM ha}^{-1} \\ 5.35 \times CropDM^{-0.442}, & CropDM > 1.5\text{t DM ha}^{-1} \end{cases} \quad (2)$$

The Soissons parameterization was based on existing parameterization of APSIM for the Australian winter wheat cultivar Marombi. We adjusted the phenology parameters of vernalization sensitivity (set to 4.5), photoperiod sensitivity (set to 4.7) and thermal time to floral initiation (set to 400 °Cd) and a biomass allocation parameter (number of grains per gram of stem, set to 35 grains per gram of stem) for Soissons, using parameters obtained from full factorial design. This was done by testing all triplets of phenology parameters for a broad range of possible parameter values. We searched for the parameters values which best fitted anthesis date and leaf area index in the 35 trials (i.e. best coefficient of determination, coefficient of variation and root mean square error).

The model was also tested for biomass, N concentration in above-ground biomass, grain yield and NNI. The model was further evaluated for the cultivar Soissons using the MET of the core collection as an independent data set.

2.4. Environment characterization

Crop N stress in the core collection MET was characterized according to (i) the level of inputs (HN vs LN) as a factor, (ii) the amount of N fertilizer applied to the crops (Nf fertilizer, kg/ha) as a covariate, (iii) the sum of N fertilizer and soil inorganic N at measured crop emergence (N supply available, kg/ha) as a covariate, or (iv) crop model based indicators of plant N satisfaction (NNI) developed below. NNI was calculated daily from tillering (growth stage (GS) 21; (Zadoks et al., 1974)) onwards, estimated from 900 °Cd before anthesis to anthesis (GS65) using Eqs. (1) and (2) with *CropN* and *CropDM* simulated by the APSIM-Wheat version 7.6 crop model for the single cultivar Soissons. To ensure the best estimation of N indices for specific stages, the simulations were adjusted according to the observed anthesis date in each trial. However, potential differences in stress patterns experienced by different cultivars of the core collection, e.g. due to differences in phenology, were not considered in this study. Three crop model based indicators for N stress were defined. The first one NNIm-a, the integrated NNI between meiosis (GS38) and anthesis GS65, measured the intensity of stress occurring during the period when grain number is determined, and was defined as the area delimited by the NNI curve below 1. The second crop model based indicator was the NNI at anthesis (NNIa). The third indicator was calculated by clustering the curves of NNI over thermal time between GS22 and GS61, capping at 1. Using the CLARA algorithm in the R package cluster (R Development Core Team, 2003), a cluster analysis of all the environments of the core collection MET was performed on values of NNI, centered at anthesis and averaged every 50 °Cd, in a similar way as previously described for drought stress indices (Chapman et al., 2000; Chenu et al., 2013).

The six N environment characterizations can be divided into two types of N predictors, factors or covariates, which requires two types of model, i.e. factor analysis or regression, respectively (Table 2).

2.5. Explanatory power of characterizations of N stress

We compared 6 interaction statistical models: one for each of the 6 different N stress characterizations. The genotype x N characterization effect was considered fixed, either as a factor or a covariate (Table 2). The additive model was used as a reference. In all these models, we modeled the precocity of each genotype in each environment as a covariate. The sum of square of the residuals, the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) were calculated (Akaike, 1973; Schwarz, 1978), and an analysis of variance (ANOVA) was performed to test the effect of the interaction terms.

Table 2
Characteristics of the nitrogen (N) stress indicators. NNI is the nitrogen nutrition index.

N characterization	Definition	Defined from crop model	Type of variable
Level of input	Low or high N input	No	Factor
Nf	Amount of N fertilizer applied to the crop	No	Covariate
Nav	N supply available is the sum of Nf and inorganic N in soil	No	Covariate
Environment cluster	Group of environments according to the time course of the NNI from −900 °Cdays to anthesis	Yes	Factor
NNIa	NNI at anthesis	Yes	Covariate
NNIm-a	Integrated NNI between meiosis and anthesis	Yes	Covariate

2.6. Crop model characterization of intra-year G × E interaction effects

Knowing that the climatic conditions in 2007 and 2008 were very different, we aimed to explore the effect of year on the variability of G × E interactions, and thus focused on intra-year G × E interaction effects. The objective was to evaluate precisely how much G × N stress contributed to the total G × E interactions per year, and assess how much of the interaction with the N stress indicators could be captured each year. We modeled a G × E interaction term by year as a random effect identically distributed each year. We compared the variance of the G × E interaction each year between the 7 models, one model for each N stress indicator and one for the global G × E.

2.7. Genomic predictions for the different characterizations of N stress

Three genomic models were developed to assess the predictive ability of the N stress characterizations (Eq. (2), Supplementary Table S1).

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{Z_G u_G} + \mathbf{N_N} + \mathbf{Z_{GN} u_{GN}} + \mathbf{Z_E u_E} + \mathbf{e} \text{ (Model 1)}$$

Genomic predictions of the additive values $\mathbf{u_G}$ were modeled using the genomic BLUP model (gBLUP, Model a in Supplementary Table S1) (Habier et al., 2013; Henderson, 1975). For the qualitative N stress characterization (Level of input, Environment clusters), $\mathbf{N_N}$ represents the fixed effect of the classification of environments, and its interactions $\mathbf{u_{GN}}$ with the genotypes is modeled as a genomic effect with a variance per class of environments (Model b in Supplementary Table S1). For the quantitative N stress characterizations (Nf, Na, NNIa, NNIm-a), $\mathbf{N_N}$ represents a regression to the N stress covariate($\mathbf{N_N} = \beta \mathbf{x_E}$, Model c in Supplementary Table S1). Genomic predictions of the response to a given quantitative characterization of N stress $\mathbf{x_E}$ were performed using a random regression with a genomic kinship as in the FR-gBLUP model ($\mathbf{u_{GN}} = \mathbf{b_G} \circ \mathbf{x_E}$,Model c in Supplementary Table S1; Ly et al., in press). For more details, see Supplementary Table S1.

The variances were estimated using Restricted Maximum Likelihood with ASReml in R (Gilmour et al., 2006).

2.8. Assessment of predictive ability of the different genomic prediction models

The prediction quality of the different models was assessed by cross-validations (Kohavi, 1995). We used three different 4-fold cross-validation schemes using 75% of the data in the training set and 25% in the validation set, sampling different combinations of tested or untested genotypes in tested or untested environments where (i) 25% of the random genotype and environment combinations were excluded from the training of the model but used for the model testing (CVrandom); (ii) 25% of the genotypes were randomly excluded from the training set to be used in the validation set (CVnewG); (iii) three environments out of the 12 were excluded from the model training but used to test it (CVnewE); or (iv) some genotypes and environments were completely excluded from the training of the model (CVnewGE). These 4 cross-validation strategies mimic different scenarios of prediction. CVrandom mimics the prediction of individuals which have already been seen in other environments, CVnewG the prediction of unknown individuals in known environments, CVnewE the prediction of known individuals in unobserved environments, and CVnewGE the prediction of unknown individuals in unobserved environments. The cross-validations were repeated 10 times. We calculated the prediction accuracy within each environment (location × year × treatment combination) as the Pearson correlation between the observation $\mathbf{y}$ and the predictions $\hat{\mathbf{y}}$: $cor(\mathbf{y}, \hat{\mathbf{y}})$where $\hat{\mathbf{y}} = \hat{\mathbf{u}}_G$ for models without interaction (Model a), or $\hat{\mathbf{y}} = \hat{\mathbf{u}}_G + \hat{\mathbf{u}}_{GN}$ for models considering interaction Model (b and c).

2.9. Simulation of experimental data

We aimed to assess the effect of the variance of the genetic reaction norms to the environmental covariate on the gain in prediction accuracy in this particular dataset. For this purpose, we simulated data according to the FR-gBLUP model defined above and in Supplementary Table S1, so that they followed the same normal distribution as the observed phenotypic data adjusted for blocks with variance parameters estimated by the FR-gBLUP for NNIa.

Because of the absence of replications, the complete GxE effect could not be estimated. The FR-gBLUP here only modeled a fraction of the GxE interactions: the linear reaction norms to the environmental covariate, which required less degrees of freedom. The residual variance here thus included GxE interactions which are not explained by the reaction norms.

Since the purpose of this analysis was to evaluate the impact of increasing importance of the reaction norms variance in this dataset, all following simulation analysis set the residual variance to the one estimated from phenotypic adjusted data.

Concerning the covariance between intercepts and slopes, it was set to 0, for the sake of simplicity. We set different levels of variance of the reaction norms to represent different levels of GxE: × 0.05, × 0.15, or × 0.25 times the additive genetic variance. For each value of variance of the reaction norms, we simulated virtual phenotypes 20 times from the given distribution. For each sampling, we ran cross-validation analysis to measure the prediction accuracy gains in the different cross-validation cases. Twenty simulation runs for each ratio were sufficient to achieve stable prediction accuracies.

3. Results

3.1. Calibration and testing of the crop model to characterize N stress

The APSIM-Wheat crop model was used to estimate crop N indices, which were not measured in the trial. To ensure the quality of the simulations, the model was first calibrated and tested for the reference variety Soissons against data from a set of 35 trials (2 locations, 7 years, various N fertilization levels). Simulations slightly under-estimated crop biomass ($y = 326.9 + 0.78x$), grain number ($y = 4256.0 + 0.6x$) and yield ($y = 956.2 + 0.65x$) (Fig. 1b, e, f). But the model allowed accurate estimations of anthesis date (RMSE = 0.72 days; Fig. 1 a), and importantly N biomass at anthesis (RMSE = 3.89 g/m^2; Fig. 1c) and NNI at anthesis (RMSE = 0.16; Fig. 1d).

Using the genotypic parameters for Soissons that were calibrated with the 35 trials, the model was then tested against data from the independent set of core collection METs. Overall the model accurately

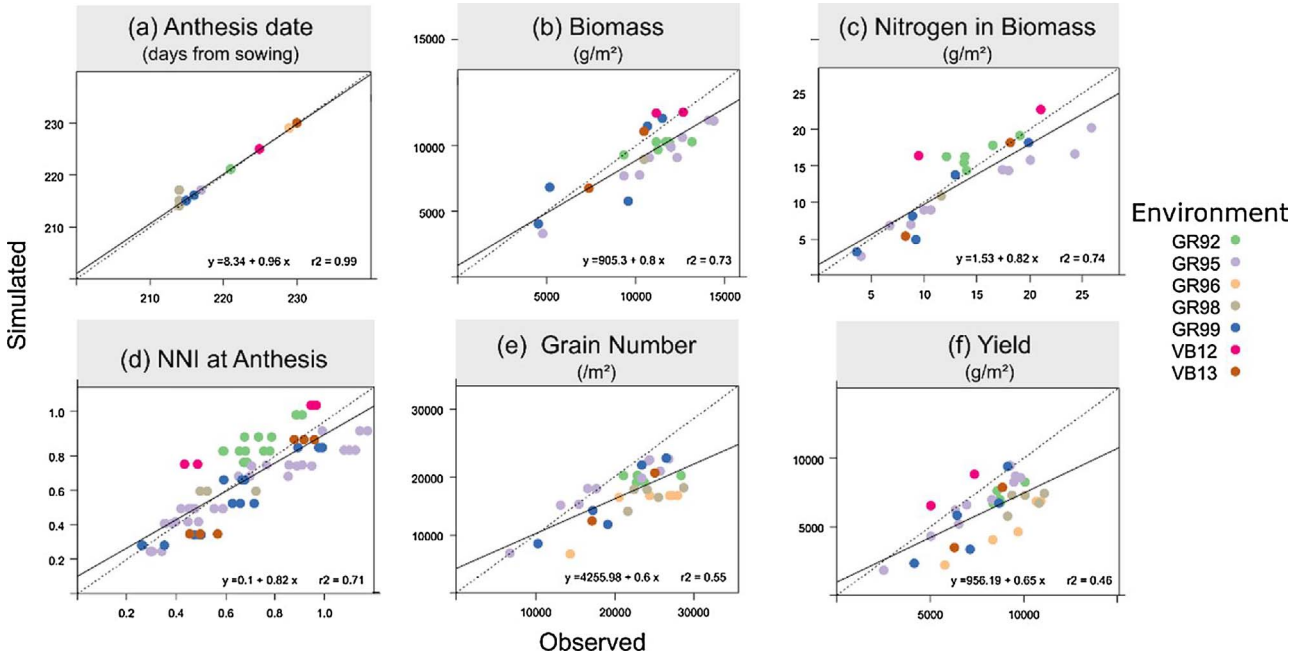

Fig. 1. Comparison of APSIM simulations and observations in the calibration data set for (a) anthesis date, (b) above-ground biomass at anthesis, (c) N in above-ground biomass at anthesis, (d) NNI at anthesis, (e) final grain number and (f) final grain yield. See Materials and Methods for details of environment code names.

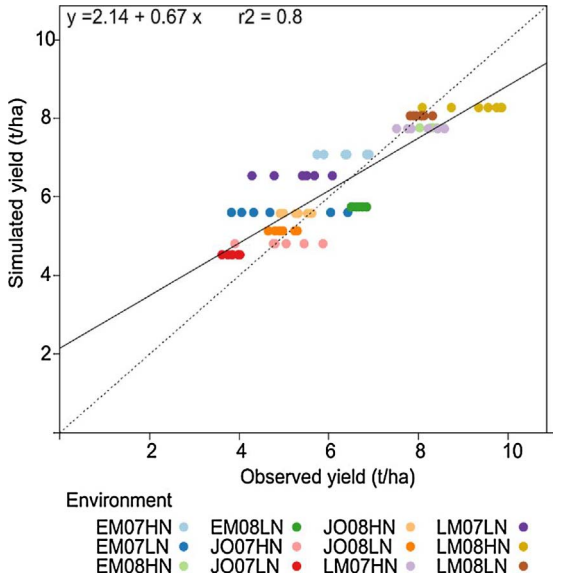

Fig. 2. Comparison of APSIM simulations and observations of yield in the core collection trials. See Materials and Methods for details of environment code names.

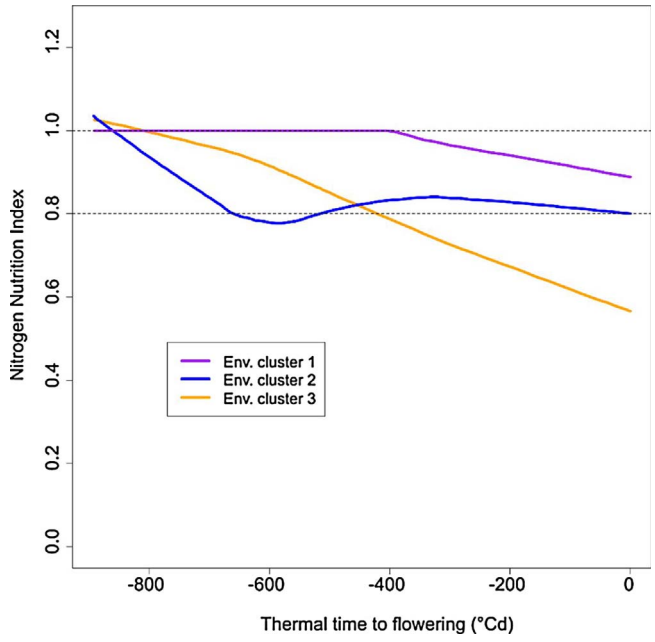

Fig. 3. Three environment clusters of the core collection MET according to the changes in NNI throughout the pre-anthesis development of the crop. Data from all the environments were centered at anthesis and averaged every 50 °Cd and clustered in three environment clusters. NNI is shown as a function of thermal time relative to anthesis. An NNI value of 1 indicates no N stress, while 0 indicates full N stress. Curves correspond to the average NNI across the different environments of a cluster smoothed using the R function lowess. The environments include in each cluster are specified in Table 3.

predicted grain yield (RMSE = 2497.5 g/m^2; Fig. 2) and so was used to characterize N stress in the core collection MET.

3.2. A diverse core collection grown in contrasting conditions

A diverse range of 194 accessions from a core collection were assessed under contrasting input levels over two years in three locations in France. Yield, grain number and heading varied greatly across genotypes, and were all highly impacted by year, location and N treatment (Supplementary Fig. S1).

Some genotypes showed extreme variations differing by over a month in heading date, by 15,000 grains m^{-2} in grain number and by over 6 t ha^{-1} in yield, which illustrates how diverse the collection is. Low-input treatments significantly decreased yield and grain number, as expected, and high variations were also observed between years, particularly as the 2007 season had more radiation, higher temperatures and more rainfall than 2008, except in Joze (Supplementary Table S2).

3.3. Environment characterization of N stress in the core collection MET

APSIM-Wheat was used to characterize N stress in the 12 MET of the core collection. Computed N indices (Table 3) reflect the diversity in the

Table 3
Real and modeled N stress indicators. Nf is the amount of N fertilizer applied. Nav is the amount of N supply available from fertilizer and inorganic N in the soil. Environment clusters 1–3 (see Fig. 3), NNIa, the NNI at anthesis, and NNIm-a, the integrated NNI between meiosis and anthesis, were defined using the simulated NNI from the crop model APSIM.

Environment	Nf (kg ha^{-1})	Nav (kg ha^{-1})	Environment cluster	NNIa	NNIm-a
EM08HN	160	218	1	0.93	0.02
LM08LN	40	109	1	0.83	0.08
LM08HN	160	229	1	0.83	0.08
LM07HN	153	148	1	0.75	0.23
EM08LN	40	98	1	0.65	0.29
EM07HN	153	196	2	0.78	0.18
JO07HN	125	225	3	0.66	0.28
JO08HN	130	200	3	0.66	0.3
EM07LN	80	123	3	0.63	0.34
LM07LN	80	148	3	0.61	0.37
JO07LN	90	190	3	0.57	0.39
JO08LN	90	160	3	0.57	0.39

Table 4
Model selection criteria for statistical models of grain number according to different N characterizations. AIC, Aikaike information criterion; BIC, Bayesian information criterion. Nf is the amount of N fertilizer. Nav is the amount of N supply available from fertilization and inorganic N in the soil. Environment clusters (described in Fig. 3 and Table 3), N nutrition index at anthesis (NNIa), and the integrated NNI between meiosis and anthesis (NNIm-a) were defined using APSIM crop model simulations.

N characterization	p-value of interaction effect	Sum squares interaction/ Sum squares residuals	Residuals sum of squares	AIC	BIC
None (additive model)	–	–	4607	8907	10095
Level of Input	0.9532	0.08	4272	9114	11429
Nf	0.949	0.08	4271	9112	11428
Nav	0.9997	0.06	4330	9144	11459
Environment cluster	1.01E-08	0.31	3510	9004	12449
NNIa	< 2.2E-16	0.23	3578	8677	10991
NNIm-a	< 2.2E-16	0.25	3698	8758	11073

N nutrition status that occurred in these trials. NNI at anthesis varied from 0.57 (moderately severe N stress) to 0.93 (almost no stress), with, as expected, higher values for high-input conditions than for low-input conditions within each trial (Table 3). However, the stress was lower in some environments with low-input conditions than in other environments with high-input conditions, highlighting the effects that year, location, soil properties can have on the crop N status.

The timing of the N stress varied across trials and the clustering of NNI curves over thermal time (Supplementary Fig. S2) allowed us to identify three environment clusters (EC) representing the pre-anthesis temporal variations (Fig. 4). EC1 corresponded to the highest level of NNI and was characterized by a non-stress level of NNI (NNI = 1) up to 400 °Cd before anthesis that declined slowly afterwards (Fig. 4). EC1 therefore encompassed environments with no or only slight pre-anthesis N stress. EC2 related to the trial EM07LN where N stress occurred early but was then relieved by supplemental fertilization at jointing (Fig. 4). Finally, EC3 was characterized by early N stress that increased steadily over time up to anthesis (Fig. 4).

3.4. Model selection criteria favor crop model based indicators to characterize G × N stress interactions

Modeling the fixed effects of the interaction between genotypes and N stress indicators reduced the residuals compared to those of the additive model (Table 4). Crop model based indicators (NNIa, NNIm-a, Environment cluster) captured G × N stress interaction better than the other N indicators such as the level of input or the amount of N available in the soil (Nf, Nav). Indeed, only interactions with crop model indicators were significant (P = 0.05). Besides, both the sum of squares of the residuals and AIC (which account for the number of parameters estimated) indicated that the models with crop model based N characterization were better at capturing the phenotypic variability of the MET (Table 4). BIC (which penalizes the number of parameters estimated more strongly than AIC) favored the additive model, as it was the simplest model (i.e. with fewer parameters), and preferred the interaction models using the crop model indicators NNIa and NNIm-a. BIC strongly penalized the environment cluster characterization, as it required more parameters for estimates (one for each genotype in each cluster of environments compared to only one for each genotype in the factorial regression).

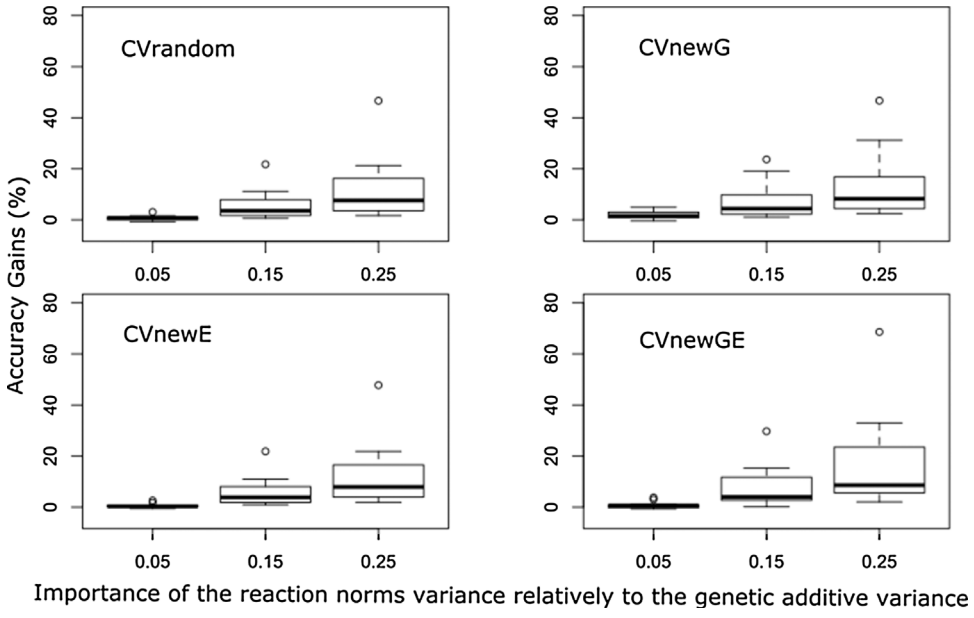

Fig. 4. Effect of the importance of the ratio of GxE variance relatively to the genetic additive variance on prediction accuracy gains in the FR-gBLUP model on simulated data.

Table 5
Variance components of interaction for model tested for each year on grain number data from the core collection MET. Models and abbreviations are described in Materials and Methods. The first row shows an estimate of G × E variance by year and the other rows show how much of this variance is explained by each method. G is the genotypic variances. and 2008 respectively. G × N the variance of the genotype × N stress characterization interaction effects. Nf is amount of N fertilizer applied. Nav is the amount of N supply available from fertilization and inorganic N in the soil. Environment clusters (described in Fig. 3 and Table 3), N nutrition index (NNI) at anthesis (NNIa), and the integrated NNI between meiosis and anthesis (NNIm-a) were defined using the simulated NNI from the APSIM crop model.

N characterization	Variance Components						
	G in 2007	GxN in 2007	GxN/G Ratio in 2007	G in 2008	GxN in 2008	GxN/G Ratio in 2008	Residuals
Environment	3.58 ± 0.39	0.03 ± 0.08	0.01	4.29 ± 0.48	0.93 ± 0.13	0.22	1.44 ± 0.09
Level of input	3.50 ± 0.39	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	5.66×10^{-8}	4.36 ± 0.48	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	4.54×10^{-8}	1.96 ± 0.06
Nf	3.50 ± 0.39	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	5.66×10^{-8}	4.36 ± 0.48	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	4.54×10^{-8}	1.96 ± 0.06
Nav	3.50 ± 0.39	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	5.66×10^{-8}	4.36 ± 0.48	$1.98 \times 10^{-7} \pm 6.19 \times 10^{-9}$	4.54×10^{-8}	1.96 ± 0.06
Environment cluster	*Not enough degrees of freedom*						
NNIa	3.54 ± 0.39	$1.70 \times 10^{-7} \pm 5.57 \times 10^{-9}$	4.80×10^{-8}	4.23 ± 0.46	0.32 ± 0.05	0.08	1.68 ± 0.06
NNIm-a	3.55 ± 0.39	$1.63 \times 10^{-7} \pm 5.35 \times 10^{-9}$	4.60×10^{-8}	4.14 ± 0.45	0.42 ± 0.06	0.10	1.61 ± 0.05

3.5. Ability of crop model indicators to capture the differences in G × E interaction variances per year

Modeling the G × E interactions by year showed that G × E variance was much higher in 2008 than in 2007 (Table 5). Even though similar N fertilization regimes were applied in 2007 and 2008 (Table 1), cooler temperature in 2008 slowed crop development which allowed the development of greater biomass and yield potential (Supplementary Table S2, Supplementary Fig. S1), but also generated greater G × E interactions in response to low inputs (Table 5). The variances per year of the genotypic interaction with classic N stress characterization (Level of input, Nf, and Nav) failed to capture the G × E interactions signal in either year. By contrast, modeling the interaction between genotype and crop model indicators for N stress captured the high G × E signal in 2008, and even the lower one in 2007.

3.6. Genomic prediction gains by modeling the interaction with N stress using crop model indicators

Genomic predictions of the response to crop model based environment characterizations (NNIa, NNIm-a and Environment cluster) were compared to genomic predictions using basic characterizations (Level of inputs, Nf or Nav). The crop model based indicators captured up to almost 10 times more interaction signal, from 3.9% to 6.9% of the additive genetic variance compared to the basic indicators which captured only 0.4–0.9% of the genetic additive variance (Table 6). This decreased the error variance and the environmental variance, with virtually no change in the genetic variance but a greater G × E interaction variance. Overall, the environment effect was captured better with crop model based indicators.

The estimation of prediction accuracies using a testing set which contained either similar individuals and environments (CVrandom), or no individuals (CVnewG) or environments (CVnewE), or neither individuals nor environments (CVnewGE) in common with the training set showed that all models (additive and G × N models) performed accurately (Supplementary Table 3). Accuracies of the additive model were high and ranged from 0.715 to 0.882 in CVrandom, from 0.417 to 0.568 in CVnewG, from 0.728 to 0.89 in CVnewE and from 0.401 to 0.556 in CVnewGE (Supplementary Table S3), which is consistent with the fact that phenotypic variance was mainly explained by the genetic variance, and to a lesser extent by the G × E variance (Table 5). Simulations results showed that in such a dataset with this given residual variance, increasing importance of GxN variance would increase accuracies gain (Fig. 4). On the real data, the G × Level of input, Nf or Nav interaction had similar prediction accuracies than the additive model in all cross-validations. By contrast, by using crop model covariates (NNIa, NNIm-a and Environment cluster) in the genomic models (b and c, Supp. Table S1), the prediction accuracies were slightly higher than those obtained with the additive model (a, Supp. Table 1) in CVrandom, and quite similar in CVnewG, CVnewE on average across all environments (Supplementary Table S3). Using environmental cluster, prediction accuracies were higher in CVnewGE. In 2008 particularly (i.e. when G × E interactions were relatively high; Table 5), prediction accuracy gains with crop model covariates in the FR-gBLUP model were higher than the additive or G × basic N stress characterization models compared to the additive model in CVrandom (e.g. 0.78% accuracy gains instead of −0.28% in 2007).

4. Discussion

4.1. Crop growth model provides N stress indicators well suited for modeling G × E interactions

With current policies to reduce fertilizer use to limit nitrate leaching

Table 6
Variance components of the genomic models considering the response of grain number to N stress. G refers to the genomic additive effect, E is the environmental effect and. G × N represents the interaction variance, which corresponds to the G × Group of environment variance in Model b or the variance of the genomic reaction norms to the environmental covariate of N stress in Model c. For model c in particular, there is also a covariance representing the relation between the additive genomic additive effects (intercepts) and the reaction norms (slopes). More details on models and abbreviations are given in the Supplementary Table S1. Nf is amount of N fertilizer. Nav is the amount of N supply available from fertilizer and inorganic N in the soil. Environment cluster (see Fig. 3 and Table 3), N nutrition index (NNI) at anthesis (NNIa), and the integrated NNI between meiosis and anthesis (NNIm-a) were defined using the simulated NNI from the APSIM crop model.

N characterization	Model	Variance of G	Covariance between G and GxN	Variance of GxN	Variance of E	Variance of Residuals
Additive	a	2.25 ± 0.25	–	–	7.69 ± 3.29	2.06 ± 0.06
Level of Input	b	2.24 ± 0.25	–	0.013 ± 0.01	6.74 ± 3.02	2.05 ± 0.06
Nf	c	2.26 ± 0.25	0.22 ± 0.04	0.02 ± 0.01	6.75 ± 3.02	2.02 ± 0.06
Nav	c	2.26 ± 0.25	0.13 ± 0.04	0.008 ± 0.006	6.75 ± 3.03	2.04 ± 0.06
Environment cluster	b	2.22 ± 0.25	–	0.15 ± 0.04	4.62 ± 2.18	1.90 ± 0.06
NNIa	c	2.28 ± 0.25	0.37 ± 0.06	0.11 ± 0.02	3.65 ± 1.64	1.85 ± 0.06
NNIm-a	c	2.29 ± 0.25	−0.37 ± 0.06	0.13 ± 0.03	3.37 ± 1.51	1.83 ± 0.06

and greenhouse gas emissions, it is becoming important to characterize N stress in the field as a prerequisite for breeding wheat varieties more adapted to limited N supply (Justes et al., 1994; Laperche et al., 2007). Despite the availability of crop models to simulate N status in wheat crops (Holzworth et al., 2014; Jamieson and Semenov, 2000; Jeuffroy and Recous, 1999), they have been rarely used for environment characterization of N stress in germplasm evaluation trials, with the notable exception of the work of Lacaze and Roumet (2004). We aimed to evaluate the ability of crop model simulations to assess the N stress experienced by crops in field trials, taking account of the soil conditions, the climatic conditions, and N applications in order to allow the N characterization of MET and to possibly unravel G × E interactions associated with N limitations. Here, crop modeling was successfully used to estimate N indicators, when measured (Fig. 1d, Table 3). The estimation of such indicators in trials where they were not measured allowed the characterization of genotype × N stress interactions (Table 4) and better understanding of G × E interactions. In the core collection MET studied, crop model based indicators of N stress were good predictors of the environmental effect (Tables 6–7) and the N-specific interactions captured a substantial part of the total G × E interactions (Table 4). Overall, the indicators based on crop model simulations for the cultivar Soissons characterized N stress better than basic information such as the treatment (Level of input) or the amount of available soil N (Nav; Table 3). Some environments with high N inputs were actually more stressful than others with limited N input (Table 3), as environmental factors (e.g. temperature, radiation, rainfall) must have greatly influenced how the level of input impacted crops.

Across all cross-validations, NNI at anthesis (NNIa) and NNI between meiosis and anthesis (NNIm-a) were the crop model N environmental covariates which performed the best at capturing some of the G × E interactions on grain number. Both reflect N stress at crucial stages when grain number is determined. However, the environment characterization of N stress using NNI, as proposed by (Lecomte, 2008), is rarely done as it requires time-consuming, destructive, and expensive measurements. Simulating NNI with crop models presents major advantages for assessing the N nutrition status of trials. In addition, simulating trials with crop models can be used to characterize different stresses and their interactions, and could be used for multiple purposes by breeding programs (Cooper et al., 2014; Chenu, 2015),

4.2. How much gain in prediction accuracy can be expected from G × E modeling?

In terms of genomic predictions, the accuracies of the statistical model were expected to be very high with the additive model due to the high genetic variance observed for this dataset. When also considering the interaction with N stress, the model was slightly improved, although not in all trials. The differences in prediction accuracies between models (additive model vs G × E models) were small, since the across-locations heritability (Cullis et al., 2006) calculated for grain number was very high (0.96) and the variance of the G × N was relatively low (less than 10% of the genetic additive variance in all the tested models). Despite significant genotype × management system effects observed within the same trials, their variance only represented 6.3% of the genetic additive effect for grain number (Bordes et al., 2013). Overall, even though the experiments were designed to have a wide range of N stress, the tested population came from a core collection (Balfourier et al., 2007) which has a very high genetic variability in terms of yield and grain number, but an unexpectedly low G × N variance compared to the genetic additive variance (Table 6). The ratio of slopes to intercepts variance was 0.048 in our dataset. For a ratio value of 0.05, simulations study with the same residual variance showed indeed no gain in accuracies. However, simulations of higher ratios showed that with greater GxN variance we would have been able to predict this signal (Fig. 4).

Despite the low level of G × N interactions observed, the average prediction accuracy across all trials was increased when using genomic prediction models that include crop model N indicators (NNIa, NNIm-a, and Environment cluster) for the cross-validation CVrandom, i.e. when random genotype and environment combinations were excluded from the training set (Supplementary Table S3). In addition, greater gains in accuracy (+ 0.78% in CVrandom) were achieved for 2008, i.e. when most G × E and G × N interactions occurred. In studies with greater G × E variance, higher prediction gains are expected and have been observed. For instance, an average prediction gain of 11.1% in accuracy was found in wheat trials where the G × E variance represented 63% of the genetic additive variance (compared to 0.4%-9% in our study), and where the prediction accuracies for the additive model were lower than 0.5 (whereas in our MET almost all were above this value) (Heslot et al., 2014). Overall, the dataset used in this study was characterized by a very high genetic variation and an atypically low G × E interaction, so greater gain in accuracy could be made with more standard populations (e.g. advanced breeding lines) and with models that take into account the overall G × E instead of the reaction norms to N stress only.

4.3. Benefits of combining genomics and crop modeling to predict G × E interactions

Handling G × E interactions has always been a challenge for plant breeding. Different methodologies have been developed to identify genetic controls underlying these complex interactions. Numerous studies have identified QTL that control trait variations between contrasting treatments or in MET. However, such QTL can be highly context-dependent and difficult to deploy for breeding purposes (Reymond et al., 2004; Richards et al., 2010). To tackle this challenge, modeling approaches have been used to dissect complex traits into simpler traits with less dependency on context. Some ecophysiological and process-based crop models have been developed with this aim (Hammer et al., 2006; Tardieu, 2003), and QTL for their parameters have been mapped to identify environmentally-stable genetic controls (Bogard et al., 2014; Chenu et al., 2009; Reymond et al., 2003; Yin et al., 2003). An approach to integrate crop growth models with whole genome prediction of ecophysiologic parameters was recently highlighted as promising (Technow et al., 2015). However, the latter approach is opposite to the one we present here. Technow et al. (2015) used genomic predictions to estimate GEBV of genetic parameters of the CGM, then ran the model with these “tailored parameters” to predict genotype performance in environments. Instead, we ran CGM parameterized for a single “average” genotype to estimate CGM-derived stress indicators, then used genomic markers to estimate reaction norms of genotypes to these indicators. Cooper et al. (2016) used this approach on real maize data, using 106 doubled-haploids from a single cross evaluated in two water-stress environments. Prediction accuracy was slightly improved (e.g. 0.82 vs 0.78 with gBLUP) in their estimation test, but not in their test set. However, the small population analyzed and its narrow genetic basis can explain this discrepancy. With our proposed approach we obtained significant improvement in explaining G × E (compared to usual stress indicators) and prediction accuracy of GS models including a random regression on a CGM stress indicator was almost always better than gBLUP accuracy. In parallel, statistical methods have been developed to dissect the overall G × E interactions in genomic selection (Burgueño et al., 2012; Heslot et al., 2014; Jarquín et al., 2014). Their approaches mainly aim to deal with the high dimensionality of G × E, using factor analytic model (Burgueño et al., 2012), or soft rules methods (Heslot et al., 2014), or an environmental similarity matrix (Jarquín et al., 2014). Some improvement in prediction accuracy was reported, mainly when using random cross-validation, i.e. all genotypes and all environments included in the training set (Jarquín et al., 2014). Because the multiple environmental covariates which will become relevant in the future are difficult to predict, we chose to focus our study on a single stress, N limitation, which was imposed by contrasting

experimental treatments. Here, we demonstrated the benefit of using crop modeling to characterize environments and unravel G × E interactions for N stress, as Chenu et al. (2011) did for drought in MET for breeding in Australia, and Heslot et al. (2014) did for temperature, radiation and rainfall related indices in MET for breeding in France.

The greater the G × E interactions for the stress considered, the more likely we are to obtain distinctive genomic predictions for the reaction norms to the stress. Using diversity panels, crop model characterization of N stress allowed genomic predictions of the reaction norms to N stress to be made when the G × E signal was high, in this case in 2008 trials. In the literature, using elite or advanced breeding material where G × E interactions represented a large proportion (about 20% to 40%) of the total variance, factorial regression models with appropriate environmental covariate(s) allowed large variations in the G × E interactions to be explained (van Eeuwijk et al., 1995; Voltas et al., 2005; Lopes et al., 2012; Ciancaleoni et al., 2016, Ly et al., in press). Furthermore, modeling of the genomic response to drought has achieved 1.6% to 26.2% prediction accuracy gains (Ly et al., in press). We believe that using crop models to characterize environments promises to improve the genomic predictions of the response to an environmental stress, and thus the adaptation of the varieties, provided that the variance of the reaction norms is large enough. In addition, in MET with larger G × E due to several stresses, even more substantial gains could made in predicting the global genomic G × E interactions.

5. Conclusion

The use of crop model based stress indicators proved powerful in explaining a significant proportion of G × E interaction in multi-environment wheat trials. Integrating these indicators as covariates in our recently developed FR-gBLUP model improved the genomic prediction accuracy in MET where G × E was observed. While this study focused on a dataset with great genetic variability and atypically low G × E interaction, the method developed promises to enhancing breeding programs with a genomic selection strategy integrating G × E interactions.

Funding

The experimental work was partly supported by the Enterprise Competitiveness Fund Project “Semences de Demain”. This work was funded by the INRA metaprogram SELGEN and the Auvergne Region through funding to DL during her PhD.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2017.09.024.

References

Abbate, P., Andrade, F., Culot, J., 1995. The effects of radiation and nitrogen on number of grains in wheat. J. Agric. Sci. 124 (3), 351–360. http://dx.doi.org/10.1017/S0021859600073317.

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov, B.N., Csáki, F. (Eds.), 2nd International Symposium on Information Theory. Tsahkadsor, Armenia, USSR, September 2–8, 1971. Akadémiai Kiadó, Budapest, pp. 267–281.

Balfourier, F., Charmet, G., 1991. Relationships between agronomic characters and eco-geographical factors in a collection of French perennial ryegrass populations. Agronomie 11, 645–657. http://dx.doi.org/10.1051/agro:19910802.

Balfourier, F., Roussel, V., Strelchenko, P., Exbrayat-Vinson, F., Sourdille, P., Boutet, G., Koenig, J., Ravel, C., Mitrofanova, O., Beckert, M., Charmet, G., 2007. A worldwide bread wheat core collection arrayed in a 384-well plate. Theor. Appl. Genet. 114, 1265–1275. http://dx.doi.org/10.1007/s00122-007-0517-1.

Berger, J.D., Turner, N.C., 2007. The Ecology of Chickpea: evolution, distribution, stresses and adaptation from an agro-climatic perspective. In: Yadav, S., Redden, R., Chen, W., Sharma, B. (Eds.), Chickpea Breeding and Management. CAB International, Wallingford, pp. 47–71.

Bogard, M., Ravel, C., Paux, E., Bordes, J., Balfourier, F., Chapman, S.C., Le Gouis, J., Allard, V., 2014. Predictions of heading date in bread wheat (Triticum aestivum L.) using QTL-based parameters of an ecophysiological model. J. Exp. Bot. 65, 5849–5865. http://dx.doi.org/10.1093/jxb/eru328.

Bordes, J., Ravel, C., Jaubertie, J.P., Duperrier, B., Gardet, O., Heumez, E., Pissavy, a L., Charmet, G., Le Gouis, J., Balfourier, F., 2013. Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection. Theor. Appl. Genet. 126, 805–822. http://dx.doi.org/10.1007/s00122-012-2019-z.

Brisson, N., Gate, P., Gouache, D., Charmet, G., Oury, F.-X., Huard, F., 2010. Why are wheat yields stagnating in Europe? A comprehensive data analysis for France. F. Crop. Res. 119, 201–212. http://dx.doi.org/10.1016/j.fcr.2010.07.012.

Burgueño, J., de los Campos, G., Weigel, K., Crossa, 2012. Genomic prediction of breeding values when modeling genotype × environment interaction using pedigree and dense molecular markers. Crop Sci. 52, 707. http://dx.doi.org/10.2135/cropsci2011.06.0299.

Chapman, S.C., Cooper, M., Butler, D.G., Henzell, R.G., 2000a. Genotype by environment interactions affecting grain sorghum. I. Characteristics that confound interpretation of hybrid yield. Aust. J. Agric. Res. 51, 197. http://dx.doi.org/10.1071/AR99020.

Chapman, S.C., Hammer, G.L., Butler, D.G., Cooper, M., 2000b. Genotype by environment interactions affecting grain sorghum. Temporal sequences and spatial patterns in the target population of environments. Aust. J. Agric. Res. 51, 223. http://dx.doi.org/10.1071/AR99022.

Chauhan, Y.S., Rachaputi, R.C.N., 2014. Defining agro-ecological regions for field crops in variable target production environments: a case study on mungbean in the northern grains region of Australia. Agric. For. Meteorol. 194, 207–217. http://dx.doi.org/10.1016/j.agrformet.2014.04.007.

Chauhan, Y.S., Solomon, K.F., Rodriguez, D., 2013. Characterization of north-eastern Australian environments using APSIM for increasing rainfed maize production. F. Crop. Res. 144, 245–255. http://dx.doi.org/10.1016/j.fcr.2013.01.018.

Chenu, K., Chapman, S.C., Tardieu, F., McLean, G., Welcker, C., Hammer, G.L., 2009. Simulating the yield impacts of organ-level quantitative trait loci associated with drought response in maize: a gene-to-phenotype modeling approach. Genetics 183, 1507–1523. http://dx.doi.org/10.1534/genetics.109.105429.

Chenu, K., Cooper, M., Hammer, G.L., Mathews, K.L., Dreccer, M.F., Chapman, S.C., 2011. Environment characterization as an aid to wheat improvement: interpreting genotype-environment interactions by modelling water-deficit patterns in North-Eastern Australia. J. Exp. Bot. 62, 1743–1755. http://dx.doi.org/10.1093/jxb/erq459.

Chenu, K., Deihimfard, R., Chapman, S.C., 2013. Large-scale characterization of drought pattern: a continent-wide modelling approach applied to the Australian wheatbelt-spatial and temporal trends. New Phytol. 198, 801–820. http://dx.doi.org/10.1111/nph.12192.

Chenu, K., 2015. Characterising the crop environments - Nature, significance and applications. In: Sadras, V., Calderini, D. (Eds.), Crop Physiology. Applications for Genetic Improvement and Agronomy. Academic Press, pp. 321–348. http://dx.doi.org/10.1016/B978-0-12-417104-6.00013-3.

Ciancaleoni, S., Onofri, A., Torricelli, R., Negri, V., 2016. Broccoli yield response to environmental factors in sustainable agriculture. Eur. J. Agron. 72, 1–9. http://dx.doi.org/10.1016/j.eja.2015.09.009.

Cooper, M., Technow, F., Messina, C., Gho, C., Totir, R., 2016. Use of crop growth models with whole genome prediction: application to a maize multienvironment trial. Crp Sci. 56, 2141–2156. http://dx.doi.org/10.2135/vropsci2015.08.0512.

Cormier, F., Faure, S., Dubreuil, P., Heumez, E., Beauchêne, K., Lafarge, S., Praud, S., Le Gouis, J., 2013. A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (Triticum aestivum L.). Theor. Appl. Genet. 126, 3035–3048. http://dx.doi.org/10.1007/s00122-013-2191-9.

Cormier, F., Foulkes, J., Hirel, B., Gouache, D., Moënne-Loccoz, Y., Le Gouis, J., 2016. Breeding for increased nitrogen-use efficiency: a review for wheat (T. aestivum L.). Plant Breed. 135, 255–278. http://dx.doi.org/10.1111/pbr.12371.

Cullis, B.R., Smith, a. B., Coombes, N.E., 2006. On the design of early generation variety trials with correlated data. J. Agric. Biol. Environ. Stat. 11, 381–393. http://dx.doi.org/10.1198/108571106X154443.

Denmead, O.T., Shaw, R.H., 1960. The effects of soil moisture stress at different stages of growth on the development and yield of corn. Agron. J. 52, 272–274. http://dx.doi.org/10.2134/agronj1960.00021962005200050010x.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2006. ASReml User Guide Release 2.0. Hemel Hempstead, HP 1 1ES, UK.

Habash, D.Z., Bernard, S., Schondelmaier, J., Weyen, J., Quarrie, S.A., 2007. The genetics of nitrogen use in hexaploid wheat: n utilisation, development and yield. Theor. Appl. Genet. 114, 403–419. http://dx.doi.org/10.1007/s00122-006-0429-5.

Habier, D., Fernando, R.L., Garrick, D.J., 2013. Genomic BLUP decoded: a look into the black box of genomic prediction. Genetics 194, 597–607. http://dx.doi.org/10.1534/genetics.113.152207.

Hammer, G.L., Muchow, R.C., 1994. Assessing climatic risk to sorghum production in water-limited subtropical environments I: Development and testing of a simulation model. F. Crop. Res. 36, 221–234.

Hammer, G., Cooper, M., Tardieu, F., Welch, S., Walsh, B., van Eeuwijk, F., Chapman, S., Podlich, D., 2006. Models for navigating biological complexity in breeding improved crop plants. Trends Plant Sci. 11, 587–593. http://dx.doi.org/10.1016/j.tplants.2006.10.006.

Harrison, M.T., Tardieu, F., Dong, Z., Messina, C.D., Hammer, G.L., 2014. Characterizing drought stress and trait influence on maize yield under current and future conditions. Glob. Change Biol. 20, 867–878. http://dx.doi.org/10.1111/gcb.12381.

Henderson, C.R., 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics 31, 423. http://dx.doi.org/10.2307/2529430.

Heslot, N., Akdemir, D., Sorrells, M.E., Jannink, J.-L., 2014. Integrating environmental covariates and crop modeling into the genomic selection framework to predict genotype by environment interactions. Theor. Appl. Genet. 127, 463–480. http://dx.doi.

org/10.1007/s00122-013-2231-5.
Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58, 2369–2387. http://dx.doi.org/10.1093/jxb/erm097.
Holzworth, D.P., Huth, N.I., deVoil, P.G., Zurcher, E.J., Herrmann, N.I., McLean, G., Chenu, K., van Oosterom, E.J., Snow, V., Murphy, C., Moore, A.D., Brown, H., Whish, J.P.M., Verrall, S., Fainges, J., Bell, L.W., Peake, A.S., Poulton, P.L., Hochman, Z., Thorburn, P.J., Gaydon, D.S., Dalgliesh, N.P., Rodriguez, D., Cox, H., Chapman, S., Doherty, A., Teixeira, E., Sharp, J., Cichota, R., Vogeler, I., Li, F.Y., Wang, E., Hammer, G.L., Robertson, M.J., Dimes, J.P., Whitbread, A.M., Hunt, J., van Rees, H., McClelland, T., Carberry, P.S., Hargreaves, J.N.G., MacLeod, N., McDonald, C., Harsdorf, J., Wedgwood, S., Keating, B.a., 2014. APSIM – Evolution towards a new generation of agricultural systems simulation. Environ. Model. Softw. 62, 327–350. http://dx.doi.org/10.1016/j.envsoft.2014.07.009.
Jamieson, P.D., Semenov, M.A., 2000. Modelling nitrogen uptake and redistribution in wheat. F. Crop. Res. 68, 21–29. http://dx.doi.org/10.1016/S0378-4290(00)00103-9.
Jarquín, D., Crossa, J., Lacaze, X., Du Cheyron, P., Daucourt, J., Lorgeou, J., Piraux, F., Guerreiro, L., Pérez, P., Calus, M., Burgueño, J., de los Campos, G., 2014. A reaction norm model for genomic selection using high-dimensional genomic and environmental data. Theor. Appl. Genet. 127, 595–607. http://dx.doi.org/10.1007/s00122-013-2243-1.
Jeuffroy, M.-H., Bouchard, C., 1999. Intensity and duration of nitrogen deficiency on wheat grain number. Crop Sci. 39, 1385–1393.
Jeuffroy, M.H., Recous, S., 1999. Azodyn: a simple model simulating the date of nitrogen deficiency for decision support in wheat fertilization. Eur. J. Agron. 10, 129–144. http://dx.doi.org/10.1016/S1161-0301(98)00059-8.
Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huche, L., 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74, 397–4007.
Kohavi, R., 1995. A study of cross-Validation and bootstrap for accuracy estimation and model selection. International Joint Conference on Artificial Intelligence.
Löffler, C.M., Wei, J., Fast, T., Gogerty, J., Langton, S., Bergman, M., Merrill, B., Cooper, M., 2005. Classification of maize environments using crop simulation and geographic information systems. Crop Sci. 45, 1708. http://dx.doi.org/10.2135/cropsci2004.0370.
Lacaze, X., Roumet, P., 2004. Environment characterisation for the interpretation of environmental effect and genotype x environment interaction. Theor. Appl. Genet. 109, 1632–1640. http://dx.doi.org/10.1007/s00122-004-1786-6.
Lande, R., Thompson, R., 1990. Efficiency of marker-Assisted selection in the improvement of quantitative traits. Genetics 124, 743–756.
Laperche, A., Le Gouis, J., Hanocq, E., Brancourt-Hulmel, M., 2007. Modelling nitrogen stress with probe genotypes to assess genetic parameters and genetic determinism of winter wheat tolerance to nitrogen constraint. Euphytica 161, 259–271. http://dx.doi.org/10.1007/s10681-007-9433-3.
Lecomte, C., 2008. L'évaluation expérimentale des innovations variétales Proposition d'outils d'analyse de l'interaction génotype-milieu adaptés à la diversité des besoins et des contraintes des acteurs de la filière semences. Institut National Agronomique Paris-Grignon.
Lemaire, G., Gastal, F., 1997. N Uptake and Distribution in Plant Canopies. Diagnosis of The Nitrogen Status in Crops. Springer Berlin Heidelberg, Heidelberg, Germany, pp. 3–43. http://dx.doi.org/10.1007/978-3-642-60684-7_1.
Lobell, D.B., Hammer, G.L., Chenu, K., Zheng, B., McLean, G., Chapman, S.C., 2015. The shifting influence of drought and heat stress for crops in northeast Australia. Glob. Change Biol. 21, 4115–4127. http://dx.doi.org/10.1111/gcb.13022.
Lopes, M.S., Reynolds, M.P., Jalal-Kamali, M.R., Moussa, M., Feltaous, Y., Tahir, I.S.A., Barma, N., Vargas, M., Mannes, Y., Baum, M., 2012. The yield correlations of selectable physiological traits in a population of advanced spring wheat lines grown in warm and drought environments. F. Crop. Res. 128, 129–136. http://dx.doi.org/10.1016/j.fcr.2011.12.017.
Ly, D., Huet, S., Gauffreteau, A., Rincent, R., Touzy, G., Mini, A., Jannink, J-L., Cormier, F., Paux, E., Lafarge, S., Legouis J., Charmet, G., Whole-genome prediction of reaction norms to environmental stress in bread wheat (*Triticum aestivum* L.) by genomic random regression, F. Crop. Res. (in press).
Meuwissen, T.H., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819–1829.
Meynard, J.M., Limaux, F., 1987. Prévision des rendements et conduite de la fertilidsation azotée: cas du blé d'hiver. compte rendu de l'Académie Française d'Agriculture.
Ortiz, R., Sayre, K.D., Govaerts, B., Gupta, R., Subbarao, G.V., Ban, T., Hodson, D., Dixon, J.M., Iván Ortiz-Monasterio, J., Reynolds, M., 2008. Climate change: can wheat beat the heat? Agric. Ecosyst. Environ. 126, 46–58. http://dx.doi.org/10.1016/j.agee.2008.01.019.
Paux, E., Faure, S., Choulet, F., Roger, D., Gauthier, V., Martinant, J.P., Sourdille, P., Balfourier, F., Le Paslier, M.C., Chauveau, A., Cakir, M., Gandon, B., Feuillet, C., 2010. Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. Plant Biotechnol. J. 8, 196–210. http://dx.doi.org/10.1111/j.1467-7652.2009.00477.x.
Pollak, L., Corbett, J.D., 1993. Using GIS datasets to classify maize-growing regions in Mexico and central america. Agron. J. 85, 1133–1139.
R Development Core Team, 2003. R: a Language and Environment for Statistical Computing.
Reymond, M., Muller, B., Leonardi, A., Charcosset, A., Tardieu, F., 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. Plant Physiol. 131, 664–675. http://dx.doi.org/10.1104/pp.013839.soil.
Reymond, M., Muller, B., Tardieu, F., 2004. Dealing with the genotype x environment interaction via a modelling approach: a comparison of QTLs of maize leaf length or width with QTLs of model parameters. J. Exp. Bot. 55, 2461–2472. http://dx.doi.org/10.1093/jxb/erh200.
Richards, R., Rebetzke, G., Watt, M., Condon, A. (Tony), Spielmeyer, W., Dolferus, R., 2010. Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. Funct. Plant Biol. 37, 85–97. http://dx.doi.org/10.1071/FP09219.
Rutkoski, J.E., Poland, J., Jannink, J.-L., Sorrells, M.E., 2013. Imputation of unordered markers and the impact on genomic selection accuracy. G3 (Bethesda) 3, 427–439. http://dx.doi.org/10.1534/g3.112.005363.
Schwarz, G., 1978. Estimating the dimansion of a model. Ann. Stat. 6, 461–464.
Tardieu, F., 2003. Virtual plants: modelling as a tool for the genomics of tolerance to water deficit. Trends Plant Sci. 8, 9–14. http://dx.doi.org/10.1016/S1360-1385(02)00008-0.
Technow, F., Messina, C.D., Totir, L.R., Cooper, M., 2015. Integrating crop growth models with whole genome prediction through approximate bayesian computation. PLoS One 10, e0130855. http://dx.doi.org/10.1371/journal.pone.0130855.
Voltas, J., López-Córcoles, H., Borrás, G., 2005. Use of biplot analysis and factorial regression for the investigation of superior genotypes in multi-environment trials. Eur. J. Agron. 22, 309–324. http://dx.doi.org/10.1016/j.eja.2004.04.005.
Yin, X., Stam, P., Kropff, M.J., Schapendonk, A.H.C.M., 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. Agron. J. 95, 90–98. http://dx.doi.org/10.2134/agronj2003.0090.
Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421. http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x.
Zheng, B., Chenu, K., Fernanda Dreccer, M., Chapman, S.C., 2012. Breeding for the future: what are the potential impacts of future frost and heat events on sowing and flowering time requirements for Australian bread wheat (*Triticum aestivium*) varieties? Glob. Change Biol. 18, 2899–2914. http://dx.doi.org/10.1111/j.1365-2486.2012.02724.x.
Zheng, B., Chapman, S.C., Christopher, J.T., Frederiks, T.M., Chenu, K., 2015. Frost trends and their estimated impact on yield in the Australian wheatbelt. J. Exp. Bot. 66, 3611–3623. http://dx.doi.org/10.1093/jxb/erv163.
van Eeuwijk, F.A., Keizer, L.C.P., Bakker, J.J., 1995. Linear and bilinear models for the analysis of multi-environment trials: II. An application to data from the Dutch Maize Variety Trials. Euphytica 84, 9–22. http://dx.doi.org/10.1007/BF01677552.