Genomic selection in rice: empirical results and implications for breeding.

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15 Genomic Selection in Rice: Empirical Results and Implications for Breeding

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Introduction

Genomic selection (GS) has arisen from the conjunction of new high-throughput marker technologies and new statistical methods that allow the analysis of the genetic architecture of complex traits in the framework of infinitesimal model effects, instead of a model of limited numbers of quantitative trait loci (QTL) of varying effects. It refers to methods that use genome-wide dense markers, mainly single nucleotide polymorphisms (SNPs), for the prediction of genetic values with enough accuracy to allow selection on that prediction alone. It consists of (i) using all markers (often large numbers) simultaneously to build a model of genotype–phenotype relationships in a training population (TP), thus accounting also for linkage disequilibrium (LD) among markers, and (ii) using the model to predict the genomic estimate of breeding values (GEBV) of candidates in a breeding population (CP) (Meuwissen *et al.*, 2001; Heffner *et al.*, 2009). It extends the use of markers to breeding highly polygenic traits, such as yield, tolerance to abiotic stresses and resource-use efficiency.

The effectiveness of GS depends, among other factors, on the degree of correlation between the predicted GEBV and the true genetic value, i.e. the predictive ability of prediction (PA).

In practice, PA of genomic prediction is evaluated by the correlation between GEBV and the realized phenotype.

Prospects for the applications of GS in plant breeding have given rise to many studies using a simulation approach or empirical data to analyse the effects of factors that affect the PA of genomic predictions. These interrelated factors include the characteristics of TP and CP and relationship between the two populations, the characteristics of the target phenotypic trait, the characteristics of genotypic data (marker density, LD and minor allele frequency) and the prediction methods.

Characteristics of the TP that affect PA of genomic prediction include its size, its structure and its relatedness with the CP. Meuwissen (2009) showed that the size of TP depended on the effective size of the population (Ne) and the length of the genome (L) in Morgans (M). Likewise, predicting the breeding values of unrelated individuals required much larger TP than predicting individuals that are progeny of the TP. In the two cases, the optimal size of the TP would be $2 \times Ne \times L$. Simulation work of Meuwissen (2009) also showed that the optimal number of markers to predict breeding values of unrelated individuals would be $10 \times Ne \times L$. In the case of rice ($Ne = 50$, $L = 15$ M), these findings would

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imply a TP of 1500 individuals genotyped with 7500 markers, provided the markers are evenly distributed across the genome. The characteristics of the target trait reported to influence the PA of genomic predictions include its heritability, the number of QTL, the distribution of their allelic effects and frequencies, and the relative magnitude of additive and non-additive genetic variances (Hayes *et al.*, 2009; Jannink *et al.*, 2010; Howard *et al.*, 2014). Characteristics of genotypic data include marker density and distribution along the genome, the extent of LD and the minor allele frequency (MAF). The accuracy of different prediction methods depends on the above listed factors, i.e. the characteristics of the target trait, the density and distribution of the markers, the size and the structure of the TP, and the degree of relatedness between TP and CP (Heslot *et al.*, 2012; de los Campos *et al.*, 2015; Crossa *et al.*, 2017).

In order for GS to become a practical method for plant breeding, especially for major annual crops, at least three methodological issues need to be further addressed: (i) method for the establishment of the TP for making selection decisions in pedigree breeding within the progeny of biparental crosses; (ii) method to account for information available on genes/QTL involved in the determinism of complex traits; and (iii) method to account for genotype-by-environment interactions (GEI) as observed in multilocation trials of advanced breeding lines and/or in managed-environment experiments to assess tolerance to abiotic stresses (drought, extreme temperatures, salinity, etc.).

Here we present: (i) a review of empirical studies analysing factors that affect the PA of GEBV in rice, and (ii) some of the results of analysis of the above-mentioned issues (TP for pedigree breeding, accounting for GEI and trait-specific markers) by CIRAD's Genetic and Varietal Innovation team involved in the implementation of different rice breeding programmes worldwide.

Factors Affecting the Predictive Ability of Genomic Prediction in Rice

The empirical studies analysing factors that affect the predictive ability of genomic prediction in rice are listed in Table 15.1. In essence, individual empirical studies are not the most powerful tool for the analysis of effects of the inter-related factors that affect the predictive ability of genomic prediction. However, when congruent, the results of such studies provide valuable practical indications.

Characteristics of the training population

Characteristics of the TP that affect the PA of genomic predictions include its size, its structure and its relatedness with the CP. In the prediction experiments based on cross validation that we reviewed, the size of the TP was around four-fifths of the total number of entries available, which varied from 110 to 575. The resulting size of the TP was much below the theoretical value of 1500 for an *Ne* of 50. Indeed, as reported by Grenier *et al.* (2015), the *Ne* higher than 50 can be observed in a population of rather limited genetic base. These small sizes of the TP are probably one of the causes of the low PA of genomic prediction observed in all studies, even for phenotypic traits of high heritability, such as days to flowering (Table 15.1). The effect of population structure has been analysed in several studies. Using a diversity panel of 413 accessions composed of representatives of the *Oryza sativa* major genetic groups (*indica*, temperate *japonica*, tropical *japonica*, *aus*), Guo *et al.* (2014) analysed the effect of population structure. They reported that the most accurate predictions were obtained by stratified sampling of the training set, i.e. presence of representatives of each genetic group in both training and validation sets. Using the same population, Isidor *et al.* (2015) compared the PA of five algorithms of optimization of the TP (stratified sampling, mean of the coefficient of determination [CDmean], mean of predictor error variance [PEVmean], stratified CDmean [StratCDmean] and random sampling). In the presence of strong population structure, the stratified sampling showed the highest PA for all traits. Grenier *et al.* (2015) reached similar conclusions with breeding lines extracted from four synthetic populations of tropical *japonica*. Whatever the trait, identical PAs were obtained when the lines composing TP and CP were randomly sampled (without

Table 15.1. Genomic prediction studies conducted on rice.

Plant material	Phenotypic data[a]	Genotypic data[b]	Type of prediction experiment	Statistical methods[c]	Range of accuracy of GEBV[d]	Main conclusion[e]	Reference
Highly structured diversity panel of 413 accessions	15 traits of rather high heritability, including DTF, PH and protein content	36,901 SNPs (1 SNP per 10 Kb)	Cross validation	GBLUP, GBLUP-CPS	DTF: 0.44–0.66 PH: 0.50–0.75	Prediction accuracy was affected by the genomic relationship between TP and CP and by genomic heritability in the TP and CP.	Guo et al. (2014)
	8 traits including DTF, PH and GY	36,901 SNPs (1 SNP per 10 Kb)	Cross validation	GBLUP	DTF: 0.25–0.60 PH: 0.25–0.55 GY: 0.20–0.50	Maximizing the phenotypic variance captured by the training set is important for optimal performance. Stratified sampling of the training set ensures better accuracy than sampling based on the CDmean.	Isidro et al. (2015)
110 Asian cultivars	8 traits including DTF	3,071 SNPs	Cross validation	rr-BLUP, ENet, GBLUP, RKHS, RF, Lasso, BL, EBL, wBSR	DTF: 0.65–0.85	Reliability depended to a great extent on the targeted traits. Reliability was low when only a small number of cultivars were used for validation.	Onogi et al. (2015)
369 Elite breeding lines	6 traits including DTF and GY	73,147 SNPs	Cross validation	rr-BLUP, BL, RKHS, RF,	DTF: 0.35–0.65 PH: 0.15–0.35 GY: 0.10–0.30	Using one marker every 0.2 cM was sufficient for genomic selection in this collection of rice breeding material. rr-BLUP was the most efficient statistical method for GY where no marked effect of QTL was detected by GWAS.	Spindel et al. (2015)
343 S2:4 lines extracted from a synthetic population	DTF, GY and PH	8,336 SNPs 1 marker per 44.8 kb	Cross validation	rr-BLUP, GBLUP, Lasso, BL	DTF: 0.20–0.30 PH: 0.50–0.60 GY: 0.20–0.31	Accuracy of GEBV was affected by (i) relatedness between TP and CP and (ii) trait heritability and interaction between traits and all the other factors studied (prediction models, LD, MAF, composition of the TP).	Grenier et al. (2015)

Continued

Table 15.1. Continued.

Plant material	Phenotypic data[a]	Genotypic data[b]	Type of prediction experiment	Statistical methods[c]	Range of accuracy of GEBV[d]	Main conclusion[e]	Reference
575 F1 hybrids	8 traits including GY and PH	2,395,866 SNPs	Cross validation	GBLUP, GBLUP dominance effects	PH: 0.45–0.86 GY: 0.13–0.34	Model including the dominance effect provided more accurate prediction, particularly in multi-traits scenario for a low-heritability target trait, with highly correlated auxiliary traits.	Wang *et al*. (2017)
Diversity panel of 284 accessions + 97 elite lines derived from crosses between 31 accessions of the panel	DTF, NI and PW	43,686 SNPs	Progeny prediction	BayesB, GBLUP, RKHS	DTF: 0.58–0.65 PW: 0.55–0.62	The diversity panel provides accurate genomic predictions for complex traits in the progenies of biparental crosses involving members of the panel.	Ben Hassen *et al*. (2017)
			Progeny prediction and multi-environment prediction	Multi-environment models, GBLUP and RKHS	DTF: 0.70–0.92 PW: 0.55–0.85	Genomic prediction accounting for G×E interactions offers an effective framework for breeding simultaneously for adaptation to an abiotic stress and performance under normal cropping conditions in rice.	Ben Hassen *et al*. (2018)
Diversity panel of 280 accessions	DTF, GY and PH	250,000 SNPs	Cross validation and multi-environment prediction	Multi-environment models, GBLUP and RKHS	DTF: 0.60–0.93 GY: 0.40–0.85 PH: 0.55–0.85	Selection of trait-specific markers and multi-environment models improve genomic predictive ability in rice.	Bhandari *et al*. (2019)
Diversity panel of 225 accessions + 95 elite lines	Arsenic content in the flag-leaf (FL-As) and in the cargo grain (CG-As)	22,370 SNPs	Across populations	BayesB, GBLUP and RKHS	FL-As: 0.35–0.45 CG-As: 0.45–0.55	Genomic prediction offers the most effective marker assisted breeding approach for ability to prevent arsenic accumulation in rice grains.	Frouin *et al*. (2019)

[a]DTF: days to flowering; PH: plant height; GY: grain yield; NI: nitrogen index; and PW: panicle weight.
[b]SNP: single nucleotide polymorphism.
[c]GBLUP: genomic best linear unbiased prediction; rr-BLUP: ridge regression best linear unbiased prediction; ENet: elastic net; RKHS: reproducing kernel Hilbert space regression; RF: Random forest; BL: Bayesian lasso; EBL: extended Bayesian lasso; and wBSR: weighted Bayesian shrinkage regression.
[d]GEBV: genomic estimate of breeding value.
[e]TP: training population; CP: candidates in a breeding population; QTL: quantitative trait locus/loci; GWAS: genome-wide association studies; LD: linkage disequilibrium; and MAF: minor allele frequency.

considering their membership in one of the four subpopulations) or when TP and CP were composed of a balanced share of each of the four subpopulations. On the other hand, significantly lower PA was observed when the TP comprised all the lines of three of the subpopulations and the CP comprised all the lines of the fourth subpopulation.

The importance of relatedness between the TP and the CP was further confirmed by Ben Hassen *et al.* (2017) in their progeny prediction experiment. They showed that the size of the TP (284 accessions including 31 accessions that were the parents of CP) could be reduced to one-third without a significant decrease in PA, if the accessions making up this one-third were the most related to the accessions of the CP and included the 31 parents.

Effect of trait characteristics

In most rice genomic prediction experiments, the PAs for traits, such as days to flowering (DTF) and plant height (PH), are higher than that for grain yield (GY) or its proxy, panicle weight (Table 15.1). Almost all those experiments also report much higher heritability for DTF and PH than for GY, confirming the positive relationship between trait heritability and the PA of genomic prediction. For instance, Grenier *et al.* (2015) reported highly significant differences for PA between PH and GY, in their population of 343 S2:4 lines extracted from a synthetic *japonica* population. In their experiment, the heritability of PH and GY was 0.58 and 0.29, respectively. Likewise, Guo *et al.* (2014), using a diversity panel of 413 accessions, reported variation for PA ranging from 0.44 to 0.84 according to phenotypic traits and attributed the variation to differences in traits heritability. Less straightforward indications are available regarding the effect of the genetic architecture of the target trait. Using genotypic and phenotypic data from 300 elite *indica* lines, Spindel *et al.* (2015) reported lower PA of genomic predictions for GY compared with DTF. When the same data served for QTL mapping by GWAS, no large-effect QTL were detected for GY, whereas for DTF a single very large-effect QTL was detected.

Effect of characteristics of genotypic data (marker density, linkage disequilibrium and minor allele frequency)

The number of markers used in the rice genomic predictions experiments varied from 3071 to 2,395,866 (Table 15.1), representing densities of 8 to 560 SNPs per Mb, often much higher than the theoretical number of markers, 7500 (density of 19.5 SNPs per Mb). Analysis of the effect of marker density on the PA of genomic prediction by Spindel *et al.* (2015), using different subsets of the 73,147 SNPs available, showed that number of markers >7500 did not improve the PA of genomic predictions among a population of 363 elite *indica* lines. Grenier *et al.* (2015) reported that the largest PAs for PH and GY, among the 343 S2:4 tropical *japonica* lines, were achieved with a marker density of 13 SNPs per Mb. Bhandari *et al.* (2019) compared the PA of genomic prediction for GY, PH and DTF in a reference population of the International Rice Research Institute's (IRRI's) rainfed lowland breeding programme, using 15 incidence matrices, with the number of markers ranging from 3000 to 215,000 SNPs. The 15 matrices were established by combining five thresholds of LD ($r^2 \leq 0.25$, $r^2 \leq 0.50$, $r^2 \leq 0.75$, $r^2 \leq 0.90$ and $r^2 \leq 1$) with three thresholds of MAF ($\geq 2\%$, $\geq 5\%$ and $\geq 25\%$). Significant differences for PA were observed only for LD variation. Whatever the trait, the incidence matrices with LD value of $r^2 \leq 0.50$ led to a PA that was significantly higher than the one with $r^2 > 0.5$. It was concluded that a marker density of 27 SNPs per Mb was sufficient.

These variations of the optimal density of markers between different studies are most probably attributable to differences in the extent of LD within the plant material used in each study. The low optimal density of markers reported by Grenier *et al.* (2015) is probably attributable to the very large extent of LD ($r^2 = 0.59$ at pairwise distance between markers of 0–25 kb and $r^2 = 0.2$ at distance of 0.9–1.5 Mb) among the S3:4 lines belonging to the tropical *japonica* genetic group, known for its large LD. The much higher optimal density of markers reported by Bhandari *et al.* (2019) should be attributed to the rather low extent of LD ($r^2 = 0.103$ at pairwise distances between 0 and 25 kb) among the diversity panel composed of accessions belonging to the *indica* and *aus* genetic groups.

Effect of prediction methods

At least ten different methods were used across all studies. Genomic best linear unbiased prediction (GBLUP) was the most used method. Studies generally compared two to four methods, but Onogi *et al*. (2015) and Grenier *et al*. (2015) compared up to nine methods. Using eight phenological and morphological traits of 110 rice cultivars, mainly developed in Japan, Onogi *et al*. (2015) compared the performances of nine genomic prediction methods: GBLUP, reproducing kernel Hilbert space regression (RKHS), Lasso, elastic net, random forest, Bayesian lasso, extended Bayesian lasso, weighted Bayesian shrinkage regression and the average of all methods. GBLUP was the most accurate for one trait, RKHS and the average of all methods for two traits, and random forest for three traits. The methods were also compared through simulation. Conditions considered in the simulation experiments included factors related to traits (number of QTL and heritability) and to TP (size and extent of LD). The Bayesian lasso, the extended Bayesian lasso and the averaging methods showed stable performance across the simulated scenarios, whereas the other methods, except weighted Bayesian shrinkage regression (which performed poorly in most scenarios), had specific areas of applicability. Similar interactions between prediction methods and phenotypic traits were reported by Grenier *et al*. (2015) and Spindel *et al*. (2015).

Designing Training Population for Pedigree Breeding

An important question in the application of GS in the context of pedigree breeding among the progeny of biparental crosses is how the TP should be constructed to predict progeny from individual crosses. Early attempts to answer this question relative to maize breeding have explored mainly options where the TP was constructed from multiple related or unrelated small biparental families. For instance, the TP was composed of full-sib doubled-haploid (DH) lines that formed the CP (Goddard and Hayes, 2009; Lorenzana and Bernardo, 2009), or half-sib DH lines of the CP, or combinations of full-sib,

half-sib and non-related lines (Meuwissen, 2009; Riedelsheimer *et al*., 2013). More recently, a second approach was investigated in a number of crops. It consists of using a reference set to train the prediction model and in using this model to predict the performances of progenies from biparental crosses between members of the reference set. For instance, Hofheinz *et al*. (2012) used a reference set of 310 inbred sugar beet lines to predict the test cross value of 56 inbred progenies derived from eight crosses between six lines of the reference set, and reported average prediction accuracy of 0.79 for sugar content. Sallam *et al*. (2015) used a training set of 168 barley lines and five sets of 96 progeny lines representative of the breeding lines developed in five consecutive years (the training set included the parents of the progeny sets) and reported a prediction accuracy of around 0.50 for grain yield. Likewise, Gezan *et al*. (2017) used a panel representative of the University of Florida's strawberry breeding programme and sets of progenies derived from the circular mating of 31 members of the panel and reported a prediction accuracy ranging from 0.16 to 0.77, depending on the traits and model fitting method used.

In rice, the first empirical evaluation of performances of GS for pedigree breeding in the progeny of biparental crosses was reported by our team (Ben Hassen *et al*., 2017). The TP was represented by the reference population of the Consiglio Ricerche in Agricoltura (CRA; Vercelli, Italy) rice breeding programme composed of 284 accessions belonging to the *japonica* group, and the CP was composed of 97 advanced (F_5–F_7) inbred lines derived from 36 biparental crosses involving 31 accessions of the TP. The target traits for both TP and CP were DTF, panicle weight (PW) and the nitrogen balance index (NI). Six scenarios, representing different degrees of relatedness between the training set and the progeny set and different sizes of the training set, and three prediction methods, were considered (Table 15.2). In addition, among the six scenarios, three (S1, S2 and S3) were implemented with two different methods of selection of individuals in the training set. Under the first method (a), the training set was composed of accessions of the TP with the lowest average pairwise Euclidian distances with the 31 parental lines of the CP. Under the second method (b), accessions of the training set were

Table 15.2. Scenarios for genomic prediction across generations.

Scenario	Training population	Candidate population
S1a	31 parents	97 progenies
S1b	31 accessions selected using the CDmean method	
S2a	58 related accessions	
S2b	58 accessions selected using the CDmean method	
S3a	31 parents + 58 related accessions	
S3b	89 accessions selected using the CDmean method	
S4	31 parents + 252 accessions	
S5	252 accessions, excluding the parents	
S6	100 random sampling of 31 accessions, excluding the parents	

selected from among the 284 accessions of TP, using the CDmean method of optimization of the training set (Rincent *et al.*, 2012). The selection criterion under CDmean is based on the prediction error variance (PEV) derived from the realized additive relationship matrix–best linear unbiased prediction model. Under the CDmean method, a dedicated training set was selected for each phenotypic trait to account for trait heritability.

The predictive ability of our progeny predictions among the 97 advanced lines of CP was, on average, 0.51 for FL, 0.52 for NI and 0.54 for PW. However, it varied greatly with the composition of the training set (Fig. 15.1). The lower mean PA observed under scenario S1a, compared with scenarios S3a and S4, shows that, in addition to relatedness between the training set and CP, the size of the training set also matters, and even distant accessions can positively contribute to PA of predictions. The results of scenario S2a demonstrate that high PA can be achieved without the presence of the parental lines in the training set, provided it is composed of individuals closely related to the parental lines. The highest PA observed under scenario S4 suggests there is still room for optimization of the size and the composition of the training set; for instance, by selection of the closely related TP individuals, proportional to the contribution of each parental line to the composition of the CP. The almost equal PAs observed in S3a and S4 suggests that, beyond a certain threshold of size of the training set composed of accessions closely related to the CP, the inclusion of less closely related individuals does not improve the PA of prediction. Comparison of PA obtained under scenarios S1a, S2a and S3a, with the accuracy obtained with the corresponding CDmean-based

training set (S1b, S2b and S3b) showed almost no gain in predictive ability for DTF and PW, and an almost systematic gain in predictive ability of about 0.1 for NI (data not shown). This is probably attributable to the fact that our scenario for optimization of the training set was also based on relatedness between the training set and the parental lines of the CP. Lastly, but importantly, rather high accuracies (up to 0.7) were obtained among the full-sib lines of individual crosses. However, the number of progenies per cross and the number of crosses analysed were too small to draw general conclusions.

Similar results were obtained in another progeny prediction experiment targeting the improvement of the ability to prevent the accumulation of arsenic (As) in rice grain (Frouin *et al.*, 2019). The problem affects many rice-growing countries and is attributable to the presence of a high concentration of As in the paddy fields (Brammer and Ravenscroft, 2009; Meharg *et al.*, 2009). In this experiment, the TP was composed of 228 *japonica* accessions representing the European Rice Core Collection (Courtois *et al.*, 2012) and the CP was composed of 95 advanced breeding lines developed by the *Centre Français du Riz* (CFR) for the Camargue region, France. The concentration of As in the flag-leaf (FL-As) and in the cargo grain (CG-As) was investigated in a field trial with soil As concentration of $10\,mg\,kg^{-1}$. The predictive ability of genomic prediction across populations was evaluated under three scenarios of composition of the training set. Under the first scenario (S1), the training set included all 228 accessions of TP. Under S2, the training set was composed of 100 accessions of the TP, with the lowest average pairwise Euclidian distances with the 95 lines of the CP. Under S3,
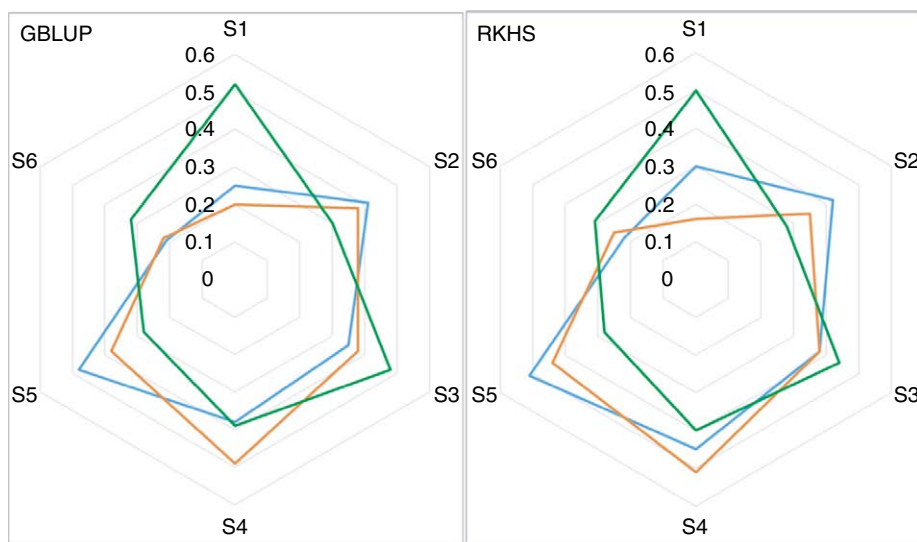
Fig. 15.1. Accuracy of genomic prediction of progeny phenotype for days to flowering (blue), nitrogen balance index (orange) and 100 panicles weight (green), obtained with two statistical methods, genomic best linear unbiased prediction (GBLUP) and reproducing kernel Hilbert space regression (RKHS), under six scenarios (S1–S6) of composition of the training set. See Table 15.2 for details of the six scenarios.

100 accessions of the training set were selected from among the 228 accessions of TP, using the CDmean method.

Under the S1 scenario, the PA of GEBV of the 95 lines of CP was, on average, 0.43 for FL-As and 0.48 for CG-As (Fig. 15.2). The PAs were much lower under S2, compared with S1. Under S3, the average PA was slightly higher than under S1 for CG-As (0.49), and much lower than under S1 for FL-As (0.34). The two prediction methods implemented (GBLUP and RKHS) provided similar levels of PA. However, there was some interaction between prediction methods and phenotypic traits. Translation of the PA observed for CG-As under S3, into average phenotypic performances of CP lines selected on the basis of their GEBV indicates that, for a selection rate of 10%, the difference in genetic gain between phenotypic selection and GEBV-based selection was approximately 5% (Frouin *et al.*, 2019).

Integrating Trait-specific Marker Selection

A common feature among almost all published studies on genomic prediction is the use of markers selected on the basis of a variety of criteria except association with the target trait. Prediction models are trained and GEBV are computed using the same set of markers for all the phenotypic traits the breeding programmes is targeting, whatever their genetic architecture. Zhang *et al.* (2010) simulated the predictive ability of different genomic prediction methods trained with a relationship matrix built with markers of equal effect (infinitesimal model) and with the same set of markers with weighted effects. Genomic prediction with markers of weighed effect had higher predictive ability. Similar improvement in predictive ability of genomic prediction for complex traits was reported by Zhang *et al.* (2010), when a trait-specific relationship matrix was built using results of genome-wide association studies (GWAS) available in the literature.

We evaluated the effectiveness of trait-specific marker selection, using a reference population of 204 rainfed lowland accessions with 148,916 SNPs and phenotyped for DTF, PH and GY under three managed environments, E1, E2 and E3. E1 corresponded to the standard lowland rice cultivation, without stress. E2 corresponded to standard lowland rice cultivation associated with application of drought stress at
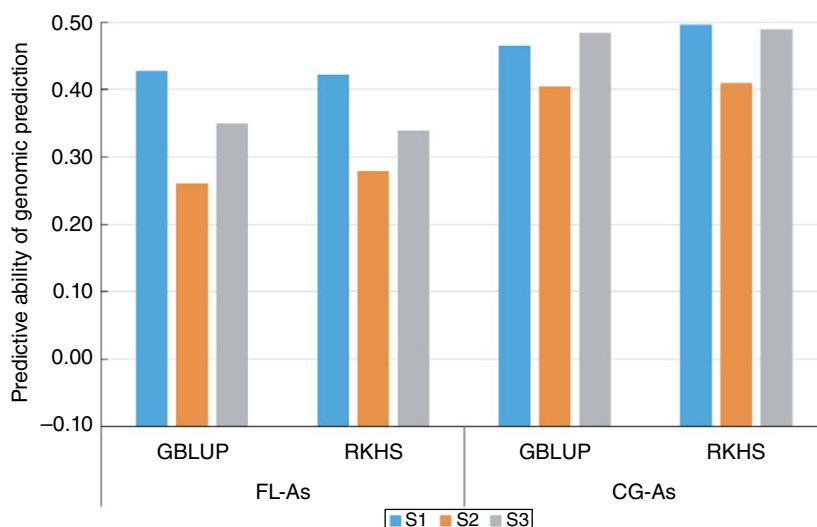
Fig. 15.2. Predictive ability of genomic prediction of the arsenic concentration in the flag leaf (FL-As) and in the cargo grain (CG-As), of the progeny population obtained with two statistical methods, genomic best linear unbiased prediction (GBLUP) and reproducing kernel Hilbert space regression (RKHS), under three scenarios of composition of the training set. S1: TP = 228 accessions; S2: TP = 100 accessions most related with the 95 lines of the CP; S3: TP = 100 accessions selected among the 228, using the CDmean method.

the reproductive stage. E3 corresponded to standard cultivation of upland rice with drought stress applied at the reproductive stage (Bhandari *et al.*, 2019). First, LD-based marker selection was performed with a threshold of $r \le 0.5$ and MAF $\ge 5\%$. It yielded 28,091 SNPs. Then, subsets of 28,091 trait-specific SNPs were selected for each trait under each drought environment, using results of GWAS performed with the complete genotypic dataset of 148,916 SNPs. Two scenarios of trait-specific marker selection were considered: (i) implementation of the GWAS experiments with the entire 204 accessions (GWAS-derived markers), and (ii) GWAS experiments with accessions (80% of the total) that did not participate in the corresponding model training process of the genomic prediction experiment (S-GWAS). The prediction models used were GBLUP and RKHS.

Whatever the phenotypic trait, the environment and the genomic prediction method, GWAS-derived markers resulted in systematically significantly higher PA (39% on average) than their LD-derived counterpart (Fig. 15.3). Interactions between the marker selection method, the prediction method and the environment were also often highly significant.

For instance, under E1, the average PA gains with GWAS-derived markers, compared with LD-derived markers, were 29% for DTF, 49% for GY and 40% for PH.

The S-GWAS-derived markers also resulted in systematically higher PA of genomic prediction (9% on average) than the corresponding LD-derived markers. The average gain in PA, over the LD-derived markers was 11% under E1, 10% under E2 and 6% under E3. The values of Fisher's least significant difference (LSD) indicate that the gains in PA for DTF, with S-GWAS-derived markers (12% on average), were significant under each of the three environments. For PH, the PA gains (9% on average) were significant only under E1 and E3. For GY, the PA gains (6% on average) were not significant under any of the three environments.

These significant gains in PA suggest that the genetic architecture of the three phenotypic traits considered in our study deviates, to a certain degree, from the infinitesimal model, and each trait is controlled by different sets of QTL. Gains in PA with S-GWAS-based marker selection are less important but more realistic in the context of actual breeding programmes where phenotypic data are not available for individuals
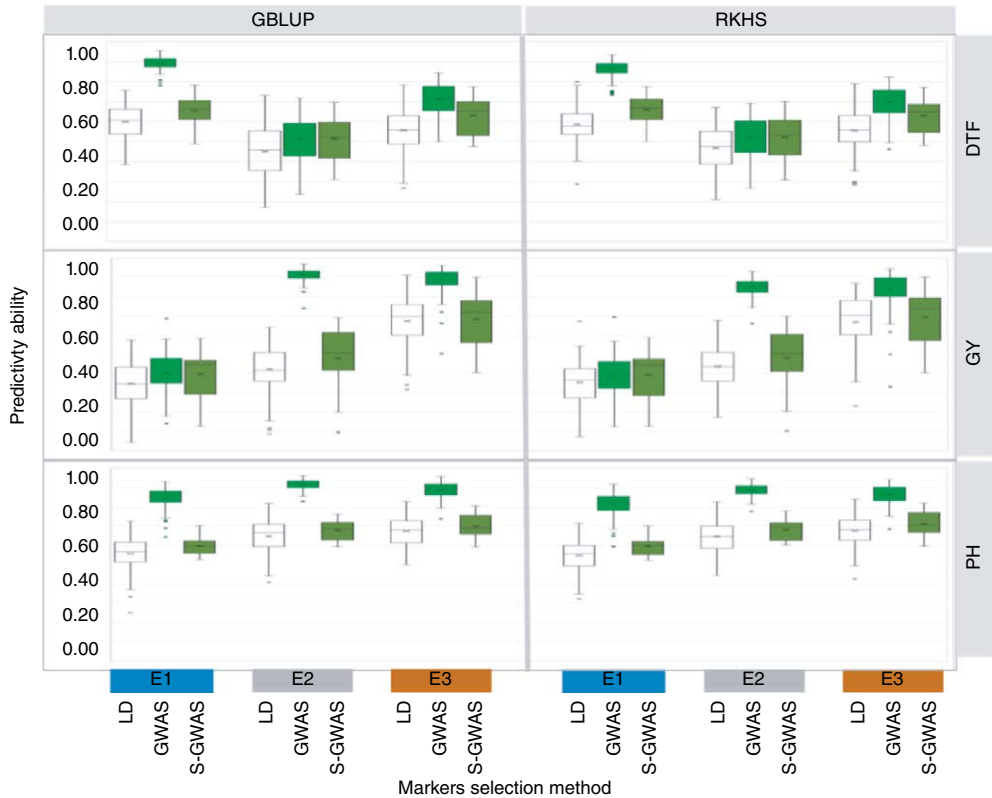
Fig. 15.3. Predictive ability of genomic prediction in cross validation experiments implemented with 28,091 single nucleotide polymorphism (SNP)-derived using three-marker selection methods: linkage disequilibrium (LD) between markers, white boxes; genome-wide association analysis with the target traits (GWAS), pale green boxes; and astringent genome wide association analysis with the target traits (S-GWAS), dark green boxes. The three traits, days to flowering (DTF), grain yield (GY) and plant height (PH), were phenotyped under three environments: rainfed lowland (E1), rainfed lowland with drought stress (E2) and upland with drought stress (E3). For each box, the mean (x) and median (horizontal bar) values are represented.

that are candidates for selection. These gains in PA, despite the limited size of our TP and the rather low heritability of the target traits, which limits the power to detect QTL, suggest that more substantial gains in PA could be achieved if more consolidated QTL information were available. Such consolidated QTL information can be built from the large number of publicly available QTL databases and SNPs detected in different linkage mapping and GWAS experiments. The QTL information could help build trait-specific genomic relationship matrices, based on the modified VanRaden genomic relationship matrix, with marker weights for each locus, as proposed by Zhang *et al.* (2014).

Accounting for GEI to Breed for Tolerance to Abiotic Stresses

In plant breeding, GEI interactions are usually assessed from multi-environment trials and expressed as a change in the relative performance of genotypes in different environments, with or without change in the ranking of the genotypes (Freeman, 1973; Cooper and Hammer, 1996). One specific case of GEI experiments is managed-environment trials that aim to assess the effect of particular environmental variables (e.g. abiotic stresses) or cropping practices (e.g. fertilizer, irrigation, etc.) that influence crop performance in the production environment concerned

(Cooper and Hammer, 1996). A still more specific case of GEI experiments is managed abiotic stress trials that aim to provide a measure of genotypic response to stress based on yield loss under stress compared with normal conditions.

Recently, a number of statistical frameworks that model GEI interactions for the purpose of genomic prediction have been proposed. First, the single-trait-single-environment GBLUP model was extended to a multi-environment context (Burgueño *et al.*, 2012). Then a GBLUP-type model using marker × environment interaction (M×E) was proposed (Lopez-Cruz *et al.*, 2015). Using a non-linear (Gaussian) kernel to model the GEI, the M×E-based approach was further developed (Cuevas *et al.*, 2016). The latest models go beyond the extension of single-environment models and propose multi-environment models based on genetic correlations between environments under two kernel methods, linear (GBLUP) and Gaussian kernel (GK) (Cuevas *et al.*, 2017). Application of these multi-environment models to data from multi-location trials of CYMMYT's maize and wheat breeding programmes confirmed their superiority over the single-environment models. The highest PA of prediction was observed with methods based on genetic correlations between environments.

In rice, we recently reported the first implementation of multi-environment genomic prediction, in the context of managed abiotic stress trials (Ben Hassen *et al.*, 2018). The above-described TP of 284 accessions was phenotyped not only under the conventional continuous flooding but also under the more water-sparing system of alternate wetting and drying. The PAs of multi-environment models were compared with the PA of single-environment models for DTF, NI and PW, under two cross validation strategies: CV1 where it is assumed that no phenotypic data are available for the CP accessions; and CV2, where it is assumed that phenotypic data for the TP accessions are available under one of the two environments. The extended GBLUP model (Lopez-Cruz *et al.*, 2015) and the extended RKHS model (Cuevas *et al.*, 2017) that integrate environmental effects were used to predict the GEBV with data from the two water-management systems. In the extended GBLUP model, the effects of m environments

and the effects of P markers are separated into two components: the main effect of the markers for all the environments and the effect of the markers for each environment. In the extended RKHS model, the mixed model is written as follows:

$$y = \mu + u + f + \varepsilon$$

where y is the response vector, μ is the vector with the intercept of each environment, u the random vector of individual genetic values, f the genetic effects associated with individuals that were not accounted for in component u, and ε the random vector of the error. u, f and ε are independent and normally distributed.

Average predictive abilities ranged from 0.48 to 0.96, depending, in decreasing importance, on the trait, the type of model (i.e. single-versus multi-environment), the cross validation strategy, the statistical model and the water-management system (Fig. 15.4). The average predictive ability was 0.77, 0.56 and 0.68 for FL, NI and PW, respectively. Whatever the trait or the water-management system, multi-environment models with the CV1 strategy performed similarly to the single-environment model. Conversely, the multi-environment models with the CV2 strategy outperformed single-environment models with an average gain of 0.27 for FL, 0.12 for NI and 0.20 for PW. Among the multi-environment prediction models, RKHS-2 performed systematically slightly better than GBLUP, with a gain in predictive ability of up to 0.02. We confirmed the superiority of multi-environment models over single-environment models, in the context of breeding for drought tolerance for the rainfed, lowland rice-growing environments (Bhandari *et al.*, 2019).

Implications for Breeding Rice

It is now widely accepted that the prediction ability for complex traits is better when using whole-genome marker prediction than when using a few markers targeting a few QTL (Crossa *et al.*, 2017; Hickey *et al.*, 2017). The general rules for the implementation of GS-based plant breeding are also well established, thanks to extensive simulation and empirical data-based analyses of the effects of factors that affect the
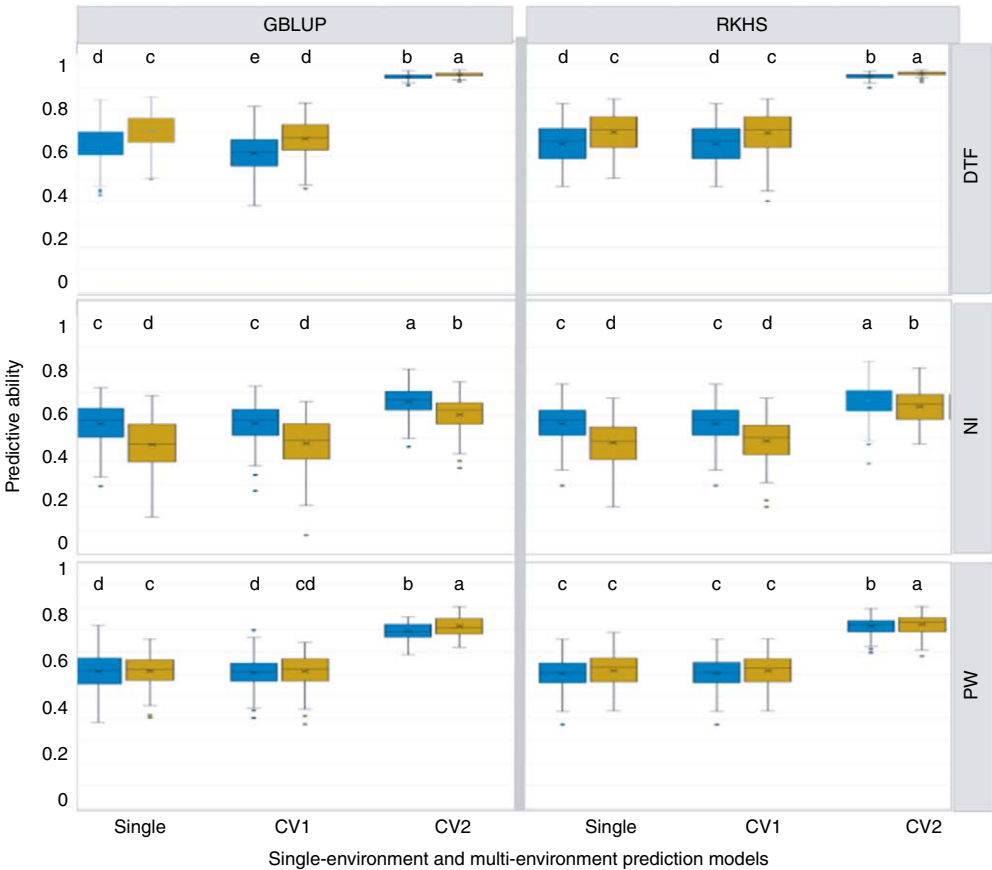
Fig. 15.4. Single-environment and multi-environment (CV1 and CV2) predictive ability in cross validation experiments in the reference population obtained with three statistical models (genomic best linear unbiased prediction [GBLUP], reproducing kernel Hilbert space regression [RKHS]). Continuous flooding and alternate wetting and drying water management conditions are in blue and orange, respectively. The three studied traits presented are: days to flowering (DTF), nitrogen balance index (NI) and panicle weight (PW). The letters in each panel represent the results of Tukey's HSD comparison of means and apply to each panel independently. The means differ significantly (*P* value < 0·05) if two boxplots have no letter in common.

PA of GEBV in all major crops, including rice. We recently undertook a number of studies using data from ongoing rice breeding programmes to draw decision-making rules for the most popular rice breeding scheme and for the rather common breeding objective of yield potential and tolerance to abiotic stresses.

Pedigree breeding within the progenies of biparental crosses extracted from a working collection or reference population (Fig. 15.5a) is the most common scheme for the improvement of complex traits in rice, as in many other autogamous crops (Bernardo, 2014). We found that using phenotypic and genotypic data from

the reference population to train the prediction model made it possible to predict performances among the first generation of advanced (F_5–F_7) progeny of a large set of biparental crosses. Thus, breeders can use this prediction approach in the framework of a pedigree breeding scheme. The approach can be associated with rapid generation advancement (in off-season nurseries or controlled environments), a practice aimed at reducing the length of the breeding cycle and hence accelerating the genetic gain per unit of time (Fig. 15.5b; Collard *et al.*, 2017). Specific optimization of the training set might be needed to obtain the best possible
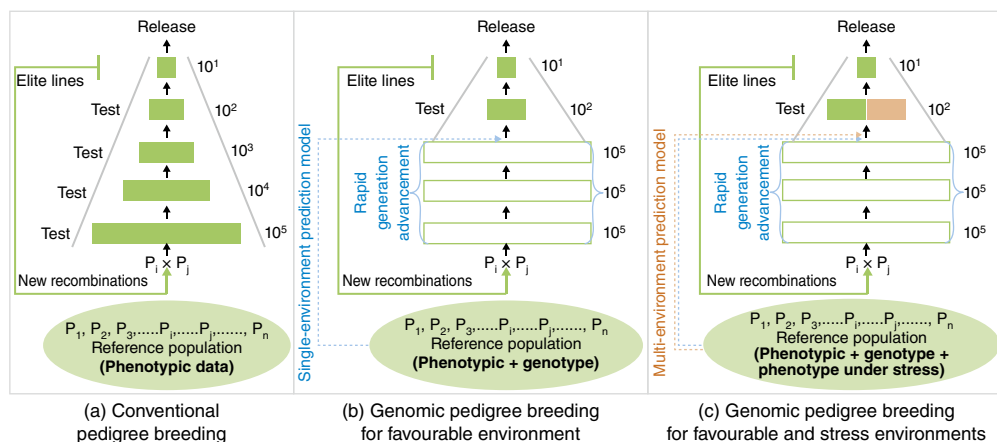
Fig. 15.5. Schematic representation of implementation of genomic selection in the framework of pedigree breeding. Shade of green colour of the reference population ellipses represents the increasing amount of phenotypic and genotypic data needed.

prediction accuracy for the progeny of each cross. The scheme can also be applied when homozygous lines from biparental crosses are produced by haplodiploidization, at least in the *japonica* genetic group, for which a high-throughput method is available (Alemanno and Guiderdoni, 1994). As the GS-based advanced line will then go through two or three cycles of phenotypic evaluation, the data collected will provide an opportunity to further refine the training model (Heffner *et al.*, 2010).

The high predictive ability of multi-environment, genomic prediction we observed, in two managed abiotic-stress case studies, paves the way for a new breeding option: conducting simultaneously direct and indirect selection for performance under both stress and non-stress environments. It requires a training population carefully phenotyped under both favourable environment and managed drought. While, in a first step of selection, the candidate population would be phenotyped only under the less expensive favourable environments (Fig. 15.5c), the selected candidate would be phenotyped under the target stress environment to ascertain their GEBV and to update the multi-environment prediction model for the next breeding cycle (Heffner *et al.*, 2009; Pszczola and Calus, 2016). The process can be implemented in the framework of the pedigree-breeding of progeny derived from biparental crosses between members of the reference population of the breeding programme, which is

used as a training population, as shown by Ben Hassen *et al.* (2018).

The effectiveness of trait-specific markers, in the context of multi-environment, model-based genomic prediction, deserves investigation using the simulation approach. Nevertheless, breeders should consider the inclusion of a limited share of trait-specific markers (especially for the most important target traits) when genotyping candidate populations.

The next step in harnessing the potential of GS in rice breeding would be the wider adoption of a population improvement scheme (Guimaraes, 2005) that allows gradual increase in frequency of favourable alleles and ensures better maintenance of QTL-marker LD along the breeding cycles, and thus the persistence of the prediction model. Such schemes associated with GS models that predict the line value of heterozygous individuals (Gallais, 1979) as early as S_0 generation would accelerate genetic gain by reducing the length of breeding cycles and providing an opportunity for increasing selection pressure. Our team has undertaken the development of such a model in the framework of the upland rice breeding programme we are running in collaboration with CIAT in Colombia, using a population improvement scheme (Grenier *et al.*, 2015). Another important step in harnessing the potential of GS in rice breeding would be connecting the genomic prediction models with ecophysiological crop models, such

as EcoMeristem (Luquet *et al*., 2006), and Sa-mara (Dingkuhn *et al*., 2016) to predict GEI for unobserved environments, and thus the per-formances and stability of the lines, based only on the genotypic information.

Summary

The increase in rice production needed to meet future demand requires new cropping systems and rice varieties with enhanced resource-use efficiency and adaptation to environmental stresses in the context of climate change. GS has the potential to accelerate the development of such varieties. We present a review of litera-ture analysing factors that affect the predictive ability of genomic prediction in rice, and an overview of the proof-of-concept studies con-ducted during the past 5 years by our team with the aim of providing rice breeders with tailored GS methods and tools. These studies involved two complementary breeding schemes (pedigree breeding and population improve-ment), mobilized different compartments of the rice genetic diversity (*indica*, tropical *japonica* and temperate *japonica*) and targeted a wide range of traits (yield potential, adaptation to alternate watering and drying, drought tolerance and exclusion of heavy metals). Issues addressed include training the population for making selec-tion decisions in pedigree breeding within the progeny of biparental crosses, accounting for information available on gene/QTL involved in the determination of complex traits and accounting for genotype-by-environment interactions. In the light of the results of these studies, we discuss a strategy for the implementation of genomic selection in the framework of pedigree breeding. We conclude on issues that need further simula-tion and empirical studies to fully harness the potential of GS.

References

Alemanno, L. and Guiderdoni, E. (1994) Increased doubled haploid plant regeneration from rice (*Oryza sativa* L.) anthers cultured on colchicine-supplemented media. *Plant Cell Reports* 13, 432–436.

Ben Hassen, M., Cao, T.-V., Bartholomé, J., Orasen, G., Colombi, C., *et al*. (2017) Rice diversity panel pro-vides accurate genomic predictions for complex traits in the progenies of biparental crosses involving members of the panel. *Theoretical and Applied Genetics* 131(2), 417–435. DOI: 10.1007/s00122-017-3011-4.

Ben Hassen, M., Bartholomé, J., Valè, G., Cao, T.-V. and Ahmadi, N. (2018) Genomic prediction accounting for genotype by environment interaction offers an effective framework for breeding simultaneously for adaptation to an abiotic stress and performance under normal cropping conditions in rice. *Genes, Genomes, Genomics* 8(9), 2319–2332. DOI: 10.1534/g3.118.200098.

Bernardo, R. (2014) *Essentials of Plant Breeding*. Stemma Press, Woodbury, Minnesota.

Bhandari, A., Bartholomé, J., Cao, T.-V., Kumari, N., Frouin, J., *et al*. (2019) Selection of trait-specific mark-ers and multi-environment models improve genomic predictive ability in rice. *PLoS ONE* 14(5), e0208871. DOI: 10.1371/JOURNAL.PONE.0208871.

Brammer, H. and Ravenscroft, P. (2009) Arsenic in groundwater: A threat to sustainable agriculture in South and South-east Asia. *Environment International* 35, 647–654.

Burgueño, J., de los Campos, G., Weigel, K. and Crossa, J. (2012) Genomic prediction of breeding values when modelling genotype × environment interaction using pedigree and dense molecular markers. *Crop Science* 52(2), 707–719.

Collard, B., Beredo, J., Lenaerts, B., Mendoza, R., Santelices, R., *et al*. (2017) Revisiting rice breeding methods – evaluating the use of rapid generation advance (RGA) for routine rice breeding. *Plant Pro-duction Science* 20(4) 337–352. DOI: 10.1080/1343943X.2017.1391705.

Cooper, M. and Hammer, G.L. (1996) *Plant Adaptation and Crop Improvement*. CAB International, Wallingford, UK.

Courtois, B., Frouin, J., Greco, R., Bruschi, G., Droc, G., *et al*. (2012) Genetic diversity and population struc-ture in a European collection of rice. *Crop Science* 52, 1663–1675.

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., *et al*. (2017) Genomic selection in plant breeding: methods, models, and perspectives. *Trends in Plant Science* 22(11), 961–975.

Cuevas, J., Crossa, J., Soberanis, V., Pérez-Elizalde, S., Pérez-Rodríguez, P., *et al*. (2016) Genomic prediction of genotype × environment interaction kernel regression models. *The Plant Genome* 9(3), 1–20. DOI: 10.3835/plantgenome2016.03.0024.

Cuevas, J., Crossa, J., Montesinos-López, O.A., Burgueño, J., Pérez-Rodríguez, P., *et al*. (2017) Bayesian genomic prediction with genotype × environment interaction kernel models. *Genes, Genomes, Genetics* 7(1), 41–53.

de los Campos, G., Hickey, J.M., Pong-Wong, R., Daetwyler, H.D. and Calus, M.P.L. (2015) Whole-genome regression and prediction methods applied to plant and animal breeding. *Genetics* 93, 327–345.

Dingkuhn, M., Kumar, U., Laza, M., Pasco, R. (2016) SAMARA: A crop model for simulating rice phenotypic plasticity. In: Ewert, F., Boote, K.J., Rötter, R.P., Thorburn, P. and Nendel, C. (eds) *Crop Modelling for Agriculture and Food Security Under Global Change. Abstracts*. International Crop Modelling Symposium, Berlin, Germany, 15–17 March 2016, pp. 45–46.

Freeman, G.H. (1973) Statistical methods for the analysis of genotype-environment interactions. *Heredity* 31(3), 339–354.

Frouin, J., Labeyrie, A., Boisnard, A., Sacchi, G.A. and Ahmadi, N. (2019) Genomic prediction offers the most effective marker assisted breeding approach for ability to prevent arsenic accumulation in rice grains. *PLoS ONE* 14(6), e0217516. DOI: 10.1371/journal.pone.0217516.

Gallais, A. (1979) The concept of varietal ability in plant breeding. *Euphytica* 28(3), 811–823.

Gezan, S.A., Osorio, L.F., Verma, S. and Whitaker, V.M. (2017) An experimental validation of genomic selection in octoploid strawberry. *Horticulture Research* 4, 16070. DOI: 10.1038/hortres.2016.70.

Goddard, M.E. and Hayes, B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nature Reviews Genetics* 10, 381–391.

Grenier, C., Cao, T.-V., Ospina, Y., Quintero, C., Châtel, M.H., *et al*. (2015) Accuracy of genomic selection in a rice synthetic population developed for recurrent selection breeding. *PLoS ONE* 10(8), e0136594. DOI: 10.1371/journal.pone.0136594.

Guimaraes, E.P. (2005) *Population Improvement: A Way of Exploiting the Rice Genetic Resources of Latin America*. FAO, Rome, pp. 56–94.

Guo, Z., Tucker, D.M., Basten, C.J., Gandhi, H., Ersoz, E., *et al*. (2014) The impact of population structure on genomic prediction in stratified populations. *Theoretical and Applied Genetics* 127, 749–762. DOI: 10.1007/s00122-013-2255-x.

Hayes, B.J., Bowman, P., Chamberlain, A., Verbyla, K. and Goddard, M. (2009) Accuracy of genomic breeding values in multi-breed dairy cattle populations. *Genetics Selection Evolution* 41, 51.

Heffner, E.L., Sorrells, M.E. and Jannink, J.-L. (2009) Genomic selection for crop improvement. *Crop Science* 49(1), 1–12.

Heffner, E.L., Lorenz, A.J., Jannink, J.-L. and Sorrells, M.E. (2010) Plant breeding with genomic selection: Gain per unit time and cost. *Crop Science* 50, 1681–1690. DOI: 10.2135/cropsci2009.11.0662.

Heslot, N., Sorrells, M.E., Jannink, J.-L. and Yang, H.P. (2012) Genomic selection in plant breeding: A comparison of models. *Crop Science* 52, 146–160.

Hickey, J.M., Chiurugwi, T., Mackay, I. and Powell, W. (2017) Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. *Nature Genetics* 49(9), 1297–1303.

Hofheinz, N., Borchardt, D., Weissleder, K. and Frisch, M. (2012) Genome based prediction of test cross performance in two subsequent breeding cycles. *Theoretical and Applied Genetics* 125, 1639–1645. DOI: 10.1007/s00122-012-1940-5.

Howard, H., Carriquiry, A.L. and Beavis, W.D. (2014) Parametric and nonparametric statistical methods for genomic selection of traits with additive and epistatic genetic architectures. *Genes, Genomes, Genomics* 4, 1027–1046.

Isidro, J., Jannink, J.-L., Akdemir, D., Poland, J., Heslot, N., *et al*. (2015) Training set optimization under population structure in genomic selection. *Theoretical and Applied Genetics* 128, 145–158. DOI: 10.1007/s00122-014-2411-y.

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

Lopez-Cruz, M., Crossa, J., Bonnett, D., Dreisigacker, S., Poland, J., *et al* (2015) Increased prediction accuracy in wheat breeding trials using a marker × environment interaction genomic selection model. *Genes, Genomes, Genetics* 5(4), 569–582.

Lorenzana, R.E. and Bernardo, R. (2009) Accuracy of genotypic value predictions for marker-based selection in biparental plant populations. *Theoretical and Applied Genetics* 120, 151–161.

Luquet, D., Dingkuhn, M., Kim Hae, K., Tambour, L. and Clément-Vidal, A. (2006) EcoMeristem, a model of morphogenesis and competition among sinks in rice: 1. Concept, validation and sensitivity analysis. *Functional Plant Biology* 33(4), 309–323. DOI: 10.1071/FP05266.

Meharg, A.A., Williams, P.N., Domako, E.A., Lawgali, Y., Deacon, C., *et al*. (2009) geographical variation in total and inoganic arsenic content of polished (white) rice. *Environmental Science & Technology* 43, 1612–1617.

Meuwissen, T.H.E. (2009) Accuracy of breeding values of 'unrelated' individuals predicted by dense SNP genotyping. *Genetics Selection Evolution* 41, 35.

Meuwissen, T.H.E., Hayes, B.J. and Goddard, M.E. (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(4), 1819–1829.

Onogi, A., Ideta, O., Inoshita, Y., Ebana, K., Yoshioka, T., *et al*. (2015) Exploring the areas of applicability of whole-genome prediction methods for Asian rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 128, 41–53. DOI: 10.1007/s00122-014-2411-y.

Pszczola, M. and Calus, M.P. (2016) Updating the reference population to achieve constant genomic prediction reliability across generations. *Animal* 10(6), 1018–1024. DOI: 10.1017/S1751731115002785.

Riedelsheimer, C., Endelman, J.F., Stange, M., Sorrells, M.E., Jannink, J.-L., *et al*. (2013) Genomic predictability of interconnected biparental maize populations. *Genetics* 194, 493–503.

Rincent, R., Laloë, D., Nicolas, S., Altmann, T., Brunel, D., *et al*. (2012) Maximizing the reliability of genomic selection by optimizing the calibration set of reference individuals: Comparison of methods in two diverse groups of maize inbreds (*Zea mays* L.). *Genetics* 192(2), 715–728. DOI: 10.1534/genetics.112.141473.

Sallam, A., Endelman, J., Jannink, J.L. and Smith, K. (2015) Assessing genomic selection prediction accuracy in a dynamic barley breeding population. *Plant Genome* 8, 1. DOI: 10.3835/plantgenome2014.05.0020.

Spindel, J., Begum, H., Virk, P., Collard, B., Redoña, E., *et al*. (2015) Genomic selection and association mapping in rice (*Oryza sativa*): Effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines. *PLoS Genetics* 11, e1004982. DOI: 10.1371/journal.pgen.1004982.

Wang, X., Li, L., Yang, Z., Zheng, X., Yu, S., *et al*. (2017) Predicting rice hybrid performance using univariate and multivariate GBLUP models based on North Carolina mating design II. *Heredity* 118, 302–310.

Zhang, Z., Liu, J., Ding, X., Bijma, P., de Koning, D.-J., *et al*. (2010) Best linear unbiased prediction of genomic breeding values using a trait-specific marker-derived relationship matrix. *PLoS ONE* 5(9), e12648. DOI: 10.1371/journal.pone.0012648.

Zhang, Z., Ober, U., Erbe, M., Zhang, H., Gao, N., *et al*. (2014) Improving the accuracy of whole genome prediction for complex traits using the results of genome wide association studies. *PLoS ONE* 9(3), e93017. DOI: 10.1371/journal.pone.0093017.