IMPLEMENTING GENOMIC SELECTION IN THE URUGUAYAN RICE BREEDING PROGRAM

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Uruguay is the top rice exporter in Latin America, and among the top eight high-quality rice exporters in the world. As Uruguayan farmers are reaching the yield potential of current varieties, new varieties with higher yield potential and milling quality must be developed. With the recent developments in genomics, rapid gains can be achieved through the integration of conventional breeding methods with genomic selection (GS). However, the best strategy for the efficient implementation of these techniques in specific breeding programs must be carefully analyzed. This work addresses some aspects of the implementation of GS in the Uruguayan breeding program and provides a model for other breeding programs of similar size and complexity. First, the impact on prediction accuracies of modeling genotype by environment interactions (G×E) was tested, and we found that modeling covariance structures that accommodate correlations between environments was beneficial for predicting yield and milling quality in both *indica* and *tropical japonica* rice. Different approaches for including weather information to assist genomic predictions were compared, and the impact of certain weather components on yield and milling quality were assessed. Modeling environmental effects by using weather variables provided an advantage in terms of prediction accuracy when predicting untested environments. Results from both genomic prediction and QTL×E analyses provided clues about the main weather variables affecting milling yield in rice grown in subtropical regions. We also tested the use of

genomic prediction for selection of parents in a *tropical japonica* rice breeding program. Starting from a population of 19 families, we evaluated several strategies for parental selection based on cross and progeny simulations to improve grain yield and milling quality traits. We also performed a field evaluation of the progeny from some of these crosses to compare genomic predictions to empirical data. Finally, a genome-wide association study was performed in order to find genomic regions associated with anther culture response in *tropical japonica* germplasm. The analysis identified 21 significant regions of the rice genome involved in callus induction and plant regeneration. Some of the same regions were reported in previous studies for anther culture response in rice. Future validations of the strategies outlined in this research will provide the foundation for future decision-making about the role that GS may play in the Uruguayan rice breeding program.

BIOGRAPHICAL SKETCH

Eliana Monteverde was born in Montevideo, Uruguay on October 27th 1982, to parents Nelly Dominguez and Juan José Monteverde. In 2006 Eliana graduated in Biology from the Facultad de Ciencias-Universidad de la República in Montevideo, with a major in Genetics. After graduation, she started working at the Department of Plant Biology, Facultad de Agronomía-Universidad de la República, as a lab technician, before starting her master studies in 2010. In 2012 she received a Master of Science degree in Genetics from Universidad de la República. With support from both Fulbright and Monsanto's Beachell-Borlaug scholarships, she moved to Ithaca in 2013, and joined Susan McCouch's lab to pursue a PhD in Plant Breeding and Genetics at Cornell University.

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CHAPTER 1:

INTRODUCTION

1.1 The importance of rice and its genetic diversity

Rice (*Oryza sativa* L.) is the primary source of nourishment for more than 1.6 billion people in the world. It is the staple food for most of Asia and many parts of Africa, providing between 20%-80% of dietary calories (Dawe et al., 2011), and is becoming increasingly important in Latin America. During the last half of the 20th century, applications of green revolution technology, along with the introduction of hybrid rice, rapidly improved rice productivity. However, over the last 50 years, rice yields have remained relatively constant while the demand for rice has increased, mostly in Asia and Africa, where consumers are also demanding higher quality grain (Zader et al., 2011; Yu et al., 2013).

Rice was domesticated independently in Asia and Africa, giving rise to *O. sativa* and *O. glaberrima*, respectively. Within *O. sativa*, two genetically distinct groups, *Indica* and *Japonica,* have been recognized since ancient times (Oka, 1988). Evidence suggests that these two varietal groups were domesticated from an ancestral species, *Oryza rufipogon*, a semi-perennial aquatic species of wild rice (Sun et al., 2002; Kovach and McCouch, 2008). During the domestication process, gene flow occurred between these domesticated populations despite geographic and biological barriers, with phenotypic consequences for both groups (Kovach et al. 2007; McCouch et al. 2012). In addition to the *Indica* and *Japonica* varietal groups, five major sub-populations can be distinguished (*aus* and *indica* within the *Indica* group; *tropical japonica* and *temperate japonica* within the *Japonica* group*;* and *aromatic,* an admixed

subpopulation that falls between *Indica* and *Japonica*) (Garris et al., 2005; Huang et al., 2012; Civan et al. 2015). *O. glaberrima* was domesticated from *O. barthii* in West Africa and forms an independent gene pool that does not cross readily with *O. sativa.* Together, these two species form the basis of modern breeding efforts in Asia, Africa and the Americas.

1.2 Rice production in Uruguay

Uruguay is located in South America (34.5° S, 56° W) and grows about 180,000 ha of irrigated long-grain rice in rotation with soybean and grazing pastures, which are mainly for beef cattle. Rice is grown mainly in the north, northeast, and eastern part of the country, with most of the production concentrated in the eastern region (Figure 1.1). Production requires high investments in a variety of inputs, including certified seed, labor, machinery, and infrastructure such as irrigation systems, silos, storage rooms, and roads.

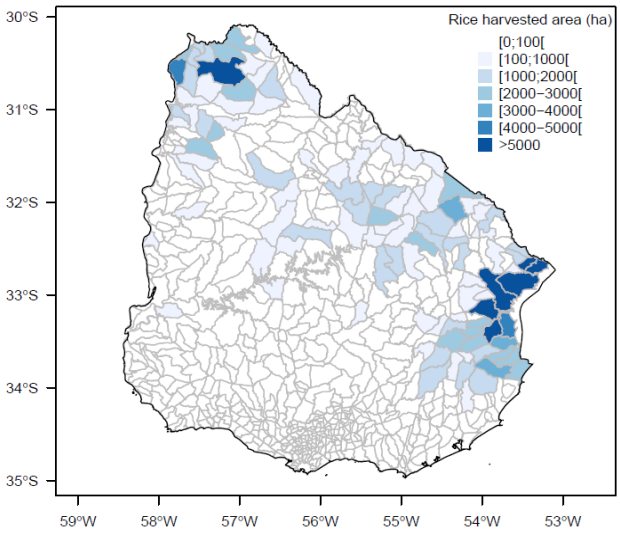

Figure 1.1: Rice harvested area distribution in Uruguay.
Source: MGAP, Census 2011.
(Taken from: www.yieldgap.org/uruguay).

The climate in Uruguay is subtropical, humid, and without large fluctuations in temperature and rainfall during the year. The maximum temperatures vary from 28 °C to 33 °C and the minimum temperatures from 2 °C to 9°C, which constrains the production of rice to one cycle per year. In the Eastern region, the growing season is short, running from October to April, with maximum temperatures of 13.9 and 26.2° C, respectively.

Rice is a major crop in Uruguay, and the adoption of optimized agronomic practices and production technologies over the last 40 years has fueled sustained increases in planted area, production and yield. Today, rice yields in Uruguay are among the highest in the world, consistently reaching 12000 tons/ha of top quality grain, and the country exports more than any other country in Latin America and is among the top eight rice exporters in the world (Battello, 2009).

The rice production system is based on a short and highly interactive value chain. Farmers are linked directly with the rice millers, who act as exporters. The two groups also share close connections with Uruguay's National Agricultural Research Institute (INIA). Furthermore, the rules regarding quality standards for rice are well-defined. Agronomists associated with the rice millers and representatives from INIA visit farmers' fields frequently. They share advice and the latest advances, but also gather information from farmers regarding research needs or inputs. The Rice Growers Association (ACA) and Rice Millers Association (GMA) negotiate rice prices each year under a frame agreement based on export prices, without government intervention. This means that the competitiveness of the rice sector relies on technology adoption, high grain yield and quality, and variety identity for all exported rice (Blanco et al., 2010).

1.3 Rice breeding program

Most of the rice research in Uruguay is conducted by the rice breeding program at INIA, which does studies on breeding, weeds, pest and disease management, irrigation, physiology, nutrition, soil management, and sustainability of rice cropping systems. Public rice breeding started in Uruguay in 1971. Early work in rice breeding started with *tropical japonica* varieties that were introduced from the Southern United States. *Indica* varieties were later introduced from the International Rice Research Institute (IRRI) and the International Center for Tropical Agriculture (CIAT), but since 1997 the Latin American Foundation for Irrigated Rice (FLAR) has been the main source of *indica* germplasm (Blanco et al. 2003; Blanco et al. 2010). Since the beginning of the program, the main breeding objectives have been focused on developing long- and medium-grain *tropical japonica* and *indica* varieties. Subsequently, other breeding objectives were introduced, such as developing Clearfield® varieties, short-grained *temperate japonica* cultivars, *aromatic* germplasm and hybrids. Three varieties released by INIA represent 85% of the total cultivated area today: El Paso 144 (43%, *indica* variety), INIA Olimar (26%, *indica* variety) and INIA Tacuarí (15%, *tropical japonica*). INIA licenses varieties to a consortium that represents seed production departments of the rice industry (GMA) and farmers (ACA). This arrangement ensures ample distribution of high quality seed for available varieties.

1.4 Selection methodology

Crossing: Each year an average of 100 crosses are carried out by the breeding program at Paso de la Laguna Experimental Station (UEPL), INIA-Treinta y Tres,

Uruguay (33°15′S, 54°25′W). Rice panicles are manually emasculated and pollinated in the lab.

Simplified individual selection: Crossing is followed by 5-6 cycles (i.e. years) of selection whereby lines are planted in 10m rows and undergo a process of visual selection. Lines that do not comply with the minimum agronomic and quality requirements (i.e. disease resistance, general morphology, grain type, photoperiod, etc.) will be discarded.

Preliminary, intermediate and advanced trials: About 500 F6 lines are planted in 6-row plots (3.5m long, spacing: 0.20m) in a randomized complete block design with 2-3 replications, and evaluated for yield, milling and cooking quality, and stem and sheath diseases. This process takes a year for each trial (3 years total), and 50% of the lines are discarded every year. Preliminary and intermediate trials are carried out in one location (Paso de la Laguna-Treinta y Tres, East), and advanced trials are carried out in two locations (Paso Farías-Artigas, North).

After advanced trials, the most prominent lines (usually ~4-5 per year) will be sent to the national network of evaluation of rice cultivars for further evaluation in additional locations for two years. The best lines (~1-2) will be sent to the national seed institute (INASE) for further evaluation and seed purification and multiplication. After this process, the chosen lines will then be released as new varieties.

1.5 Milling quality of Uruguayan rice

Most of the rice produced in Uruguay (~95%) is exported, with the main markets being Brazil, Peru, Iran, and Iraq. Milling quality represents a major breeding objective since it dictates the price in the market. The Uruguayan rice sector targets a high-value export market. Strict production and milling standards are applied to ensure

consistent quality. Breeding efforts to improve milling quality started in the early 1990s by introducing premium quality *temperate japonica* short grain varieties, mainly from Japan. These varieties were crossed with local *tropical japonica* material and new high quality and high yielding long and medium grain varieties have been released.

Rice is usually harvested at moisture contents between 13% to 22%. Harvested rice is also called "rough rice", since the hull is still attached. Rice above 14.4% moisture is not safe for long-term storage and must be dried to ≤14.4%. In the mill, dried rough rice is first cleaned to remove foreign material, and dehulled to produce "brown rice." Immediately after hulling, brown rice is milled to remove the bran layer and the germ by friction and/or abrasive action, which results in "polished" or "white" rice. The resulting white rice represents the "milled rice yield" (MY), and is expressed as a percentage of the original dried rough rice mass. White rice is comprised of head rice (defined as those kernels retaining three-fourths or more of their original length) and broken kernels. After the broken kernels are removed, only "head rice" remains. "Head rice yield" (HR) is the mass of head rice expressed as a percentage of the original rough rice mass. Broken kernels produced during milling are generally the result of immature, chalky, or fissured kernels; all of which are weak and break due to the forces applied to kernels when removing the bran. Chalky grains not only increase kernel breakage, but also affect consumer acceptance.

The rules regarding quality standards for rice are well defined. Farmers and millers sign a production contract every year, which includes a private agreement on the price the farmer will receive per weight of rough rice. Law 321/988 among other conditions, establishes the following parameters for penalizations and bonuses for milling quality:

Baseline values for milling quality:

- Milling yield: 70%
- Head rice: 58%
- Chalkiness: 6%

1. For milling yield (%): A bonus (or penalty) of 0.5% over the total price per each 1% above (or below) the baseline value.
2. For head rice: A bonus (or penalty) of 0.5% over the total price per each 1% above (or below) the baseline value.
3. For chalkiness: A penalty of 0.5% over the total price per each 1% above the baseline value.

1.6 The evolution of grain yield

Between 1930 and 1970 grain yields in Uruguay were relatively low and stable averaging 3.25 t ha^{-1}. The adoption of improved varieties and agronomic practices resulted in a rate of increase of rice yield of 90 kg ha^{-1} per year between 1970 and 2010 (Figure 1.2), with a rate of increase of 142 kg ha^{-1} per year for the 1990-2010 period. Today, the average yield is 9000 Kg ha^{-1}. The 36% yield increase between 1970 and 1990 was driven by the adoption of the variety Bluebelle from the United States, and improved agronomic practices. Subsequent replacement of Bluebelle by the high yielding Uruguayan cultivars El Paso 144, INIA Tacuarí and INIA Olimar led to a 67% yield increase between 1990 and 2010 (Blanco et al., 2010). Further increases in rice productivity will be hard to achieve because average yield obtained by farmers is about 70% of the estimated environmental yield potential (10.9 t/ha) (Pérez de Vida 2010, 2011).

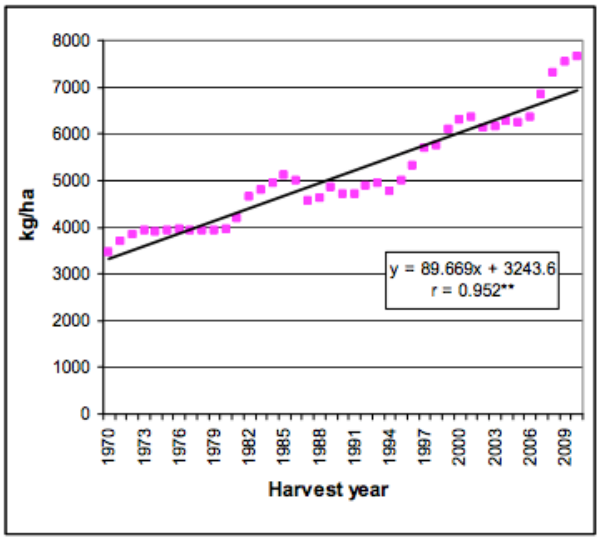

Figure 1.2: Evolution of grain yield in Uruguay from 1970 to 2010. Taken from Blanco et al., 2010.

The adoption of improved cultural practices throughout the country has narrowed the gap between yield in farmers' fields, and achievable yield potential. The percentage of farmers with yields higher than 9 t ha^{-1} increased from 4% in the 1990s to 18% in the 2000s (Blanco et al. 2010). This scenario represents a major challenge to rice breeders to accelerate cultivar development and further improve yield potential.

1.7 Accelerating genetic gain in the Uruguayan breeding program

One of the primary objectives of the current study is to evaluate ways to increase the rate of genetic gain in the Uruguayan rice breeding program. With only one rice cycle per year, the release of a new variety typically takes ~10 years. Considering the fundamentals of selection theory, we consult the formula for genetic gain (ΔG), also known as the "breeder's equation":

$$\Delta G = \left(ih^2\sigma_p\right)/L$$

where i is the selection intensity, h^2 is the trait heritability, σ_p is the square root of the phenotypic variance, and L is the length of the breeding cycle or generation interval (Ceccarelli, 2015). According to this equation, one of the simplest ways to increase genetic gain is to reduce the time of the breeding cycle.

There are several widely accepted breeding methods to develop new varieties of self-pollinated crops: pedigree, bulk, single seed descent (SSD), and doubled haploid (DH) (Mackill et al., 1996; Poelhman & Sleper, 1995). The pedigree method has been the most popular method used in rice breeding, followed by the bulk breeding method (Khush & Virk, 2005; Collard et al., 2013; Collard et al., 2017). The SSD method is used to fix lines during early generations, making them homozygous; the method generally refers to the repeated use of a single seed per line (i.e. one seed from a single plant) from a segregating population to advance the line one generation at a time by self-pollination until 'fixed lines' are generated (~F6 generation). Typically, plants are grown in a greenhouse facility and several generations or cycles are completed each year, advancing the material from the F2 to the F6 generation within a shorter time period that would be possibly under normal field conditions. For this reason, this method has also been referred to as 'rapid generation advance' (RGA). The other method used to rapidly fix segregating material is the DH method, which requires a tissue culture-based stage but can fix lines (i.e. make them genetically homozygous) in a single step. Both DH and RGA technologies can, in theory, boost the rate of genetic gain by reducing either the interval between generations, or the number of cycles needed to obtain fixed genotypes, upon which selection can be imposed.

Recent advances in genotyping technology have permitted cost-effective scoring of genome-wide marker polymorphisms (Davey et al., 2011; He et al., 2014), and offer new opportunities for novel breeding technologies that take advantage of detailed

genomic information. Among these new technologies, genomic selection (GS) has emerged as a promising method for plant breeding (Crossa et al. 2017). GS represents a strategy for selecting favorable individuals from a population using dense, genome-wide marker coverage to predict genomic estimated breeding values (GEBVs; Meuwissen et al., 2001). This method is based on the utilization of a model that has been previously "trained" on a subset of the study population ("training population") using both phenotypic and genome-wide genotypic data. The model differentially weights all SNPs in the dataset, reflecting the presence of both large and small-effect QTL in the training population, and enables calculation of GEBVs for the entire study population using only genotypic data. The GEBVs are then used to select the most desirable individuals from the study population for advancing in the breeding program. Because GS does not require phenotypic data on the study population in order to select candidates, it can shorten breeding cycle length and thereby increase gains per unit time (Heffner et al., 2010). In this study, we aimed to investigate the potential of GS to accelerate the rate of genetic gain and augment the success of the Uruguayan rice breeding program.

The widespread adoption of improved, high-quality varieties and sound agronomic practices have successfully positioned Uruguay among the top, high-quality rice exporters in the world. As farmers are beginning to realize the yield potential of current varieties, new varieties with higher yield potential and milling quality must be developed. Taking advantage of recent developments in genomics and bioinformatics, rapid genetic gains can theoretically be achieved via the integration of GS with conventional breeding methods, including the use of RGA and DH technologies. However, the best strategy for efficiently implementing these approaches in the existing Uruguayan breeding program must be carefully analyzed.

1.8 Thesis structure

This thesis deals with some critical aspects of the implementation of genomic selection in the Uruguayan breeding program. Chapter 2 evaluates the use of genomic selection when accommodating data from multiple environments. Prediction accuracies for yield and milling quality traits when modeling G×E using covariance structures that differ in their ability to borrow information among environments are compared, and results are discussed in the context of the application of these models in other small plant breeding programs. This chapter was published as an original research article in Crop Science: Monteverde E, JE Rosas, P Blanco, F Pérez de Vida, V Bonnecarrère, G Quero, L Gutiérrez, S McCouch. 2018. Multienvironment models increase prediction accuracy of complex traits in advanced breeding lines of rice. Crop Sci 58: 1519-1530.

Chapter 3 moves further into multi-environment modeling by exploring ways to fit weather information into genomic selection models, and to identify which weather components most significantly affect yield and milling quality traits in rice grown in subtropical environments. This chapter was submitted to G3, and has been accepted with minor revisions: Monteverde E, L Gutiérrez, P Blanco, F Pérez de Vida, JE Rosas, V Bonnecarrère, G Quero, S McCouch (2019). Integrating molecular markers and environmental covariates to interpret genotype by environment interaction in rice (*O. sativa L.*) grown in temperate areas. G3 (accepted pending revision).

In chapter 4, we evaluate the use of genomic prediction to select parents in a *tropical japonica* breeding program. Starting from a population of 19 F4 families, we discuss several strategies for parental selection based on cross and progeny simulations to improve grain yield and milling quality traits. We also performed a field evaluation of the DH progeny from some of these crosses to compare GS predictions to empirical

data from the field. The implications of using DH lines in the Uruguayan breeding program are also discussed.

Given the high variability found for anther culture response in our breeding lines, Chapter 5 applies a GWAS analysis to identify QTL associated with the variability of anther culture response in the Uruguayan *tropical japonica* germplasm.

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CHAPTER 2:

MULTI-ENVIRONMENT MODELS INCREASE PREDICTION ACCURACY OF COMPLEX TRAITS IN ADVANCED BREEDING LINES OF RICE (*O. sativa*)

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My contributions to this paper: I generated all the genotypic data, performed all of the analyses, and wrote the paper.

Contributions by others: The two breeding populations were originally developed in Uruguay under the direction of L. Gutiérrez and V. Bonnecarrère for a GWAS project on grain traits as reported in Quero et al. (2018). The phenotypic data was generated by P. Blanco and F. Pérez de Vida.

2.1 ABSTRACT

Genotype by environment interaction (G×E) is the differential response of genotypes in different environments and represents a major challenge for breeders. Genotype-by-year-interaction (G×Y) is a relevant component of G×E and accounting for it is an important strategy for identifying lines with stable and superior performance across years. In this study, we compared the prediction accuracy of modeling G×Y using covariance structures that differ in their ability to accommodate correlation among environments. We present the use of these approaches in two different rice (*Oryza sativa* L.) breeding populations (*indica* and *tropical japonica*), for predicting grain yield (GY), plant height (PH) and three milling quality traits: milling yield (MY), percent head rice (PHR), and grain chalkiness (GC), under different cross-validation scenarios. We also compared model performance in the context of global predictions (i.e. predictions across years). Most of the benefits of multienvironment models come from modeling genetic correlations between environments when predicting performance of lines that have been tested in some environments but not others (CV2). For predicting

the performance of newly developed lines (CV1) modeling between environment correlations has no effect compared to considering environments independently. Response to selection of multienvironment models when modeling covariance structures that accommodate covariances between environments was always beneficial when predicting the performance of lines across years. Also, we show that for some traits high prediction accuracies can be obtained in untested years, which is important for resource allocation in small breeding programs.

2.2 INTRODUCTION

Improving selection efficiency and rate of genetic gain in plant breeding is essential to meet the growing global demand for food, feed, fiber and fuel. Rice is a staple food that supplies essential calories for approximately a quarter of the world's population. In some of the major rice-consuming countries there has been a shift from quantity to quality in terms of consumer demand for rice (Yu et al., 2013; Hsiaoping, 2005; Zader, 2011). Thus, grain quality must be a major breeding objective not only for high quality rice exporting countries, but also for most major rice-consuming countries. An increase in rice production has to be concomitant with an increase in grain quality.

Common to many traits of interest to breeders, yield and grain quality traits are controlled by many genes. The most promising way to improve yield and quality traits is through the application of new genomic tools and statistical approaches such as Genomic Selection (GS) (Meuwissen et al., 2001) that is designed to enhance the rate of genetic gain for complex traits (Heffner et al., 2009; Jannink et al., 2010). Initially, GS methods were focused on single-trait, single-environment analyses, but one major challenge for breeders is the differential response of genotypes in different environments, known as genotype by environment interaction (G×E). G×E can affect

trait heritability and line ranking over environments, frequently affecting decision-making. For this reason, GS approaches capable of modeling G×E have increasingly gained popularity.

Several models accounting for G×E have been proposed to characterize the mean response of genotypes across environments, with the objective of developing locally adapted genotypes. Regression analysis (Yates and Cochran, 1938; Finlay and Wilkinson, 1963) measures the relative genotypic performance and stability across multiple locations. Multiplicative models combine univariate and multivariate approaches for reducing the dimensions of data and facilitating interpretation and selection decisions (Gauch, 1988; Smith et al., 2005). The most commonly used multiplicative model is the additive main effect and multiplicative interaction model (AMMI; Gauch, 1992) that combines ANOVA for estimating genotype and environment main effects and singular value decomposition (SVD) to partition the G×E component. The use of mixed model approaches was later introduced to improve flexibility and to allow different correlation structures among environments (Piepho, 1998; Piepho and Möring, 2005; Burgueño et al., 2008; Burgueño et al, 2012).

Modeling covariance matrices to account for G×E allows borrowing information among correlated environments. Curnow (1998) showed that using correlated information about treatment effects was beneficial for selecting the best treatment effects. Atlin (2000) took up this idea to study the response to selection in a subdivided target region. Piepho and Möring (2005) applied this approach on a BLUP framework and showed that when there are different target regions in a breeding program, models that fit a genetic correlation across sub-regions showed better performance than only using data from the particular target region or using a global average value for genotypic performance.

Mixed models that allow the incorporation of a genetic covariance matrix calculated either from pedigree or molecular marker data, rather than assuming independence among genotypes, also improve the estimation of genetic effects (VanRaden, 2008). The advantage of using genetic covariance matrices in G×E mixed models is that the model relates genotypes across locations even if lines are not present in all locations. Burgueño et al. (2012) were the first to combine genetic and environmental covariance matrices using different covariance structures to model the environmental component. They showed that modeling both genetic and environmental covariances improved predictions in the target environment for genotypes that have already been evaluated in correlated environments.

If we consider G×E in the context of genotype-by-year interactions (G×Y), identifying lines with stable and superior performance across years is an important challenge for breeders. In this context, breeders are not only interested in predicting performance in future seasons (local predictions), pointing to the importance of repeatable G×Y interactions, but also in predicting rankings across years, taking G×Y as part of the noise (global predictions). The ability of models to predict G×Y in this context is crucial for crop improvement in current and future climate change scenarios. This is likely to be especially beneficial for rice breeding programs that are focused on high yield and quality, given that both components are highly affected by environmental conditions (Cameron and Wang, 2005; Chen et al., 2012; Lyman et al. 2013).

The objective of our study was to compare the effect on prediction accuracy of different types of prediction models for G×Y interaction. The models compared in this study differ in whether they allow borrowing of information across years or not, for both local and global prediction scenarios. We also compared the effect of using two different kernel regression approaches to construct the genetic covariance matrix: a linear kernel

(GBLUP) which assumes that allelic effects are additive, and a non-linear Reproducing Kernel Hilbert Spaces (RKHS) approach, which takes into account non-additive as well as additive effects between genes. We present the use of these approaches in two different rice-breeding populations (*indica* and *tropical japonica*), which have been evaluated for five different traits across three years in a single location.

2.3 MATERIALS AND METHODS

2.3.1 Phenotypic data

Experimental design

The data used in this study belongs to the National Institute of Agricultural Research (INIA-Uruguay) and consists of three years of phenotypic data from field trials (2010, 2011, 2012) collected from 309 *tropical japonica* and 327 *indica* elite rice lines in one location, Paso de la Laguna Experimental Station (UEPL), Treinta y Tres, Uruguay (33°15′S, 54°25′W). These two breeding populations were originally developed for a GWAS project for grain me traits, and results from that study have been reported by Quero et al. (2018).

Each year, the *tropical japonica* and *indica* populations were planted independently in replicated trials in six-row plots using an augmented randomized complete block design with two or three replications, and included two Uruguayan cultivars as checks for each population (El Paso 144 and INIA Olimar for the *indica* dataset, and EEA-404 and INIA Tacuari for the *tropical japonica* dataset, http://www.inase.org.uy/Sitio/RegistroNacionalCultivares/Default.aspx). Trials were conducted under irrigated conditions using appropriate pest and weed control.

The agronomic traits analyzed each year were grain yield (GY of paddy rice in

kilograms per hectare) and plant height (PH measured in cm from the soil surface to the tip of the flag leaf). The grain quality traits measured were yield after milling (MY measured in grams, as the weight of grain recovered after milling divided by the weight of rough rice before milling, using a 100g sample of rough rice), percentage of head rice recovery (PHR measured in grams, as the weight of whole milled kernels, divided by the weight of rough rice, using a 100g sample of rough rice), and the percentage of grain chalkiness (GC measured as % of chalky kernels in a subsample of 50 g of total milled rice, where the area of chalk -core, white back or white belly- was larger than half the kernel area based on visual inspection) (Supplemental Table S1). The grain quality traits were measured as described in Quero et al. (2018) and in Siebenmorgen et al. (2012).

Genotype data for both datasets were obtained using genotyping-by-sequencing (GBS). SNP calling was performed using the TASSEL 3.0 GBS pipeline (Bradbury et al. 2017), and SNPs were aligned to the Nipponbare reference genome MSU version 7.0 using Bowtie 2 (Langmead and Salzberg, 2012). Imputation of missing data was performed with the FILLIN algorithm implemented in TASSEL 5.0 (Swarts et al. 2008) for both datasets separately. The GBS datasets were filtered to retain markers with <50% missing data after imputation, and a minor allele frequency MAF< 0.05, as reported by Quero et al. (2018). The final *indica* dataset contained 92,430 markers and the *tropical japonica* dataset had 44,598 markers. The final datasets was transformed to numeric coding (-1, 0, 1 for class I homozygotes, heterozygotes, and class II homozygotes respectively) to facilitate statistical analysis.

2.3.2 Statistical models

The Best Linear Unbiased Estimators (BLUEs) were calculated on a per line basis for each year/trait separately. The model used to calculate BLUEs for each year is:

$$y_{ijkl} = \mu + b_i + g_j + r_{k(i)} + c_{l(i)} + \varepsilon_{ijkl}$$

where y_{ijkl} is the trait score, μ is the overall mean, b_i is the random block effect with $b_i \sim N\left(0,\sigma_b^2\right)$, g_j is the genotypic effect, $r_{k(i)}$ and $c_{l(i)}$ are the random row and column effects nested within blocks with $r_{k(i)} \sim N\left(0,\sigma_r^2\right)$ and $c_{l(i)} = N\left(0,\sigma_c^2\right)$, and ε_{ijkl} are the model residuals with $\varepsilon_{ijkl} \sim N(0,\sigma_\varepsilon^2)$ where σ_ε^2 is the error variance.

Broad–sense heritability for each environment was calculated on a per line basis as $H^2 = \sigma_g^2 / \left(\sigma_g^2 + \frac{\sigma_e^2}{r}\right)$, where σ_g^2 is the variance among rice lines, and r is the number of replicates.

Prediction models

The general model for the G×E interaction is given by the following equation that fits the data for n lines $(i = 1,\ldots,n)$ in m environments $(j = 1,\ldots,m)$ in the following way:

$$\boldsymbol{y} = \boldsymbol{\mu} + \boldsymbol{u} + \boldsymbol{\varepsilon}$$

where $\boldsymbol{y} = \left(\boldsymbol{y}_1',\ldots,\boldsymbol{y}_j',\ldots,\boldsymbol{y}_m'\right)'$ is the vector of the response variable of the BLUE values for the jth environment; $\boldsymbol{\mu} = \left(\mathbf{1}_{n_1}'\mu_1,\ldots,\mathbf{1}_{n_j}'\mu_j,\ldots,\mathbf{1}_{n_m}'\mu_m\right)'$, where $\boldsymbol{\mu}$ represents the vector of intercepts for each environment and $\mathbf{1}_{nj}$ is a vector of ones of order n_j; $\boldsymbol{u} = \left(\boldsymbol{u}_1',\ldots,\boldsymbol{u}_j',\ldots,\boldsymbol{u}_m'\right)'$ is the random vector of genetic values with $\boldsymbol{u}_j \sim N\left(0,\boldsymbol{\Sigma}\right)$, where $\boldsymbol{\Sigma}$ is a genotypic covariance matrix between environments; and $\boldsymbol{\varepsilon} = \left(\boldsymbol{\varepsilon}_1,\ldots,\boldsymbol{\varepsilon}_j,\ldots,\boldsymbol{\varepsilon}_m\right)'$ is the vector of random residuals with $\boldsymbol{\varepsilon} \sim N\left(0,\boldsymbol{R}\otimes\boldsymbol{I}_n\right)$. In this study, $\boldsymbol{R}$ takes a diagonal

form, where each environment has its own residual variance. $\boldsymbol{I}_n$ is an identity matrix of order n, and $\otimes$ is the Kronecker product.

This general mixed model can be used to predict genotypes that have not been evaluated in either a particular environment or in any environment, via the $\boldsymbol{\Sigma}$ covariance matrix, that allows borrowing information from evaluated lines and/or environments. In this study, we discuss two different models for the structure of $\boldsymbol{\Sigma}$ (diagonal and unstructured) and their implications for predicting genotype performance under different scenarios.

Models for $\boldsymbol{\Sigma}$

The genetic covariance matrix $\boldsymbol{\Sigma}$ can be decomposed into a genomic related matrix K and an environment related matrix U_E. When the number of individuals is the same in each environment $\left(n_j = n_{j'} = n\right)$, as is the case of this study, $\Sigma = U_E \otimes K$, where $\otimes$ is the Kronecker product.

There are several structures for modeling matrix U_E in mixed models (Burgueño et al. 2012; Malosetti, et al. 2016). In this study, we will use two contrasting structures for the U_E matrix that differ as to whether the information between environments can be borrowed or not. The diagonal (U_{DIAG}) structure fits a separate variance component per environment assuming no genetic correlations between environments. The diagonal values of the diagonal matrix are thus variance components for each environment, with off-diagonal elements equal to zero.

The most general way to model covariances between environments is the unstructured $m \times m$ covariance matrix (U_{UN}):

$$U_{UN} = \begin{bmatrix} \sigma^2_{u_1} & \cdots & \sigma_{u_1 u_m} \\ \vdots & \ddots & \vdots \\ \sigma_{u_m u_1} & \cdots & \sigma^2_{u_m} \end{bmatrix}$$

where the j^{th} diagonal element of the variance-covariance matrix is the additive genetic variance $\sigma^2_{u_m}$ within the j^{th} environment, and the off-diagonal element $\sigma_{u_j u_{j'}}$ is the genetic covariance between environments *j* and *j'*.

Genomic kernels

The matrix K is a genetic relationship matrix that can be derived either from pedigree information or from molecular marker data. In this study, we compared the effect of two different kernels K on genotype predictions. Model (1) uses the linear kernel, also known as GBLUP where $K = G = (XX'/p)$, where X represents the matrix of p centered and standardized molecular markers in the j^{th} environment.

Model (2) uses the Gaussian Kernel (GK) as covariance structure. The GK used in this work was $K(x_i, x_{i'}) = \exp(-hd^2_{ii'})$ where $d^2_{ii'}$ is the Euclidean distance between individuals i and i' based on molecular markers, and h is the bandwidth parameter that controls the rate of decay of the values of K. Following Crossa et al. (2010), we consider $h = 2/d^2_m$, where d^2_m is the sample median $d^2_{ii'}$.

Model implementation

All the models tested in this work were fitted using R (R Core Team, 2016). The ME models were fitted using the Multi Trait Model (MTM) software developed by de los Campos and Grüneberg (2016) that also uses a Bayesian approach and assumes that $\boldsymbol{K}$ is the same in all environments. This package assumes that the prior distribution for

$\boldsymbol{u}$ is multivariate normal with mean zero and a variance-covariance matrix $U_E \otimes K$, that is, $p(\boldsymbol{u} \mid \boldsymbol{U_E}, \boldsymbol{K}) = N(\boldsymbol{u} \mid 0, \boldsymbol{U_E} \otimes \boldsymbol{K})$. The intercepts μ_j are assigned a flat prior, and the prior distribution for $\boldsymbol{U_E}$ is an inverse Wishart $p(\boldsymbol{U_E} \mid \boldsymbol{S_0}, df_0) = W^{-1}(\boldsymbol{S_0}, df_0)$, where the parameters are a scale matrix S_0 equal to an identity matrix of order m (number of environments), and degrees of freedom $df_0 = m$. The error variances for each environment $\left(\sigma^2_{\varepsilon_j}\right)$ are the diagonal elements of $\boldsymbol{R}$ and are assigned a scaled inverse χ^2 distribution with degrees of freedom and scaled factor equal to 1.

The MTM package uses Markov chain Monte Carlo (MCMC) and the Gibbs sampler to fit the models. In this work we used 55,000 iterations with a burn-in of 5,000 and a thinning of 5 for the Gibbs sampler. For the ME models, the posterior variance-covariance matrices between environments were also calculated for each model using the full datasets.

Assessing prediction accuracy

Prediction accuracies for each trait for both the U_{DIAG} and U_{UN} models were assessed using 50 training-validation (TRN-VAL) random partitions. We used two different cross-validation (CV) designs (Burgueño et al., 2012). The CV1 mimics the situation when newly developed genotypes have not been tested in any environments; in this scenario, 30% of the lines were missing in all the three environments and were treated as unknown. In this case, predictions for the missing lines are based in the phenotypic records of the other lines that were tested. The CV2 design mimics the situation where some lines were evaluated in some environments, but are missing in others. For the two CV scenarios we assigned individuals to the TRN and VAL sets by using the same procedure as Lopez-Cruz et al. (2015).

In each random partition, the Pearson's correlations (r) between the predicted and the observed values were computed. As in Malosetti et al. 2016, and to comply with the normality assumption, the Fisher's z transformation was used, where $z = \frac{1}{2}\left(\ln\left(\frac{1+r}{1-r}\right)\right)$. Mean prediction accuracies were presented using the original scale after back transformation $r = \frac{\exp(2z)-1}{\exp(2z)+1}$.

Another prediction problem studied here was that of predicting future seasons and was denoted as the "leave-one-year-out". This prediction was performed by using two years in the training population to predict a third year where no phenotypic data was collected.

Response to selection under global prediction scenario

When dealing with G×Y, it is interesting to explore the line performance across years (global prediction). For the global prediction scenario we consider the response to selection as a way of comparing the predictive ability of the two covariance matrices (U_{DIAG} and U_{UN}) and the two genetic kernels (GBLUP and GK), as reported by Piepho and Möring (2005).

In the global scenario, the genotypic value of the i^{th} genotype accounting for all the evaluated years is:

$$g_i = v_1 g_{i1} + v_2 g_{i2} + \ldots + v_m g_{im} = \boldsymbol{v}' \boldsymbol{g}_i$$

where $v_r \ (r = 1, \cdots, m)$, with $\boldsymbol{v} = (v_1, v_2, \ldots, v_m)'$ is some environment weighting parameter such as the relative growing areas in the m subregions as discussed in Piepho and Möring (2005), or the inverse of the heritability, as evaluated in the present study.

As in Piepho and Möhring (2005), we regard the estimator $\tilde{\boldsymbol{L}}_i = \boldsymbol{w}'(\boldsymbol{y}_i - \mu)$ to be an indirect trait, in the special case that $\boldsymbol{w}' = \boldsymbol{v}'\boldsymbol{W}$, where $\boldsymbol{L}_i = \boldsymbol{v}'\boldsymbol{g}_i$ and assuming $\tilde{\boldsymbol{g}}_i$ is the BLUP of genetic effects with $\tilde{\boldsymbol{g}}_i = \boldsymbol{W}(\boldsymbol{y} - \mu)$ and $\boldsymbol{W} = U_E(U_E + \Sigma_e)^{-1}$. We formulate the selection response as a correlated response to selection as:

$$R = i\rho_g h\sqrt{\text{var}\, L_i}$$

where ρ_g is the genetic correlation between $\boldsymbol{L}_i$ and $\tilde{\boldsymbol{L}}_i$ and can be calculated as:

$$\rho_g = \frac{\boldsymbol{v}'\boldsymbol{U}_E\boldsymbol{w}}{\sqrt{(\boldsymbol{v}'\boldsymbol{U}_E\boldsymbol{v})(\boldsymbol{w}'\boldsymbol{U}_E\boldsymbol{w})}},$$

i is the selection intensity and h is the square-root of the heritability (Falconer and Mackay, 2001), that can be calculated as:

$h = \sqrt{\dfrac{\boldsymbol{w}'\boldsymbol{U}_E\boldsymbol{w}}{\boldsymbol{w}'(\boldsymbol{U}_E + \Sigma_e)\boldsymbol{w}}}$ (Piepho and Möring, 2005).

To evaluate different methods we consider the ratio of R values, since both the selection intensity i and the $\sqrt{\text{var}(L_i)}$ terms cancel out. By this way, the ratio R_{UN}/R_{DIAG} is calculated as:

$$R_{UN} / R_{DIAG} = \frac{\rho_{UN} h_{UN}}{\rho_{DIAG} h_{DIAG}}$$

where ρ_{UN} and ρ_{DIAG}, and h_{UN} and h_{DIAG}, are the ρ_g and h values calculated by substituting U_E with U_{UN} and U_{DIAG}, respectively.

2.4 RESULTS

2.4.1 Descriptive statistics

Box-plots of the five traits (GY, MY, PHR, GC and PH) in each of the years (2010-2012) for the *indica* and the *tropical japonica* datasets are shown in Figure 1. For the *indica* dataset, the trait distributions for each year are similar, though we see a slight increase for GY and decrease for PH in 2012 (Figure 2.1A). For the *tropical japonica* dataset, 2011 was a bad year for GY, showing the lowest value for this trait. On the other hand, 2012 was the worst year in terms of grain quality and PH, showing the lowest values for MY, PHR and PH, and the highest for GC (Figure 2.1B). ANOVA analyses performed on each trait and each population on both datasets confirmed significant G×Y interaction (Supplementary Tables 2.2 and 2.3).

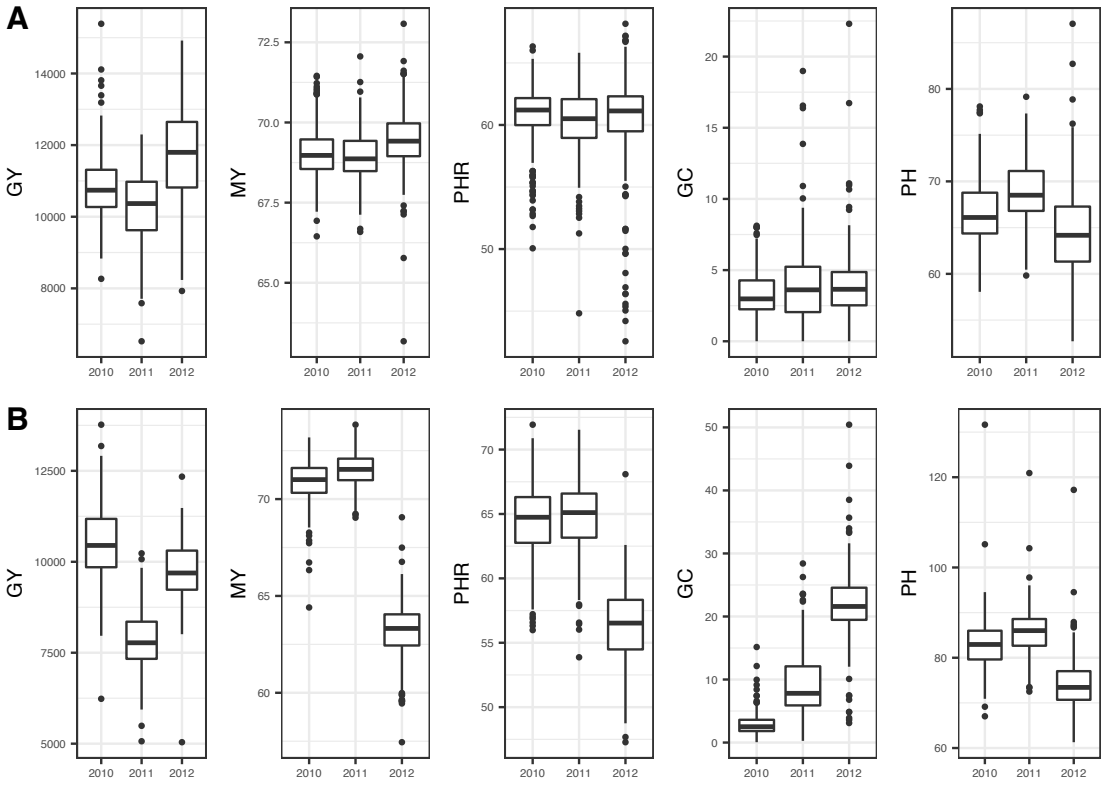

Figure 2.1: Box-plot of Grain Yield (GY) (Kg ha^{-1}), Milling yield (MY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) (cm) for A) *indica* and B) *tropical japonica* rice (Oryza sativa L.) breeding populations, measured in three different environments: years 2010, 2011, 2012.

Trait heritabilities by year were intermediate to high for both datasets. For the *indica* dataset the lowest values were MY in 2012 (0.42) and GY in 2010 (0.46), while for *tropical japonica* the lowest values were GY in 2010 (0.43) and GC in 2011 (0.41) (Table 2.1).

Table 2.1: Trait heritabilities by year for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *indica* and *tropical japonica* Uruguayan breeding populations.

indica					
Year	GY	MY	PHR	GC	PH
2010	0.46	0.78	0.86	0.73	0.49
2011	0.60	0.69	0.78	0.59	0.66
2012	0.68	0.42	0.71	0.69	0.58
tropical japonica					
Year	GY	MY	PHR	GC	PH
2010	0.43	0.69	0.71	0.59	0.62
2011	0.57	0.77	0.85	0.41	0.62
2012	0.70	0.71	0.79	0.75	0.79

2.4.2 Prediction accuracies for U_{DIAG} and U_{UN} models under different CV scenarios

For the *indica* dataset, among all the prediction models tested with the CV1 and CV2 methods, the relative advantage of using the U_{UN} covariance matrix over the U_{DIAG} was evident for most traits only when the CV2 method was used, though GY was an exception (Figure 2.2). There were no significant differences between CV1 and CV2 cross-validations when the U_{DIAG} was used. As expected, mean prediction accuracies for CV1 were lower compared to those obtained with CV2 for $U_{UN,}$ since this scenario benefits from borrowing information across environments (Figure 2.2).

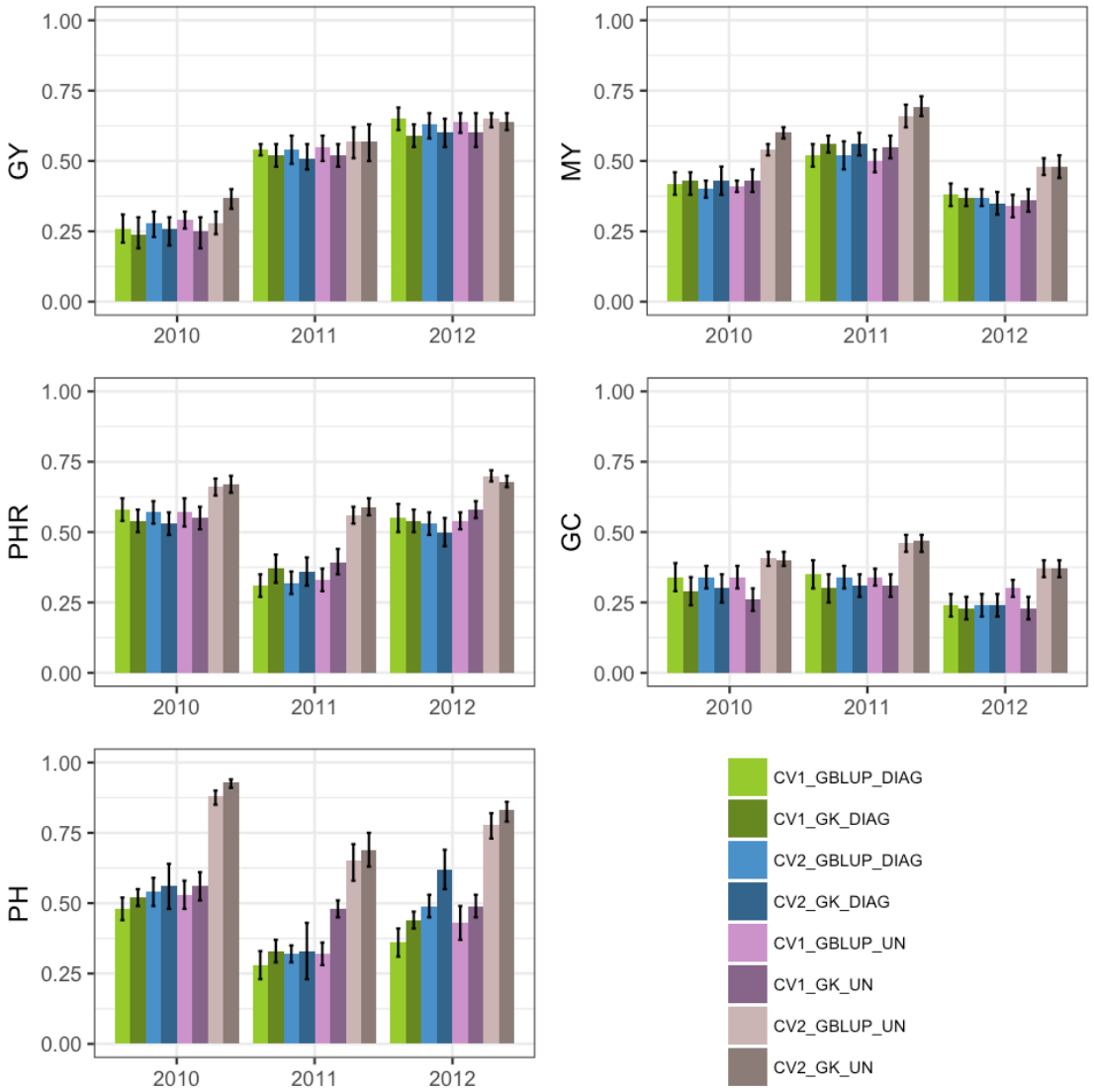

Figure 2.2: Mean prediction accuracies (50 Training-Validation populations) for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) for Multienvironment models using GBLUP and GK kernels under cross-validation CV1 and CV2 with diagonal covariance matrices (DIAG) (CV1-GBLUP_DIAG, CV1-GK_DIAG, CV2-GBLUP_DIAG, CV2-GBLUP_DIAG) and unstructured covariance matrices (UN) (CV1-GBLUP_UN, CV1-GK_UN, CV2-GBLUP_UN, CV2-GK_UN) for the *indica* rice breeding population.

Regarding the two different kernel methods, there were no big differences between the GBLUP and GK kernels in general.

Phenotypic correlations among environments for both datasets were all moderate to high and positive (Supplementary Table 2.4). In general, PH showed the highest correlation for both *indica* and *tropical japonica*, while GY for *indica* showed the lowest correlations. Estimated genetic variance within environments (diagonal) and covariances and correlations between environments (off diagonals) were higher for GK than for GBLUP for all traits, except GC (Supplementary Table 2.5).

Among all traits, GY showed the lowest gains from modeling the U_{UN} covariance structure under CV2, compared to U_{DIAG} (Figure 2.2). GY also showed generally low variance-covariance values and correlations when compared to other traits (Supplemental Tables 2.4 and 2.5). On the other hand, PH was the trait that showed the highest gain from U_{UN} *vs.* U_{DIAG} for CV2 (Figure 2.2) and the highest genetic variance-covariance values and correlations among all traits (Supplemental Tables 2.4 and 2.5).

For the *tropical japonica* dataset, CV2-GK method when using U_{UN} matrix was either better or not different than the other methods (Figure 2.3). For this dataset, PH was again the trait that showed the highest gains from prediction when modeling covariance between environments under CV2.

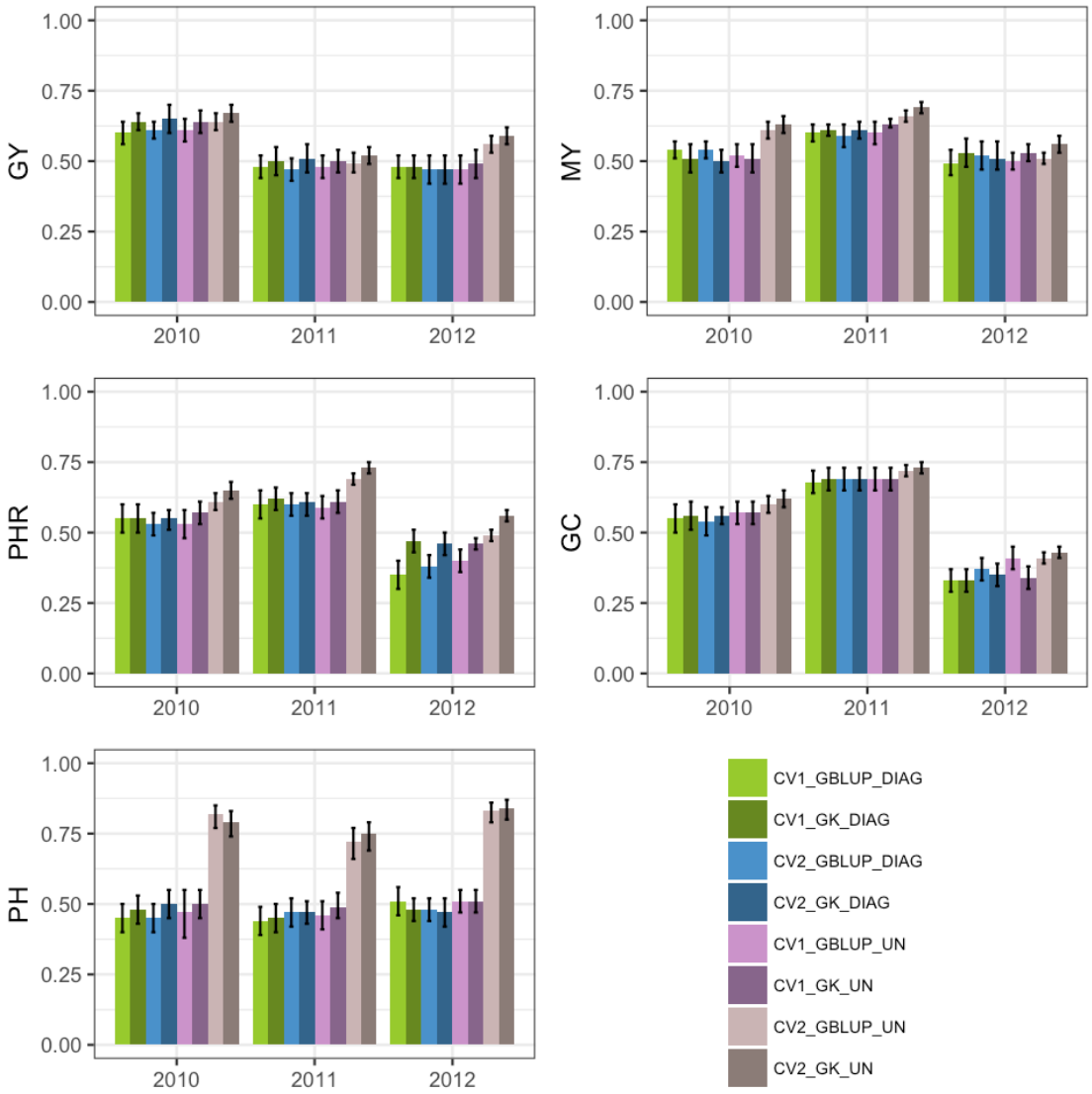

Figure 2.3: Mean prediction accuracies (50 Training-Validation populations) for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) for Multienvironment models using GBLUP and GK kernels under cross-validation CV1 and CV2 with diagonal covariance matrices (DIAG) (CV1-GBLUP_DIAG, CV1-GK_DIAG, CV2-GBLUP_DIAG, CV2-GBLUP_DIAG) and unstructured covariance matrices (UN) (CV1-GBLUP_UN, CV1-GK_UN, CV2-GBLUP_UN, CV2-GK_UN) for the *tropical japonica* rice breeding population.

There were no big differences when predicting with the GBLUP vs. the GK kernel (Figure 2.3). Supplementary Table 2.6 shows that the elements of U_E for the GK are larger than those for the GBLUP method, and the opposite occurred with the diagonal elements of the R matrices. The results of the performance of the models in untested environments (leave-one-year-out) are shown in Table 1.2. The advantage of modeling U_{UN} over U_{DIAG} is more evident for those traits that show a higher correlation between environments, such as MY, PHR and PH in *indica*, and PHR and PH in *tropical japonica*. In all cases prediction accuracies for U_{UN} were either equal or higher than those obtained with U_{DIAG}.

Table 2.2: Correlation between the observed and predicted values of the leave-one-year-out prediction problem, for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *indica* and *tropical japonica* Uruguayan breeding populations.

U_{DIAG}												
indica		GBLUP						GK				
	Predicted year	GY	MY	PHR	GC	PH	Predicted year	GY	MY	PHR	GC	PH
	2010	0.41	0.50	0.43	0.30	0.79	2010	0.34	0.49	0.42	0.28	0.79
	2011	0.52	0.60	0.46	0.38	0.54	2011	0.40	0.61	0.46	0.45	0.56
	2012	0.52	0.39	0.50	0.37	0.63	2012	0.35	0.38	0.49	0.50	0.64
tropical japonica		GBLUP						GK				
	Predicted year	GY	MY	PHR	GC	PH	Predicted year	GY	MY	PHR	GC	PH
	2010	0.52	0.49	0.52	0.44	0.72	2010	0.52	0.47	0.52	0.38	0.71
	2011	0.40	0.51	0.57	0.60	0.64	2011	0.40	0.48	0.60	0.42	0.64
	2012	0.47	0.30	0.43	0.40	0.74	2012	0.48	0.31	0.47	0.38	0.73
U_{UN}												
indica		GBLUP						GK				
	Predicted year	GY	MY	PHR	GC	PH	Predicted year	GY	MY	PHR	GC	PH
	2010	0.43	0.56	0.50	0.33	0.88	2010	0.35	0.58	0.51	0.35	0.91
	2011	0.55	0.67	0.51	0.40	0.63	2011	0.43	0.67	0.52	0.43	0.64
	2012	0.57	0.47	0.58	0.40	0.73	2012	0.38	0.47	0.51	0.38	0.74
tropical japonica		GBLUP						GK				
	Predicted year	GY	MY	PHR	GC	PH	Predicted year	GY	MY	PHR	GC	PH
	2010	0.60	0.54	0.60	0.47	0.79	2010	0.57	0.54	0.59	0.35	0.80
	2011	0.45	0.55	0.65	0.61	0.73	2011	0.45	0.56	0.66	0.43	0.73
	2012	0.51	0.34	0.50	0.42	0.83	2012	0.52	0.36	0.53	0.38	0.80

2.4.3 Estimation for Global Adaptation in the *indica* and *tropical japonica* datasets

To study the gain from borrowing information between environments in the global adaptation scenario, we calculated the ratio of the correlated response to selection obtained from modeling U_{UN} and U_{DIAG}. We assumed two different weighting scenarios: 1- by growing area, with subregions of equal size where $\boldsymbol{v} = (v_1, v_2, v_3)$ with $v_r = 1/3$ and 2- by using the inverse of the heritability, where $\boldsymbol{v} = (v_1, v_2, v_3)$ with $v_r = 1/H_r^2$. In both cases $\boldsymbol{w} = \boldsymbol{v}'\boldsymbol{W}$, where $\boldsymbol{W}$ is either U_{UN} or U_{DIAG} and R_{UN} and R_{DIAG} are the responses calculated with both matrices respectively. Ratios R_{UN}/R_{DIAG} are shown in Table 1.3.

Table 2.3: Response to selection for global adaptation scenario for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *indica* and *tropical japonica* Uruguayan breeding populations. Response measured as ratio R_{UN}/R_{DIAG} where R_{UN} is the response to selection when information between environments is borrowed (Unstructured matrix) and R_{DIAG} is the response to selection when information between environments is not borrowed (Diagonal matrix).

indica	GBLUP					GK				
v	GY	MY	PHR	GC	PH	GY	MY	PHR	GC	PH
Growth area	1.652	1.502	1.114	1.281	1.500	1.202	1.187	1.136	1.060	1.500
$1/H^2$	1.710	1.594	1.110	1.286	1.510	1.200	1.195	1.130	1.058	1.510
tropical japonica	GBLUP					GK				
v	GY	MY	PHR	GC	PH	GY	MY	PHR	GC	PH
Growth area	1.485	1.372	1.628	1.255	1.153	1.085	1.080	1.085	1.078	1.078
$1/H^2$	1.481	1.371	1.609	1.237	1.157	1.080	1.088	1.088	1.082	1.078

The results show that for both weighting scenarios modeling a covariance structure that allows for borrowing information across environments is always beneficial, in both datasets (Table 2.3). The traits that showed highest R_{UN}/R_{DIAG} ratios in the *indica* dataset were GY, MY and PH, with gains above 50%, while in *tropical japonica* the traits with highest R_{UN}/R_{DIAG} ratios were GY, MY and PHR.

For both datasets the relative gains from borrowing information across years are higher for the GBLUP kernel than when the GK kernel is used (Table 2.3).

2.5 DISCUSSION

One of the major challenges in plant breeding is the differential response of genotypes in different environments, known as G×E interaction. Finding lines with predictable G×E across seasons (genotype x year interactions, G×Y) is becoming an increasingly pressing issue due to the highly variable and unpredictable weather conditions in current and future climate change scenarios. Several approaches for modeling G×E have been proposed in the literature (Burgueño et al., 2012; Jarquin et al., 2014; Heslot et al. 2014; Lopez-Cruz et al., 2015), and all confirm the relative benefits of using multi-environment models in comparison to single-environment analyses.

In this work, we used a multi-environment modeling approach for GS where the genetic effects of rice lines were calculated using the Kronecker product of genetic variance-covariance matrices between environments (U_E) and marker-genomic relationship matrices calculated with two different kernel methods, GBLUP and GK. We compared the effect of using two different covariance structures for the U_E matrix: (i) a covariance structure that fits a separate variance component per environment and does not allow borrowing information across environments (U_{DIAG}) and (ii) a

covariance structure that has a variance component for each environment and a separate covariance parameter for each pair of environments (U_{UN}).

These prediction models were implemented as reported in previous studies (Burgueño et al., 2012; Malosetti et al., 2016; Cuevas et al., 2017). Prediction accuracies from both multi-environment models were calculated for five traits with different genetic architectures and medium to high heritabilities: grain yield, three different grain quality traits and plant height, all measured in three different years (2010, 2011 and 2012). The two datasets used in this study were an *indica* and a *tropical japonica* rice breeding population belonging to the Uruguayan National Rice Breeding Program. Population structure and GWAS analyses on these same two populations and the three grain quality traits analyzed in this study were previously reported (Quero et al., 2017). In our dataset, the environments represent three years of evaluation (2010, 2011 and 2012), so G×E is considered as G×Y. In this context we performed both local predictions, by predicting the traits for each of the tested years, and global predictions, by predicting line performance across years, taking G×Y as part of the noise. For local prediction we considered three different cross-validation scenarios: (i) CV1, where we predicted the performance of untested lines on tested environments, (ii) CV2, where we predicted the performance of tested lines in tested environments, and (iii) leave-one-year-out, where we predicted tested lines in untested environments. For global prediction we compared the correlated response to selection calculated from models using both covariance structures (U_{UN} and U_{DIAG}), based on the method reported by Piepho and Möring (2005).

GS models capable of accounting for multi-environment data have been demonstrated to increase prediction accuracies relative to single-environment analyses (Lopez-Cruz et al., 2015; Malosetti et al. 2016; Lado et al., 2016; Saint Pierre et al.,

2016; Bandeira e Sousa et al., 2017; Cuevas et al. 2017). In our study, we found that multi-environment models that allow borrowing information across environments were superior to or not different from those that do not exploit genetic covariance between environments. We also found that the gains in prediction accuracy from models that exploit the genetic covariance between environments depend on the cross-validation method employed. This was expected, as when using phenotypic information from tested genotypes in already tested environments (CV2) we are better exploiting the correlation between environments. On the other hand, under CV1 the information between environments flows in a more indirect fashion through the kinship relatedness matrix only. These results were in accordance with previous studies that used similar cross-validation designs (Burgueño et al., 2012; Jarquin et al., 2014; Zhang et al., 2014; Crossa et al., 2016; Malosetti et al., 2016; Saint Pierre et al., 2016; Bandeira e Sousa et al., 2017). For this reason, it is always beneficial to use as much data as possible to train our models, and to partition our resources by phenotyping as many different genotypes as possible, and spreading them across the tested environments, rather than fully phenotyping only a part of the population, as also suggested by Malosetti et al. (2016). This could be helpful for resource allocation in a small breeding program like the Uruguayan breeding program.

Previous studies in wheat, maize and rice have demonstrated that ME GS models can give improved prediction accuracies by borrowing information across highly correlated environments (Crossa et al., 2014; Lado, et al., 2016; Saint Pierre et al. 2016; Spindel et al., 2016). For these ME models, the genetic correlations between environments depend in part on the off-diagonal values of matrix U_{UN}. When these values are close to zero, the U_{UN} matrix tends to U_{DIAG}, producing prediction accuracies similar to those obtained by using U_{DIAG} models. When the off-diagonal values of U_{UN}

are moderate to high (either positive or negative), the correlations between environments will be far from zero, allowing the borrowing of information across environments. In this case, the U_{UN} models for those environments will perform better than the U_{DIAG} models under CV2.

In our study, the phenotypic and genotypic correlations between environments were all positive, and moderate to high. This led to higher prediction accuracies in all environments using the U_{UN} models in comparison to U_{DIAG} models when using cross-validation method CV2. PH was the trait that showed the highest genetic and phenotypic correlations between environments for the two datasets. On the other hand, traits with lower genetic covariance between environments (off-diagonal values of U_{UN}) showed similar prediction accuracies between U_{DIAG} and U_{UN} models. This was the case for GY in *indica*, where genetic covariances were the lowest, leading to comparable prediction accuracies between U_{DIAG} and U_{UN} models. The accurate estimation of the genetic covariance structure between correlated environments is critical to increase prediction accuracy with ME models. Unstructured covariance matrices have $m(m+1)/2$ unique parameters, where m is the number of environments. When m is large, estimation of an unstructured covariance matrix may be unfeasible due to convergence problems. Also, the estimation of multiple covariance parameters poses the need for sufficiently large datasets to support accurate estimation of the numerous parameters involved (Denis et al., 1997; Meyer and Kirkpatrick, 2008; Meyer, 2009; Elias et al., 2016). For our two balanced datasets with only 3 environments, fitting an unstructured covariance matrix did not pose any convergence issues and worked better than a diagonal matrix model. However, as the number of environments increases, fitting a more parsimonious covariance structure (such as Factor Analytic) should be considered (Piepho, 1998; Smith et al., 2001).

Prediction of untested environments is a highly relevant topic for breeders because it allows predicting which lines will be the best performers in future testing. Another good reason to use cross validation designs such as leave-one-year-out, is to avoid the overlap between training and validating populations. Predicting for already tested environments such as in CV1 and CV2 scenarios, assures the overlap between the testing and validation populations along the environmental and genotype dimensions. This could lead to overfitting, and thus, inflation of the prediction accuracies. Here, we evaluated the predictions for different traits in untested years (leave-one-year-out), where the training set included the same genotypes as the testing set, but no phenotypic information for the year tested, and thus, avoiding the overlap of information along the environment dimension. Our analysis revealed that years where no genotypes have been evaluated could be predicted with good accuracy (over 40% in most cases) when using the U_{UN} matrix. Previous studies have remarked that predicting environments that have not yet been tested could be a difficult task, pointing to the need to establish a link between the tested and untested environments. A good way to establish this link could be using environmental covariates (Malosetti et al, 2016; Saint Pierre et al. 2016), however, positive correlation between environments is still an important factor for achieving good prediction accuracies in unobserved environments, as shown in this study. Jarquin et al. (2016) optimized training sets for genomic prediction of soybean accessions using designs similar to our leave-one-site-out with no environmental covariates, as in this study, and also showed high prediction accuracy for % protein and grain yield. High correlation among sites could be also the result of stable climate conditions, which tend to be more common in temperate areas compared to the tropics, which experience greater seasonal variation as well as more disruptive weather events (typhoons, for example). Spindel & McCouch (2016) stressed the importance of highly

correlated phenotypic and environmental data in order to achieve higher prediction accuracies and improve breeding outcomes. As long as our environments remain highly correlated, with relatively stable weather conditions, and as long as the breeders do not introduce any novel genetic diversity into their populations, prediction accuracies will remain high, as shown in this study. Another factor that contributed to the prediction accuracies observed in this study were the moderate to high heritabilities found for our traits across years in both datasets. Good agronomy practices and experimental design as long as the irrigated rice paddy system that homogenizes field conditions, improve heritability in rice compared to other crops such as wheat or maize, where water availability is an important variable affecting trait expression.

Besides prediction for specific years/environments (local prediction), another relevant scenario is to predict genotype rankings across years (global prediction). This is particularly relevant given the unpredictable weather patterns related to years. In this work we evaluated the response to selection of ME models when information is borrowed across environments using the R_{UN}/R_{DIAG} ratio. To calculate the responses we used a method previously reported by Piepho and Möring (2005), which involves a weighting scheme based on BLUP. Our conceptual framework for this global scenario assumes an extensive target region (i.e., the Uruguayan rice breeding program) that is subdivided into subregions (years), and the objective is to predict the best performing genotypes across years considering G×Y as part of the noise. We concluded that modeling covariance structures that accommodate covariances between environments is always beneficial (or at least has no penalty) when predicting the performance of lines across years, and for this reason it is always useful to consider all data. We acknowledge that our dataset is small, as it includes data from one location and only three years of testing, and that this may limit the conclusions we can draw. However, the increase in prediction accuracy observed

under CV2, and the increased response to selection when using unstructured covariance matrices strongly suggests that the addition of more information should improve the prediction ability of our U_{UN} models. A more comprehensive analysis with more years of testing and the incorporation of environmental covariates is the subject of current research.

Various studies have documented the benefits of using Gaussian kernels in genomic prediction in both single- and multi-environment settings in crops such as maize, wheat and rice (Gianola et al., 2014; Iwata et al. 2015; Onogi et al. 2015; Pérez-Elizalde et al., 2015; Spindel et al. 2015; Cuevas et al. 2016; Bandeira e Souza et al., 2017; Cuevas et al. 2017). However in our study, prediction accuracies obtained with the GK kernel were in general not significantly different than those obtained with a linear kernel (GBLUP), with a few exceptions detailed in the results section.

GS can shorten breeding cycle length and increase gains per unit time by replacing time-intensive phenotypic evaluation of complex traits with GEBVs. In temperate crops, GS can be exploited to accelerate gain from selection through the use of off-season nurseries, where phenotyping is difficult and costly. This could be beneficial for temperate rice growing countries, like Uruguay, where only one rice cycle per year is possible. There are several points where GS could be implemented in a standard pedigree selection-based rice breeding program to either avoid a cycle of phenotyping or to help to decide which lines should be advanced (or discarded) for the next generation. In general, a genomic selection step before conducting multi-environment trails is suggested, and some studies propose the incorporation of phenotypic data from preliminary yield trials into the genomic selection framework (Endelman et al., 2014; Michel et al., 2017).

Our results show that for the germplasm and environmental conditions used in this work, most of the benefits of multienvironment models come from modeling genetic correlations between environments under CV2. For predicting the performance of newly developed lines (CV1) modeling between environment correlations has no effect compared to considering environments independently. However, our study showed that for some traits high prediction accuracies can be obtained in untested years, which is important for resource allocation in small breeding programs. Additional research incorporating the use of environmental covariates to model future seasons, and bringing historical data to model climate response patterns, are promising steps to improve the prediction accuracies as a part of our effort to accelerate the rate of genetic gain for both yield and grain quality in rice breeding programs in temperate areas.

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2.7 SUPPLEMENTARY DATA

Supplementary Table 2.1: List of the traits evaluated in GS.

Trait category	Trait name	Acronym	TO†	PO‡	Trait description
Yield related	Yield	Y	TO:0000396	PO:0009001	Kg of paddy rice per hectare
	Plant Height	PH	TO:0000207	PO:0025029	Length (in cm) from the ground to the tip of the flag leaf
Grain quality	Milling yield	MY	TO:0000144	PO:0009010	Mass of total milled rice as a percentage of the original dried rough rice
	Head Rice Percentage	PHR	TO:0000222	PO:0009010	Mass of head rice after removing broken kernels as a percentage of the original dried rough rice
	Grain chalkiness	GC	TO:0000266	PO:0009089	Percentage of chalky grains in a sample of 50g of milled rice, where the area of chalk -core, white back or white belly- is larger than half the kernel area based on visual inspection

† - TO: Trait Ontology
‡ - Phenotype Ontology

Supplementary Table 2.2: Analysis of Variance for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *indica* Uruguayan breeding population.

GY					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	361	2748794618	7614390	6.9477	< 2.2e-16 ***
Year	2	1161627496	580813748	529.958	< 2.2e-16 ***
Row	35	178988365	5113953	4.6662	< 2.2e-16 ***
Column	127	502558703	3957155	3.6107	< 2.2e-16 ***
Block	88	625713798	7110384	6.4878	< 2.2e-16 ***
Genotype x Year	665	940721210	1414618	1.2908	1.320e-5 **
Residual	1812	1985882768	1095962		
MY					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	361	1665.8	4.614	2.4497	< 2.2e-16 ***
Year	2	106.2	53.102	28.1904	8.788e-13 ***
Row	35	282.3	8.064	4.2811	2.200e-15 ***
Column	127	293.9	2.314	1.2285	0.04744 *
Block	88	453.3	5.151	2.7345	8.280e-15 ***
Genotype x Year	665	1631.2	2.453	1.302	1.320e-5 **
Residual	1810	3409.5	1.884		
PHR					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	361	22023.1	61.01	9.27	< 2.2e-16 ***
Year	2	1957.6	978.81	148.7149	< 2.2e-16 ***
Row	35	2397.3	68.5	10.4068	< 2.2e-16 ***
Column	127	3413.8	26.88	4.084	< 2.2e-16 ***
Block	88	5994.8	68.12	10.3503	< 2.2e-16 ***
Genotype x Year	665	8391	12.62	1.9171	< 2.2e-16 ***
Residual	1810	11913	6.58		
GC					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	361	11132.3	30.837	5.6711	< 2.2e-16 ***
Year	2	624.9	312.447	57.4605	< 2.2e-16 ***
Row	35	1190.2	34.007	6.254	< 2.2e-16 ***
Column	127	1855.7	14.611	2.6871	< 2.2e-16 ***
Block	88	4711.1	53.535	9.8454	< 2.2e-16 ***
Genotype x Year	665	5769.7	8.676	1.5956	2.393e-14 ***
Residual	1811	9847.5	5.438		
PH					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F

Genotype	361	90045	249.4	1.5692	7.631e-09 ***
Year	2	45342	22671	1809.3	< 2.2e-16 ***
Row	35	5716	163.3	1.0274	0.425092
Column	127	10464	82.39	6.57	< 2.2e-16 ***
Block	88	14552	165.3	13.19	< 2.2e-16 ***
Genotype x Year	665	59392	89.31	7.12	< 2.2e-16 ***
Residual	1812	22699	12.53		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Supplementary Table 2.3: Analysis of Variance for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *tropical japonica* Uruguayan breeding population.

GY					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	312	1169419410	3748139	4.5461	< 2.2e-16 ***
Year	2	2805319316	1402659658	1701.292	< 2.2e-16 ***
Row	47	369490731	7861505	9.5353	< 2.2e-16 ***
Column	63	210624556	3343247	4.055	< 2.2e-16 ***
Block	44	788727622	17925628	21.7421	< 2.2e-16 ***
Genotype x Year	621	705496683	1136066	1.3779	9.454e-07 ***
Residual	1346	1109733005	824467		
MY					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	312	62725	201	61.232	< 2.2e-16 ***
Year	2	30129	15064.3	4588.206	< 2.2e-16 ***
Row	47	29401	653.4	198.996	< 2.2e-16 ***
Column	63	66331	1442	439.19	< 2.2e-16 ***
Block	46	2228	35.4	10.771	< 2.2e-16 ***
Genotype x Year	621	24931	40.1	12.228	< 2.2e-16 ***
Residual	1340	4400	3.3		
PHR					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	312	68078	218.2	35.1727	< 2.2e-16 ***
Year	2	33615	16807.3	2709.2547	< 2.2e-16 ***
Row	45	31230	694	111.8682	< 2.2e-16 ***
Column	63	73293	1593.3	256.8372	< 2.2e-16 ***
Block	46	2904	46.1	7.4316	< 2.2e-16 ***
Genotype x Year	621	28946	46.6	7.5135	< 2.2e-16 ***
Residual	1337	8294	6.2		
GC					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	312	264852	849	39.4618	< 2.2e-16 ***
Year	2	119129	59564	2768.954	< 2.2e-16 ***
Row	45	106585	2369	110.1059	< 2.2e-16 ***
Column	63	12833	204	9.4693	< 2.2e-16 ***
Block	46	106585	2369	110.1059	< 2.2e-16 ***
Genotype x Year	621	114081	184	8.5398	< 2.2e-16 ***
Residual	1337	28890	22		
PH					
Source	Df	Sum of Sq	Mean Sq	F value	Pr>F
Genotype	312	67143	215.2	12.8427	< 2.2e-16 ***
Year	2	44612	22306.1	1331.1843	< 2.2e-16 ***

Row	45	8361	181.8	10.8477	< 2.2e-16 ***
Column	63	9350	148.4	8.8568	< 2.2e-16 ***
Block	46	9407	209	12.475	< 2.2e-16 ***
Genotype x Year	621	27553	44.4	2.6478	< 2.2e-16 ***
Residual					

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Supplementary Table 2.4: Phenotypic correlations (off-diagonals) for Grain Yield (GY), Milling Yield (MY), Head Rice Percentage (PHR), Grain Chalkiness (GC), and Plant Height (PH), for *indica* (upper diagonal) and *tropical japonica* (lower diagonal) Uruguayan breeding populations.

GY					**MY**				
	indica					***indica***			
	Year	**2010**	**2011**	**2012**		**Year**	**2010**	**2011**	**2012**
tropical	**2010**	-	0.23	0.26	***tropical***	**2010**	-	0.57	0.37
japonica	**2011**	0.36	-	0.50	***japonica***	**2011**	0.54	-	0.41
	2012	0.45	0.38	-		**2012**	0.32	0.47	-
PHR					**GC**				
	indica					***indica***			
	Year	**2010**	**2011**	**2012**		**Year**	**2010**	**2011**	**2012**
tropical	**2010**	-	0.39	0.47	***tropical***	**2010**	-	0.40	0.49
japonica	**2011**	0.58	-	0.48	***japonica***	**2011**	0.49	-	0.50
	2012	0.42	0.52	-		**2012**	0.39	0.47	-
PH									
	indica								
	Year	**2010**	**2011**	**2012**					
tropical	**2010**	-	0.79	0.91					
japonica	**2011**	0.66	-	0.46					
	2012	0.78	0.71	-					

Supplementary Table 2.5: Estimates of genetic variance-covariance matrices (U_{UN}) (lower diagonal), genetic correlations (upper diagonal), and error matrices (R) with GBLUP and GK kernels, for yield (Y), milling yield (MY), head rice percentage (PHR), grain chalkiness (GC) and plant height (PH) in the *indica* rice breeding population.

Correlation matrix (upper diagonal) U_{UN} matrix (lower diagonal)				R matrix			Correlation matrix (upper diagonal) U_{UN} matrix (lower diagonal)			R matrix		
GBLUP							GK					
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
GY												
2010	0.38	0.35	0.28	0.80	-	-	0.62	0.30	0.35	0.61	-	-
2011	0.14	0.46	0.33	-	0.53	-	0.27	0.63	0.35	-	0.41	-
2012	0.11	0.19	0.37	-	-	0.51	0.27	0.30	0.50	-	-	0.42
MY												
2010	0.82	0.87	0.79	0.46	-	-	1.25	0.91	0.84	0.44	-	-
2011	0.58	0.77	0.88	-	0.39	-	0.90	1.19	0.89	-	0.36	-
2012	0.40	0.42	0.37	-	-	0.65	0.65	0.70	0.90	-	-	0.63
PHR												
2010	1.36	0.76	0.84	0.23	-	-	2.01	0.54	0.63	0.18	-	-
2011	0.92	1.61	0.73	-	0.31	-	1.14	2.15	0.70	-	0.26	-
2012	1.00	1.11	1.42	-	-	0.29	1.27	1.37	2.05	-	-	0.24
GC												
2010	0.84	0.61	0.52	0.54	-	-	0.86	0.56	0.45	0.36	-	-
2011	0.49	0.88	0.69	-	0.56	-	0.36	0.94	0.57	-	0.31	-
2012	0.43	0.58	1.03	-	-	0.42	0.31	0.42	0.96	-	-	0.28
PH												
2010	1.08	0.79	0.91	0.08	-	-	0.82	0.86	0.95	0.05	-	-
2011	0.85	1.21	0.75	-	0.16	-	0.66	0.62	0.64	-	0.17	-
2012	1.31	0.49	2.12	-	-	0.20	0.97	0.69	1.25	-	-	0.19

Supplementary Table 2.6: Estimates of genetic variance-covariance matrices (U_E) (lower diagonal), genetic correlations (upper diagonal), and error matrices (R) with GBLUP and GK kernels, for yield (Y), milling yield (MY), head rice percentage (PHR), grain chalkiness (GC) and plant height (PH) in the *tropical japonica* rice breeding population.

Correlation matrix (upper diagonal) U_{UN} matrix (lower diagonal)				R matrix			Correlation matrix (upper diagonal) U_{UN} matrix (lower diagonal)			R matrix		
GBLUP							GK					
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
GY												
2010	0.65	0.71	0.72	0.39	-	-	0.91	0.76	0.79	0.34	-	-
2011	0.29	0.52	0.63	-	0.59	-	0.37	0.87	0.73	-	0.50	-
2012	0.40	0.33	0.72	-	-	0.46	0.58	0.51	1.15	-	-	0.38
MY												
2010	0.73	0.89	0.52	0.46	-	-	0.92	0.88	0.52	0.38	-	-
2011	0.55	0.82	0.50	-	0.35	-	0.62	0.96	0.49	-	0.27	-
2012	0.40	0.45	1.02	-	-	0.33	0.44	0.45	1.14	-	-	0.27
PHR												
2010	0.82	0.89	0.75	0.45	-	-	0.70	0.81	0.65	0.27	-	-
2011	0.77	1.22	0.75	-	0.26	-	0.42	0.73	0.71	-	0.19	-
2012	0.68	0.90	1.30	-	-	0.36	0.33	0.43	0.82	-	-	0.25
GC												
2010	0.84	0.70	0.43	0.25	-	-	2.66	0.71	0.43	0.23	-	-
2011	0.43	0.85	0.68	-	0.30	-	1.40	2.44	0.69	-	0.29	-
2012	0.30	0.61	1.19	-	-	0.28	0.94	1.79	3.41	-	-	0.27
PH												
2010	1.46	0.89	0.94	0.19	-	-	2.30	0.91	0.95	0.18	-	-
2011	1.13	1.20	0.94	-	0.32	-	1.76	1.88	0.95	-	0.30	-
2012	1.30	1.15	1.44	-	-	0.19	2.04	1.80	2.23	-	-	0.19

Supplementary Table 2.7: Estimates of genetic variance-covariance matrices (U_{DIAG}), and error matrices (R) with GBLUP and GK kernels, for yield (Y), milling yield (MY), head rice percentage (PHR), grain chalkiness (GC) and plant height (PH) in the *indica* rice breeding population.

Correlation matrix U_{DIAG}				R matrix			Correlation matrix U_{DIAG}			R matrix		
GBLUP							GK					
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
GY												
2010	0.12	-	-	0.81	-	-	0.55	-	-	0.65	-	-
2011	-	0.18	-	-	0.56	-	-	0.54	-	-	0.45	-
2012	-	-	0.13	-	-	0.51	-	-	0.40	-	-	0.47
MY												
2010	0.19	-	-	0.33	-	-	0.69	-	-	0.64	-	-
2011	-	0.18	-	-	0.24	-	-	0.66	-	-	0.45	-
2012	-	-	0.17	-	-	0.57	-	-	0.66	-	-	0.47
PHR												
2010	0.72	-	-	0.21	-	-	1.10	-	-	0.16	-	-
2011	-	0.81	-	-	0.31	-	-	1.04	-	-	0.20	-
2012	-	-	0.76	-	-	0.25	-	-	1.12	-	-	0.17
GC												
2010	0.72	-	-	0.22	-	-	0.89	-	-	0.22	-	-
2011	-	0.81	-	-	0.31	-	-	1.00	-	-	0.31	-
2012	-	-	0.76	-	-	0.25	-	-	1.05	-	-	0.25
PH												
2010	0.82	-	-	0.10	-	-	0.96	-	-	0.08	-	-
2011	-	0.97	-	-	0.18	-	-	1.04	-	-	0.19	-
2012	-	-	1.02	-	-	0.21	-	-	1.87	-	-	0.22

Supplementary Table 2.8: Estimates of genetic variance-covariance matrices (U_{DIAG}), and error matrices (R) with GBLUP and GK kernels, for yield (Y), milling yield (MY), head rice percentage (PHR), grain chalkiness (GC) and plant height (PH) in the *tropical japonica* rice breeding population.

Correlation matrix U_{DIAG}				R matrix			Correlation matrix U_{DIAG}			R matrix		
GBLUP							GK					
	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
GY												
2010	0.28	-	-	0.46	-	-	0.93	-	-	0.33	-	-
2011	-	0.18	-	-	0.65	-	-	0.82	-	-	0.52	-
2012	-	-	0.31	-	-	0.54	-	-	1.11	-	-	0.39
MY												
2010	0.24	-	-	0.55	-	-	0.93	-	-	0.44	-	-
2011	-	0.30	-	-	0.43	-	-	0.99	-	-	0.33	-
2012	-	-	0.46	-	-	0.42	-	-	1.2	-	-	0.31
PHR												
2010	0.20	-	-	0.56	-	-	0.85	-	-	0.45	-	-
2011	-	0.31	-	-	0.43	-	-	1.04	-	-	0.32	-
2012	-	-	0.26	-	-	0.62	-	-	1.26	-	-	0.37
GC												
2010	0.55	-	-	0.30	-	-	1.2	-	-	0.23	-	-
2011	-	0.38	-	-	0.31	-	-	1.04	-	-	0.28	-
2012	-	-	0.40	-	-	0.55	-	-	1.26	-	-	0.29
PH												
2010	0.86	-	-	0.23	-	-	1.5	-	-	0.27	-	-
2011	-	0.45	-	-	0.45	-	-	1.22	-	-	0.36	-
2012	-	-	0.75	-	-	0.26	-	-	1.38	-	-	0.27

CHAPTER 3:

INTEGRATING MOLECULAR MARKERS AND ENVIRONMENTAL COVARIATES TO INTERPRET GENOTYPE BY ENVIRONMENT INTERACTION IN RICE (*Oryza sativa* L.) GROWN IN SUBTROPICAL AREAS

This chapter was submitted as a publication in G3: Genes, Genomes, Genetics: Monteverde E, L Gutiérrez, P Blanco, F Pérez de Vida, JE Rosas, V Bonnecarrère, G Quero, S McCouch. 2018. Integrating molecular markers and environmental covariates to interpret genotype by environment interaction in rice (Oryza sativa L.) grown in temperate areas.

My contributions to this paper: I generated all the genotypic data, performed all of the analyses, and wrote the paper.

Contributions by others: The two breeding populations were originally developed in Uruguay under the direction of L. Gutiérrez and V. Bonnecarrère for a GWAS project on grain traits as reported in Quero et al. (2018). The phenotypic data was generated by P. Blanco and F. Pérez de Vida.

3.1 ABSTRACT

Understanding the genetic and environmental basis of genotype × environment interaction (G×E) is of fundamental importance in plant breeding. If we consider G×E in the context of genotype × year interactions (G×Y), predicting which lines will have stable and superior performance across years is an important challenge for breeders. A better understanding of the factors that contribute to the overall grain yield and quality of rice (Oryza sativa L.) will lay the foundation for developing new breeding and selection strategies for combining high quality, with high yield. In this study, we used molecular marker data and environmental covariates (EC) simultaneously to predict rice yield, milling quality traits and plant height in untested environments (years), using both reaction norm models and partial least squares (PLS), in two rice breeding populations (*indica* and *tropical japonica*). We also sought to explain G×E by differential quantitative trait loci (QTL) expression in relation to EC. Our results showed that PLS

models trained with both molecular markers and EC gave better prediction accuracies than reaction norm models when predicting future years. We also detected several milling quality QTL that showed a differential expression conditional on humidity and solar radiation, providing insight for the main environmental factors affecting milling quality in subtropical rice growing areas.

3.2 INTRODUCTION

Genetic by environment interaction (G×E) could be expressed as a difference in the relative response of genotypes across diverse environments. When we consider a set of genotypes exposed to different environments, their performance will differ depending on the interaction of genetic properties with the different environmental conditions, leading to differences in variances and rank changes among genotypes (Cooper and DeLacy 1994). These rank changes represent a very important challenge for breeders due to the difficulties of selecting genotypes with stable performance over diverse environments.

Environments can be different both in time and space. For this reason, the concept of G×E embraces both interactions that take place between genotypes and a particular location (genotype by location interaction), and between genotypes and particular years (genotype by year interaction). Genotype by location interactions are usually determined by soil and climate conditions, while genotype by year interactions are characterized by plot-to-plot variability and weather conditions (Malosetti et al., 2016).

Several statistical approaches have been proposed to describe G×E in the context of classical plant breeding. The classic parametric approaches used to evaluate G×E are based on linear regression and ANOVA techniques. Linear regression analysis (Yates and Cochran 1938; Finlay and Wilkinson 1963) measures individual genotype performance over environmental means. Multiplicative models combine univariate and multivariate approaches for reducing data dimensionality and facilitate the interpretation of results (Gauch 1998; Smith et al. 2005). The most commonly used multiplicative model is the additive main effect and multiplicative interaction model (AMMI; Gauch 1992). AMMI combines univariate (ANOVA) and multivariate (singular value decomposition; SVD) techniques for estimating genotype and environment main effects, and G×E effects, respectively. Factorial regression models are another type of models that allow the modeling of genotype sensitivity to specific environmental covariates (EC) (van Eeuwijk et al. 1996; Vargas et al. 1998; Malosetti et al. 2004; Malvar et al. 2005). Linear mixed-models became very popular for the analysis of G×E since they allow different correlation structures among environments among other features (Piepho 1998; Smith et al. 2001; Burgueño et al. 2007). These covariance structures may range from a compound symmetry form, where homogeneous variance and homogeneous covariance between environments are assumed, to an unstructured form where a covariance parameter is assumed between each pair of environments and environments are assumed to have heterogeneous variances.

Recent developments in sequencing technologies and statistical modeling have made it possible to use dense genotypic information to predict phenotypic responses through genomic prediction (GP). This idea was introduced by Meuwissen et al. (2001), and provides an alternative approach to indirect selection in crop breeding. GP models were originally developed for traits evaluated in single environments, but more recently

standard GP models have been extended to account for G×E. Burgueño et al. (2012) were the first to extend genomic best linear unbiased prediction (GBLUP) to a multi-environment context, by combining genetic and environmental covariance matrices and using different covariance structures to model the environmental component. Lopez Cruz et al. (2015) proposed a marker by environment approach where marker effects and genotypic values are partitioned into main effects across environments (stability) effects that are specific to each environment (interactions).

Standard GP models can be modified to accommodate climate information in the form of EC. However, including EC in the analysis can pose some similar constraints encountered when predicting breeding values with multiple markers. As climatic and agronomic systems develop, a very high number of covariates can potentially be obtained increasing the dimensionality of the data, increasing also the possibility of being correlated with each other. Several studies have proposed different ways to deal with highly dimensional data, showing that the incorporation of explicit environmental and genetic information can improve prediction accuracies and predict performance in untested environments (Heslot et al. 2014; Jarquín et al. 2014; Malosetti et al. 2016). Jarquín et al. (2014) proposed a Bayesian reaction norm model where the main genetic and environmental effects were modeled using covariate structures as functions of molecular markers and EC respectively, and the interaction effects between markers and EC were modeled using a multiplicative operator. Heslot et al. (2014) proposed a factorial regression model, where instead of using all the available EC and molecular markers, they chose the EC that most significantly influenced the growth and development of the crop by using crop growth models (CGM). These variables were introduced in the factorial regression model along with those markers that showed the

most variable effects across environments and reducing thus, dimensionality of both markers and EC.

The partial least square regression (PLS) (Wold et al. 2001) is a generalization of multiple linear regression (MLR). PLS is a dimension reduction approach that can accommodate a large number of correlated genetic and environmental variables simultaneously, by finding one or few factors named latent variables (LV) that explain both the variance of the matrix (containing predictor variables) and the covariance between matrices and (containing response variables). PLS can be used for variable selection, in order to improve estimation/prediction performance, but also to improve model interpretation and understanding of the system studied. Another advantage of PLS is that it can be more robust against multicollinearity (Aastveit and Martens 1986). PLS models have previously been use for GP both in plant and animal breeding (Solberg et al. 2009; Long et al. 2011; Colombani et al. 2012; Iwata et al. 2015), to detect highly influential environmental and marker covariates that explain a significant proportion of the total G×E (Vargas et al. 1998; Crossa et al. 1999; Vargas et al. 1999).

Understanding the genetic basis of G×E is also necessary to gain predictive capability, and one way to do this is detecting QTLs with varying effects across different environmental conditions, or QTL by environment interaction (QTL×E). Methods usually employed to detect QTL×E have been very useful to detect QTL with differential expression across environments, but provide no explanation of the underlying environmental factors involved. When weather data are available, factorial regression models can be used to determine the extent of influence of these factors on QTL×E (Crossa et al. 1999; Campbell et all. 2004; Malosetti et al. 2004).

Rice is one of the world's most important staple food crops, constituting over 21% of the caloric intake of the world's population and up to 76% of the caloric needs

in many Asian countries (Fitzgerald et al. 2008). World markets dictate the value of rice mainly based on milling quality traits, so breeding for both high yield and quality is a major breeding objective for rice exporting countries like Uruguay.

In a previous study we showed that accounting for heterogeneous covariance parameters between pairs of environments can be beneficial for predicting yield and milling quality performance in Uruguayan rice for untested environments (Monteverde et al. 2018). In another study, Quero et al. (2018) found a set of QTL for milling yield traits in the same Uruguayan *indica* and *tropical japonica* populations. However, none of these studies tested the use of EC to both predict yield and milling quality traits in untested environments, and investigate QTL responses in specific environments. The main objectives of this study were to: 1) use molecular marker data and environmental covariates simultaneously to predict rice yield and milling quality traits in untested environments (years), and 2) Detect marker by environment covariate interactions that provide explanations of variable QTL effects across environments. Two rice breeding populations (*indica* and *tropical japonica*) were used in this study and were evaluated for grain yield, plant height and grain quality traits (head rice percentage and chalky grain percentage) across 3-5 years in Eastern Uruguay. Results from these two analyses provided clues about the main environmental variables that could be driving G×E in temperate rice-growing regions such as Uruguay.

3.3 MATERIALS AND METHODS

3.3.1 Germplasm

The germplasm consists of two rice-breeding populations, an *indica* and a *tropical japonica* population belonging to the National Institute of Agricultural Research (INIA-Uruguay). Both populations were evaluated in a single location, Paso

de la Laguna Experimental Station (UEPL), Treinta y Tres, Uruguay (33°15'S, 54°25'W) between 2009-2013.

The *indica* population consisted of 327 elite breeding lines evaluated over three years (2010-2012), and the field design consisted in a randomized complete block design with two or three replications. Trait correlations, heritabilities, and genomic prediction accuracies for this dataset were computed in previous studies (Rosas et al. 2017, Monteverde et al. 2018, Quero et al. 2018). The *tropical japonica* population consisted of 320 elite breeding lines evaluated over five years (2009-2013). The number of accessions observed each year ranged from 93 to 319, as detailed in Table 3.1. This dataset was unbalanced with non-random missing data, since ~50% genotypes were dropped from testing every year based on performance, and new genotypes were added over time. Each year, the genotypes were planted independently in replicated trials in six-row plots using an augmented randomized complete block design with two or three replications. Both *indica* and *tropical japonica* trials were conducted under irrigated conditions using appropriate pest and weed control.

Table 3.1: Description of the rice breeding lines evaluated each year and broad-sense heritabilities for each trait calculated in a line-basis. GY: grain yield, PHR: percentage of head rice, GC: percentage of chalky grains, PH: plant height.

indica					
		H^2			
Year	Lines evaluated	GY	PHR	GC	PH
2010	327	0.46[a]	0.86[a]	0.73[a]	0.49[a]
2011	327	0.60[a]	0.78[a]	0.59[a]	0.66[a]
2012	327	0.68[a]	0.71[a]	0.69[a]	0.58[a]
Tropical japonica					
2009	93	0.44	0.67	0.59	0.77
2010	292	0.68	0.71	0.59	0.62
2011	319	0.43[a]	0.85[a]	0.41[a]	0.62[a]
2012	319	0.57[a]	0.79[a]	0.75[a]	0.79[a]
2013	134	0.70	0.75	0.80	0.78

[a] Previously reported by Monteverde et al. (2018) and Rosas et al. (2017)

The agronomic traits of interest used in this study were Grain Yield (GY of paddy rice in kilograms per hectare) and Plant Height (PH measured in cm from the soil surface to the tip of the flag leaf). The grain quality traits measured were Percentage of Head Rice Recovery (PHR measured in grams, as the weight of whole milled kernels, using a 100g sample of rough rice), and the percentage of Chalky Grain (GC measured as % of chalky kernels in a subsample of 50 g of total milled rice, where the area of chalk -core, white back or white belly- was larger than half the kernel area based on visual inspection). More details about how grain quality traits were measured can be found in Quero et al. (2017) and Monteverde et al. (2018).

3.3.2 Phenotypic analysis

Phenotypic data for each trait were analyzed separately each year. The model used to calculate the best linear unbiased estimators (BLUEs) for each year was:

$$y_{ijkl} = \mu + b_i + g_j + r_{k(i)} + c_{l(i)} + \varepsilon_{ijkl}$$

where y_{ijkl} is the trait score, μ is the overall mean, b_i is the random effect of the ith block with $b_i \sim N(0,\sigma_b^2)$, where σ_b^2 is the block variance, g_j is the genotypic effect of the jth genotype, $r_{k(i)}$ and $c_{l(i)}$ are the random kth row and lth column effects nested within the ith block with $r_{k(i)} \sim N(0,\sigma_r^2)$ and $c_{l(i)} \sim N(0,\sigma_c^2)$, where σ_r^2 and σ_c^2 are the row and column variances respectively, and ε_{ijkl} is the model residual vector with $\varepsilon_{ijkl} \sim N(0,\sigma_\varepsilon^2)$ where σ_ε^2 is the error variance.

Trait heritabilities in *tropical japonica* for years 2009 and 2013 (data not yet published) was calculated on a per line basis as $H^2 = \sigma_g^2 \Big/ \left(\sigma_g^2 + \sigma_\varepsilon^2 / r\right)$, where σ_g^2 is the variance among genotypes, σ_ε^2 is the error variance, and *r* is the number of replicates.

3.3.3 Genotypic characterization

The lines were genotyped using genotyping-by-sequencing (GBS). SNP calling was performed using the TASSEL 3.0 GBS pipeline (Bradbury et al. 2017), and SNPs were aligned to the Nipponbare reference genome MSU version 7.0 (http://rice.plantbiology.msu.edu/) using Bowtie 2 (Langmead and Salzberg, 2012). Imputation of missing data was performed with the FILLIN algorithm implemented in TASSEL 5.0 (Swarts et al. 2008) for both datasets separately. The GBS datasets were filtered to retain markers with <50% missing data after imputation, and a minor allele frequency MAF>0.05, as reported by Quero et al. (2018), and Monteverde et al. (2018). The final *indica* and *tropical japonica* marker dataset consisted of 92,430 and 44,598 SNP markers respectively.

3.3.4 Derivation of EC from weather data

Daily weather data were obtained from GRAS unit from INIA (http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico). The database contains weather data from 1965 to the last calendar month completed, for all 5 INIA experimental stations in Uruguay. The variables available were related to temperature, precipitation, solar radiation, humidity, wind, and evaporation.

To compute the EC from daily weather data for each rice genotype, the plant development stage has to be determined in order to account for the differential effect that weather variables may have in different stages of crop development. This information is usually hard to obtain directly or, as in our case, not available. For this reason, the phenology of the crop was defined according to flowering time (days to 50% flowering, FT), which was measured for each line every year. With this measure, and sowing and harvest date, three main phenology stages were determined for each year,

according to Yoshida (1981): vegetative stage (of variable length, starting on sowing date), reproductive stage (starting 35 days before FT), and maturation stage (ending 30 days after FT).

Once these phenological stages are defined for each year, EC can be computed from daily weather data. Covariates with zero variance were removed from the analysis. For prediction, both markers and EC were centered by subtracting the mean, and standardized to unit variance by dividing the centered values by the standard deviation of the marker or EC. A total of 54 EC were used in both populations (18 for each developmental stage: vegetative, reproductive, and maturation), and are summarized in Table 3.2.

Table 3.2: Environmental covariates used in this study.

EC abbreviation	Explanation
ThermAmp	Thermal Amplitude (°C): Average of daily thermal amplitude calculated as (max temperature (°C) – min temperature (°C).
RelSun	Relative sunshine duration (%): Quotient between the real duration of the brightness of the sun and the possible geographical or topographic duration.
SolRad	Solar radiation (cal/cm2/day): Solar radiation calculated with the Armstrong's formula.
EfPpit	Effective Precipitation (mm): Average of daily precipitation in mm that is actually added and stored in the soil.
DegDay	Degrees Day in rice (°C): Mean of Daily average temperature minus 10 °.
RelH	Relative humidity (hs): Sum of daily amount of hours (0hs-24hs) where the relative humidity was equal to 100%.
PpitDay	Precipitation day: Sum of days where it rained.
MeanTemp	Mean Temperature in 24 hs (°C): Average of temperature over 24 hs (0-24 hs).
AvTemp	Average Temperature in 24 hs (°C): Average Temperature calculated as daily (Max+Min)/2.
MaxTemp	Maximum Temperature (°C): Average of maximum daily temperature.
MinTemp	Minimum Temperature (°C): Average of minimum daily temperature.
TankEv	Tank water evaporation (mm): Amount of evaporated water under influence of sun and wind.
Wind	Wind speed (2m/km/24hs): Distance covered by wind (in km) over

	2m height in one day.
PicheEv	Piche Evaporation (mm): Amount of evaporated water without the influence of the sun.
MinRelH	Minimum relative humidity (%): Lowest value of relative humidity for the day.
AccumPpit	Accumulated precipitation (mm): Daily accumulated precipitation.
Sunhs	Sunshine duration: Sum of total hours of sunshine per day
MinT15	Minimum temperature below 15°: Sum of the days where the minimum temperature was below 15°.

3.3.5 PLS regression

PLS regression was first introduced by Wold (1966), and was originally developed for econometrics and chemometrics. It is a multivariate statistical technique that was designed to deal with the $p >> n$ problem; i.e., when the number of explanatory variables (p) is much larger (and more highly correlated) than the number of observations (n). A brief explanation of PLS relating one response variable $(\mathbf{y})$ to a set of explanatory variables $(\mathbf{X})$ is given below, but it can be extended to more than one response variable (Boulesteix and Strimmer 2006; Wold 2001).

In PLS, the data for p explanatory variables are given by the matrix $\mathbf{X} = (\mathbf{x}_1, \ldots, \mathbf{x}_p)$, and data for the dependent variables are given by the response vector $\mathbf{y}$. Each $\mathbf{x_1}, \ldots, \mathbf{x_p}$ and $\mathbf{y}$ vectors have $n \times 1$ dimensions corresponding to the number of observations. In this work, the $\mathbf{y}$ vector contains all the observations for a given trait in different environments (years), and the columns of the $\mathbf{X}$ matrix are the variables corresponding to either markers only, or markers and EC. All variables in PLS must be centered and scaled.

PLS is based on the latent variable (LV) decomposition:

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathrm{T}} + \mathbf{E}, \quad (1)$$

$$\mathbf{y} = \mathbf{T}\mathbf{q}^{\mathrm{T}} + \mathbf{f}, \quad (2)$$

where $\mathbf{T}$ is a $n \times c$ matrix giving the LV (also called scores) for the n observations, and $\mathbf{P}$ $(p \times c)$ is a matrix of p-dimensional orthogonal vectors called X-loadings, $\mathbf{q}$ $(1 \times c)$ is a vector of scalars and , also named Y-loadings, $\mathbf{E}$ $(n \times p)$ and $\mathbf{f}$ $(n \times 1)$ are a residual matrix and vector respectively.

The LV matrix $\mathbf{T}$ that relates the $\mathbf{X}$ matrix to the vector $\mathbf{y}$ is calculated as:

$$\mathbf{T} = \mathbf{XW}, \qquad (3)$$

where $\mathbf{W}$ is a $(p \times c)$ matrix of weights. For a given matrix $\mathbf{W}$, the LV obtained by forming corresponding linear transformations of the variables in $\mathbf{X}$, $X_1,\ldots,X_p$ are denoted as $T_1,\ldots,T_c$:

$$\begin{aligned} T_1 &= w_{11}X_1 + \ldots + w_{p1}X_p \\ &\vdots \\ T_c &= w_{1c}X_1 + \ldots + w_{pc}X_p \end{aligned}$$

These LV are then used for prediction in place of the original variables. After computing the $\mathbf{T}$ matrix, $\mathbf{q}^{\mathbf{T}}$ is obtained as the least squares solution of Eq. (2):

$$\mathbf{q}^{\mathbf{T}} = \left(\mathbf{T}^{\mathbf{T}}\mathbf{T}\right)^{-1}\mathbf{T}^{\mathbf{T}}\mathbf{y}.$$

The vector $\mathbf{b}$ of regression coefficients for the model $\mathbf{y} = \mathbf{Xb} + \mathbf{f}$, to predict new responses, is calculated as:

$$\mathbf{b} = \mathbf{W}\mathbf{q}^{\mathbf{T}} = \mathbf{W}\left(\mathbf{T}^{\mathbf{T}}\mathbf{T}\right)^{-1}\mathbf{T}^{\mathbf{T}}\mathbf{y}.$$

Since regression and dimension reduction are performed simultaneously, all $\mathbf{b}$, $\mathbf{T}$, $\mathbf{W}$, $\mathbf{P}$ and $\mathbf{q}$ are part of the output. Both $\mathbf{X}$ and $\mathbf{y}$ are taken into account when calculating the LV in $\mathbf{T}$. Moreover, they are defined so that the covariance between the LV and the response is maximized.

In PLS, the optimal number of LV (c) must be determined. In this work, we used the root means squared error of prediction (RMSEP),

$$RMSEP = \sqrt{\frac{1}{10}\sum_{k=1}^{10}\left(\hat{\mathbf{y}}_k - \mathbf{y}_k\right)}$$

which was minimized with 10-fold cross-validation in the training data set and for each value of LV (Mevik and Cederkvist, 2004). In this study, two PLS models were fitted: the PLS-G model used marker covariates as predictors, and the PLS-GW model, which used both marker covariates and EC as predictors. PLS models calculations were performed with the R package "mixOmics" (Lê Cao et al. 2016).

3.3.6 Genomic Best Linear Prediction (GBLUP) and reaction norm models

Mixed linear models were used as a baseline comparison of prediction accuracies with PLS models. The models used considered the random main effects of markers (G model), the random main effects of markers and EC (G+W model), and the random main effects of markers, EC, and the interactions between them (G+W+GW model).

The G model constituted of a standard GBLUP model for the mean performance of genotypes within each set of environments, using the following model:

$$y_i = \mu + g_i + \varepsilon_i, \qquad (4)$$

where μ is the overall mean, g_i is the genotypic random effect of the ith line expressed as a regression on marker covariates of the form: $g_i = \sum_{m=1}^{p} x_{im} b_m$, where x_{im} is the genotype of the ith line at the mth marker, and b_m is the effect of the mth marker. Marker effects are considered as IID draws from normal distributions of the form $b_m \overset{IID}{\sim} N\left(0, \sigma_b^2\right)$, $(m =, \ldots, p)$.

The vector $\mathbf{g} = \mathbf{Xb}$ contains the genomic values of all the lines, and follows a multivariate normal density with null mean and covariance matrix $Cov(\mathbf{g}) = \mathbf{G}\sigma_g^2$, where $\mathbf{G}$ is a genomic relationship matrix whose entries are given by $\mathbf{G} = \mathbf{XX}^{\mathrm{T}}/p$.

As previously reported by Jarquín et al. (2014), it is possible to model the environmental effects with a random regression on the EC that describes the environmental conditions faced by each genotype, that is: $w_{ij} = \sum_{q=1}^{Q} W_{ijq}\gamma_q$, where W_{ijq} is the value of the q$^{\text{th}}$ EC evaluated in the ij$^{\text{th}}$ environment × genotype combination, γ_q is the main effect of the corresponding EC, and Q is the total number of EC. Again, we consider the effects of the EC as IID draws from normal densities, $\gamma_q \overset{IID}{\sim} N\left(0,\sigma_\gamma^2\right)$. The vector $\mathbf{w} = \mathbf{W}\gamma$ follows a multivariate normal density with null mean and a covariance matrix proportional to $\mathbf{\Omega}$ whose entries are computed the same way as those of the $\mathbf{G}$ matrix but using EC instead of markers. This covariance structure describes the similarity among environmental conditions. Then, the model becomes:

$$y_{ij} = \mu + w_{ij} + g_j + \varepsilon_{ij} \qquad (5)$$

This model also includes a marker × EC interaction term, where the covariance of the interaction is modeled by the Hadamard product of $\mathbf{Z_g G Z_g^T}$ and $\mathbf{\Omega}$, denoted as $\left[\mathbf{Z_g G Z_g^T}\right] \circ \mathbf{\Omega}$, where $\mathbf{Z}_g$ is an incidence matrix for the vector of additive genetic effects. This model extends Eq. (4) as follows:

$$y_{ij} = \mu + w_{ij} + g_j + gw_{ij} + \varepsilon_{ij}, \qquad (6)$$

with $\mathbf{w} \sim N\left(\mathbf{0}, \mathbf{\Omega}\sigma_w^2\right)$, $\mathbf{g} \sim N\left(0, \mathbf{G}\sigma_g^2\right)$, $\mathbf{gw} \sim N\left(\mathbf{0}, \left[\mathbf{Z_g G Z_g^T}\right] \circ \mathbf{\Omega}\sigma_{gw}^2\right)$, $\varepsilon \overset{IID}{\sim} N\left(0, \sigma_\varepsilon^2\right)$.

3.3.7 Assessing prediction accuracy for new environments

The prediction problem studied here was that of predicting future seasons, also denoted as "leave one environment out" prediction scenario. This prediction was performed by including phenotypic records and parameter information of either two (*indica* dataset) or four (*tropical japonica* dataset) years in the training population to predict a third (*indica* dataset) or fifth (*tropical japonica* dataset) year, where no phenotypic data were collected. Prediction accuracies obtained from both PLS and Reaction norm models were assessed by calculating the Pearson correlation between the predicted values from each model for a particular testing year, and the observed phenotypic values for that same year.

3.3.8 QTL by EC interactions

For the detection of QTL by environment interaction we used a two-step strategy as described in Gutiérrez et al. (2015). In the first step, we scanned the genome of both *indica* and *tropical japonica* subspecies to detect QTL in individual environments (single environment QTL mapping). In the second step, QTL expression across environments was regressed on environmental covariates in order to explain QTL effects in terms of sensitivities to environmental covariates (Malosetti et al. 2004; Boer et al. 2007; Malosetti et al. 2013).

For the first step, we fitted a mixed model for single environment QTL detection. The model used was the kinship model with:

$$\boldsymbol{y} = \mathbf{X}\beta + \mathbf{Z}\boldsymbol{u} + \boldsymbol{e},$$

where $\boldsymbol{y}$ is the vector of phenotypic means for that environment, $\mathbf{X}$ is the molecular marker score matrix, β is the vector of marker effects, $\mathbf{Z}$ is an incidence matrix, $\boldsymbol{u}$ is the vector of random background polygenic effects with variance $\sigma_u^2 = \mathbf{K}\sigma_G^2$ (where $\mathbf{K}$

is the kinship matrix, and σ_G^2 is the genetic variance), and $\boldsymbol{e}$ is the vector of residuals. A GWAS analysis for each dataset, trait and environment was performed using the R statistical software (R Core Team, 2017) with the package GWASpoly (Rosyara et al. 2016) fitting the additive model. For QTL determination in each environment, we used the Benjamini-Hochberg FDR (α=0.05) to control the type I error (Benjamini and Hochberg 1995).

In the second step, all marker-trait associations detected in the first step were fitted in a second mixed model testing for interaction with all available EC. This model assumes a linear relationship between the effect of the QTL and a given environmental covariate, using the model presented in Malosetti et al. (2013) given by:

$$y_{ij} = \mu + E_j + x_i(\alpha_q + \beta_q z_j + \underline{a}_{jq}) + \underline{G}_i + \underline{GE}_{ij}$$

where y_{ij} is the phenotype of individual i at environment j, μ is the general mean, E_j effect of the j[th] environment, x_i is the value of the i[th] marker predictor, α_q is the effect of the q[th] QTL in the average environment, β_q corresponds to the change of the QTL effect per unit of change of the covariable's value, and $\underline{a}_{iq}$ is the random effect corresponding to the residual (unexplained) QTL effect, with $\underline{a}_{iq} \sim N(0, \sigma_{aq}^2)$, $\underline{G}_i$ is the random remaining (not due to the QTL) genotype effect with $\underline{G}_i \sim N(0, K\sigma_G^2)$, and $\underline{GE}_{ij}$ is the remaining (random) G×E effect, with $\underline{GE}_{ij} \sim N(0, \boldsymbol{\Sigma})$. All EC were tested for interaction with Three different models for the variance-covariance matrix Σ were compared: compound symmetry (CS) where the genetic variances are homogeneous across environments ($\sigma_G^2 + \sigma_{GE}^2$) and the genetic covariances between environments are modeled by σ_G^2; heterogeneous compound symmetry (HCS), which allows for heterogeneous genetic variances across environments ($\sigma_{G_j}^2$) and a common genetic

covariance parameter σ_G^2; and the unstructured (UN) model with a specific genetic variance parameter per environment and a specific genetic covariance between environment. The different models were compared using the Bayesian information criterion (BIC) to select the optimal model (Broman and Speed 2002). We tested for the significance of the fixed terms in mixed models using Wald test at a *p* value of 0.05, following Malosetti et al. (2004). For QTLxEC interaction testing we used the Benjamini-Hochberg FDR (α=0.05) to control the type I error (Benjamini and Hochberg 1995). Mixed models for QTL×EC interaction were computed with the R package *sommer* (Covarrubias 2016).

3.4 RESULTS

3.4.1 Phenotypic data analysis

The *indica* dataset was balanced with a total of 327 lines per environment, while the *tropical japonica* dataset was unbalanced, with a total of 23 lines common to all environments (Table 3.1). Estimations of broad-sense heritability estimated on a line-mean basis per trait by year for both datasets were medium to high, with PHR having the highest values of heritability in both datasets.

Table 3.3 shows the partitioning of the observed phenotypic variance into different sources of variation for both rice datasets. In the *indica* population, PHR and GC showed the highest proportion of variance explained by G×Y, at 20.04% and 13.22%, respectively. On the other hand, the year component was the highest variance component for GY and PH (Table 3.3). In the *tropical japonica* population, the year component was the highest; it was above all components for the four traits, and much higher than for the *indica* population. In contrast, the G×Y component was lower in *tropical japonica* compared to *indica* (Table 3.3).

Table 3.3: Trait variance component estimation and proportion of the total variance explained for the four traits evaluated in Uruguayan *indica* and *tropical japonica* populations. GY: grain yield, PHR: percentage of head rice, GC: percentage of chalky grains, PH: plant height.

indica					
GY			PHR		
Group	Variance	%	Group	Variance	%
Year	496540	18.9	Year	0.0001	10.02
Genotype	379554	14.5	Genotype	0.0002	20.04
GxY	143374	5.5	GxY	0.0002	20.04
Column	55361	2.1	Column	0.000007	0.70
Row	31357	1.2	Row	0.000006	0.60
Block	516792	19.7	Block	0.0002	20.04
Residual	1000130	38.1	Residual	0.000285	28.56
GC			PH		
Group	Variance	%	Group	Variance	%
Year	0.0003	13.22	Year	12.51	9.26
Genotype	0.0004	17.62	Genotype	8.91	6.60
GxY	0.0003	13.22	GxY	5.56	4.12
Column	0.0003	13.22	Column	0.84	0.62
Row	0.00007	3.08	Row	0.92	0.68
Block	0.0005	22.03	Block	8.31	6.15
Residual	0.0004	17.62	Residual	98.02	72.57
tropical japonica					
GY			PHR		
Group	Variance	%	Group	Variance	%
Year	1988252	43.2	Year	0.001	41.36
Genotype	197961	13.2	Genotype	0.0003	12.41
GxY	99052	5.1	GxY	0.0001	4.14
Column	24932	0.3	Column	0.000008	0.33
Row	19062	0.5	Row	0.00001	0.41
Block	115775	22.7	Block	0.0006	24.81
Residual	682613	15.1	Residual	0.0004	16.54
GC			PH		
Group	Variance	%	Group	Variance	%
Year	0.005	66.32	Year	37.5	43.7
Genotype	0.0007	9.29	Genotype	18.4	21.4
GxY	0.0006	7.96	GxY	1.6	1.8
Column	0.000009	0.12	Column	0.1	0.1
Row	0.00003	0.40	Row	0.1	0.2
Block	0.0004	5.31	Block	13.3	15.5
Residual	0.0008	10.61	Residual	14.8	17.2

3.4.2 Genomic prediction of untested years

Bar plots showing prediction accuracy for the four traits in the *indica* population are shown in Figure 3.1. PLS-based methods showed higher prediction accuracies than reaction norm-based models for all traits except GC, where prediction accuracies for the PLS method using both markers and EC (PLS-GW) were the same as the reaction norm models. For PLS models, the use of EC in addition to molecular markers resulted in higher prediction accuracies in all cases, though PHR in 2011 and GC in 2012 had identical prediction accuracies for both methods. For reaction norm models, fitting the main effect of genotypes, environments and interaction (G+W+GW model) resulted in either lower or equal prediction accuracies than fitting the simpler model without the interaction term (G+W model) (Figure 3.1).

In the *tropical japonica* population, the use of PLS-based models was always better than reaction norm models, with the single exception of GY in 2010 (Figure 3.2). In all cases, including both markers and EC (PLS-GW) was better than using markers only (PLS-G). Within the reaction norm models, the G+W method was the best, with the exception of GC in 2013. Fitting a G×E component in these models resulted in lower prediction accuracies than fitting the G+W model (Figure 3.2).

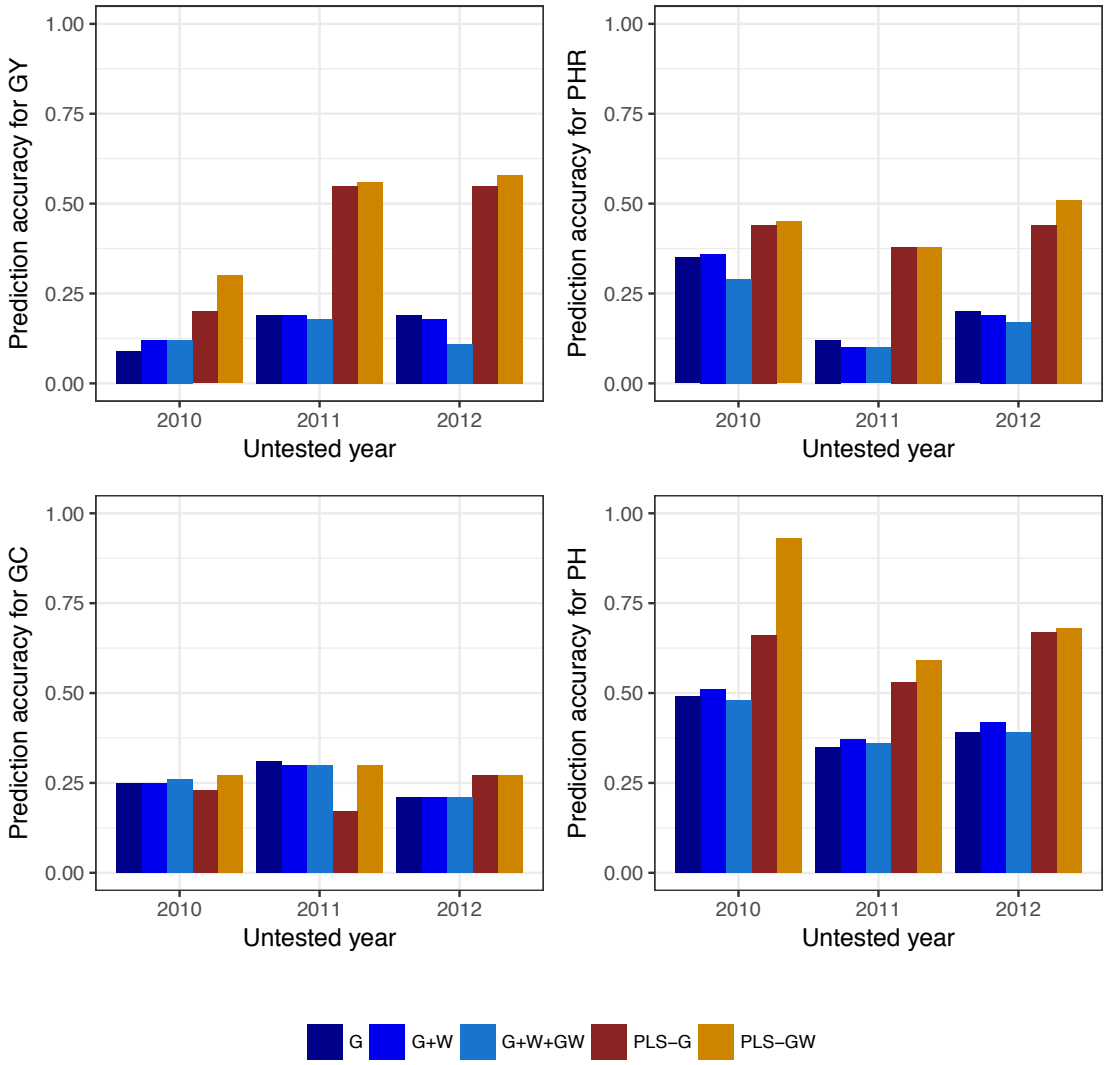

Figure 3.1: Correlations between predicted vs. observed values for Grain Yield (GY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) for predicting untested years with the G, G+W, G+W+GW, PLS-G and PLS-GW for the *indica* rice breeding population. G = genotypic main effect modeled with marker covariates, W = Environmental main effect modeled with EC, GW = interaction between genotypic and environmental effects, PLS-G = Partial least squares using marker covariates as predictors, PLS-GW = Partial least squares using marker covariates and EC as predictors.

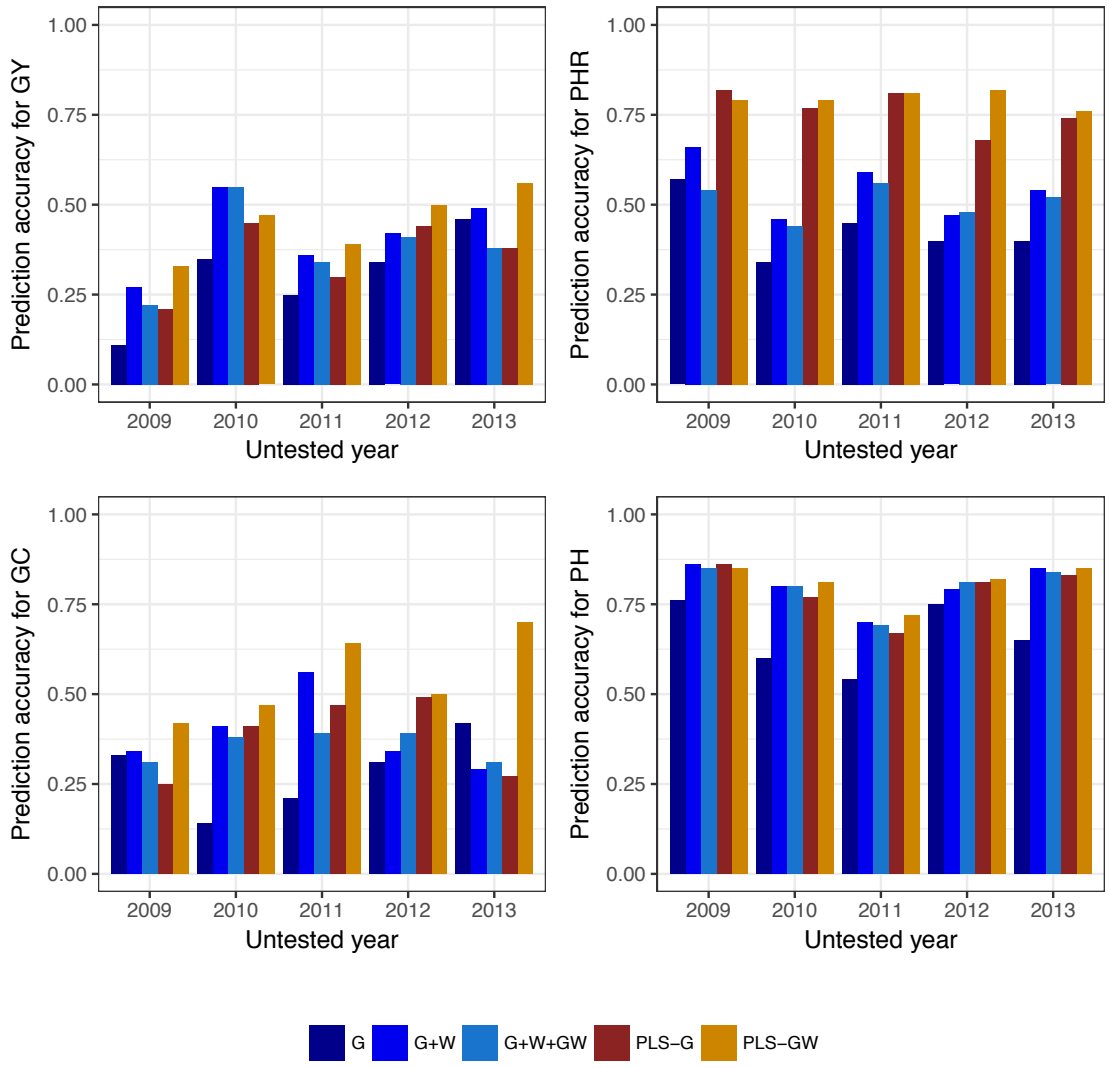

Figure 3.2: Correlations between predicted vs. observed values for Grain Yield (GY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) for predicting untested years with the G, G+W, G+W+GW, PLS-G and PLS-GW for the *tropical japonica* rice breeding population. G = genotypic main effect modeled with marker covariates, W = Environmental main effect modeled with EC, GW = interaction between genotypic and environmental effects, PLS-G = Partial least squares using marker covariates as predictors, PLS-GW = Partial least squares using marker covariates and EC as predictors.

Many of the EC used in this study were correlated. This may result in lower prediction accuracies in the reaction norm model, since it would weight the environmental covariances toward the highly correlated variables. Reaction norm models with a subset of less correlated variables were tried, resulting in very similar or even lower prediction accuracies than when using the entire set of EC (data not shown).

When running PLS with all the environments within each dataset, we can detect which variables best explain each trait by looking at the coefficients. Table 3.4 shows the ranking of coefficients for the EC variables for each trait in both datasets. For GY, variables related to temperature and humidity during flowering stage were among the most important. For PHR and GC, the 5 variables with the highest coefficients were related to temperature, humidity and solar radiation during maturation. In the *tropical japonica* dataset, variables related to humidity, solar radiation and rainfall during maturation showed the highest coefficients for PHR and GC. For GY, two variables at flowering time showed higher coefficient values than the rest: maximum temperature and wind speed (Table 3.4).

3.4.3 Detecting QTL in single environments

We searched for significant trait-marker associations in single years to find QTL to test for interactions with EC in the next step. In this first analysis, we could not find any QTL that passed the FDR threshold for GY in any environment in any population. In the case of PH, we did not find any QTL for the *indica* population, but we found one major effect QTL on chromosome 1 (position: 37,755,448 - 38,755,448 bp) that was significant in all environments in the *japonica* dataset; it corresponds to the *sd-1* gene (position: 38,363,881 bp).

We detected QTL for grain quality traits in both datasets (Table 3.5). In the *indica* population, a total of 13 QTL (chromosomes 1, 2, 3, 4, 6, 7, 10 and 11) were found for PHR, and a total of 4 QTL (chromosomes 1, 3 and 4) for GC. QTL were found only in years 2010 and 2012 for PHR, and in years 2011 and 2012 for GC. Three of the QTL were reported in a previous GWAS analysis using this same dataset (Quero et al. 2018). These QTL were: qPHR.i.2.2 (S2_24210614), qPHR.i.3.1 (S3_10247958), qGC.i.1.1 (S1_1066894). Two additional QTL were in LD with two previously reported QTL in the same study. These were qPHR.i.3.2 and qPHR.i.6.1, which were in LD with S3_15365726 and S6_829223 in our study, respectively. In the *tropical japonica* population, a total of 5 QTL were found for PHR (chromosomes 1, 2, 3, 6, and 8), and one for GC (chromosome 6) (Table 3.5). Two of these QTL were in LD with previously reported QTL: qPHR.j.3.1 with S3_1395165, and qGC.j.6.2 with S6_27402260 (Quero et al. 2018). No significant QTL were found for GY or PH for any year in either of the populations.

Table 3.4: Top 5 PLS-GW coefficients for the environmental covariates for Grain Yield (GY), Head Rice Percentage (PHR), Grain Chalkiness percentage (GC) and Plant Height (PH) for the *indica* and *tropical japonica* rice breeding populations.

indica					
		Coefficient			
Type	Variable	GY	PHR	GC	PH
Temperature	ThermAmp_V	-	0.0001251	-0.000403	-
	MinTemp_R	0.005277	-	-	-0.00751
	MeanTemp_R	0.005265	-	-	-0.00773
	ThermAmp_M	-	0.0001273	-0.000402	-
Precipitation	EfPpit_R	0.005159	-	-	-0.00723
Evaporation	TankEv_V	0.005185	-	-	-0.00772
	TankEv_M	0.0001272	-	-0.000407	-
Humidity	MinRelH_M	-	-0.0001270	0.000406	-
Radiation	SolRad_M	-	0.0001267	-0.000402	-
Wind	Wind_V	-0.005108	-	-	0.00766
tropical japonica					
		Coefficient			
Type	Variable	GY	PHR	GC	PH
Temperature	MinTemp_V	-	-	-	-0.014
	MaxTemp_V	0.0102	-	-	-0.015
	MeanTemp_V	-	-	-	-0.016
	DegDayRice_V	-	-	-	-0.016
	MaxTemp_R	-0.0125	-	-	-
	AvTemp_M	-0.0101	-	-	-
Precipitation	PpitDay_R	-	-	-	0.014
	EfPpit_M	-	0.013	0.018	-
	AccumPpit_M	-	-0.013	-	-
Evaporation	PicheEv_V	-	0.013	-	-
Humidity	MinRelH_M	-	-0.015	0.019	-
Radiation	Helhs_M	-	0.013	-0.018	-
	SolRad_M	-	-	-0.017	-
	RelHel_M	-	-	-0.017	-
Wind	Wind_V	-0.0103	-	-	-
	Wind_R	-0.0126	-	-	-

Table 3.5: Marker-trait associations for percentage of head rice (PHR) and percentage of chalky grain (GC) traits in *indica* and *tropical japonica* rice breeding populations. Chromosome position (bp), year, effect of the alternative allele, and score ($-\log_{10}$(p-value)) are shown in the table.

indica					
Marker	Chr	Position	Year	Alt allele effect (%)	Score
PHR					
S1_1015065	1	1015065	2010	-1.62	4.98
S2_24210614	2	24210614	2012	2.95	7.20
S3_8880979	3	8880979	2010	1.32	4.60
S3_10247958	3	10247958	2010	-2.12	9.97
S3_15365726	3	15365726	2010	-1.85	7.19
S4_29728982	4	29728982	2010	1.30	4.68
			2012	2.37	5.66
S6_829223	6	829223	2010	1.76	5.04
S6_11022101	6	11022101	2012	-2.10	5.30
S6_13215923	6	13215923	2010	-1.24	5.47
S6_21327503	6	21327503	2012	2.63	5.54
S7_14798606	7	14798606	2010	1.25	5.05
S10_6737554	10	6737554	2010	2.40	5.64
S11_24425810	11	24425810	2010	1.92	4.70
GC					
S1_1066894	1	1066894	2011	1.33	4.06
S1_22492066	1	22492066	2012	1.45	5.74
S3_16037360	3	16037360	2011	0.72	4.07
S4_22480721	4	22480721	2011	1.24	4.14
Tropical *japonica*					
PHR					
S1_38686312	1	38686312	2013	2.0	3.20
S2_27660046	2	27660046	2013	-1.0	4.41
S3_1395165	3	1395165	2011	1.0	6.18
S6_27834772	6	27834772	2011	-2.0	4.89
			2013	-1.0	3.44
S8_23380395	8	23380395	2013	-2.0	4.11
GC					
S6_27402260	6	27402260	2011	2.00	4.62
			2013	2.00	3.94

3.4.4 QTL × environmental covariate interactions

A decomposition of the QTL with significant QTL × environment interaction was obtained by introducing environmental covariates as explanatory variables. We first tested different covariance structures for the modeling of the G×E component and compared them using BIC (see Methods). For all QTL and traits, BIC values decreased when using the HCS matrix compared to the CS matrix. However, the HCS model already behaves quite similar to the maximally complex UN model, so the HCS was the model of choice (data not shown).. The QTL responses for the *indica* dataset are shown in Table 3.6. One QTL showed significant interaction with environmental covariates related to precipitation and humidity during the maturation stage. Marker S2_24210614 showed a negative relationship with PpitDay_M and RelH_M. The high correlation between these two variables ($r = 0.99$) explains why they show the same coefficients for the main QTL effect (α), and the interaction (β). No significant main effect was detected for this QTL (Table 3.6).

Results for regression of marker covariates on environmental covariates for the *tropical japonica* dataset are shown in Table 3.7. For GC, marker S6_27402260, located in chromosome 6, showed a significant positive response to weather covariates related to precipitation and minimum temperature, and a negative response to sunshine duration and solar radiation. This marker also showed a significant main effect (Table 3.7).

Table 3.6: QTL responses to EC for percentage of head rice (PHR) in the *indica* rice population. Suffixes R and M mean Reproductive stage, and Maturation stage respectively.

Trait	Marker	Chromosome	Position	EC	α	β
PHR	S2_24210614	2	24210614	PpitDay_M	0.09	-0.03*
				RelH_M	0.08	-0.04*
				MinTemp_R	0.10	-0.04*

α : QTL main effect
β: Slope parameter for the QTL×EC parameter
* significance level at α = 0.05

Table 3.7: QTL responses to EC for head rice percentage (PHR) and percentage of chalky grain (GC) in the *tropical japonica* rice population. Suffixes R and M mean Reproductive stage, and Maturation stage respectively.

Trait	Marker	Chromosome	Position	EC	α	β
GC	S6_27402260	6	27402260	TempMin15_M	0.2**	-0.08**
				MinTemp_M	0.3***	0.3**
				SolRad_M	0.3*	-0.2*
				PpitDay_M	0.4***	0.07**
				RelSun_M	0.4**	-0.08**
				Sunhs_M	0.4**	-0.7**

α : QTL main effect
β: Slope parameter for the QTL×EC parameter
* significance at α = 0.05
** significance at α = 0.01
*** significance at α = 0.001

3.5 DISCUSSION

In this work we proposed to characterize and interpret G×E interaction for four traits (GY, PHR, GC and PH) in two different breeding populations of rice (*indica* and *tropical japonica*) grown in a subtropical/temperate climate. In the first part of our paper, we compare the performance of different genomic prediction models that account for genotype, environment and G×E components, to predict untested years, and we identify the most influential weather covariates for our two datasets. In the second part, we map environment-specific QTL and study the environmental variables that affect their expression, in order to interpret the QTL×E effects that account for the total G×E.

3.5.1 Prediction accuracies for untested environments

Usually genomic prediction models are tested and compared using cross-validation strategies. In a multiple environment context, most studies include two basic random cross-validation schemes (Burgueño et al. 2012): CV1, which tests the performance of lines that have not been evaluated in any of the observed environments, and CV2, which tests the performance of lines that have been evaluated in some environments but not in others. These two scenarios have the disadvantage of training and validating the models with the same data, which could lead to an overestimation of the prediction accuracy the model would attain if it had been applied in an independent test dataset. Predicting new environments is a more difficult task but could represent a good validation strategy because the performance of prediction models is assessed in an independent dataset. In this work we used a cross-validation scheme for prediction in untested environments, represented by years, a component of G×E that is not easy to reproduce. This is a very relevant type of prediction for a small plant breeding program, where data from multiple locations is either limited or absent, and the need is to predict

which lines are more likely to perform better in future environments. The use of EC to model the environment component explicitly has been previously shown to increase prediction accuracies for untested environments (Malosetti et al. 2016, Jarquín et al. 2017), and this situation also applies to our work.

For prediction, we compared two modeling approaches that differ in the way that multiple and correlated variables are handled: 1) a variance components approach that allows modeling the main and interaction effects of markers and EC using covariance structures, and 2) a PLS approach that models genotype and environment effects by identifying a linear combination of all the explanatory variables, providing latent vectors that optimally predict the response variable. We found that the PLS-GW model was in all cases superior to or not different from PLS-G and reaction norm models in both datasets. Although the variance explained by the G×E component in the *indica* population was comparable in some cases to the variance explained by the genotype and/or the year main components, the proportion of variance explained jointly by the genotype, environment and G×E components, was never superior to 50% of the total variance. This could explain the lower prediction accuracies obtained in this population compared to the *japonica* population. It is possible that the EC used in this study explained only a limited proportion of the across environment interaction in the *indica* dataset, and for this reason reaction norm models, when fitting covariance matrices for the environment and marker by environment interaction, did not improve prediction accuracies in comparison to the simpler GBLUP model. In the *japonica* population, the proportion of the total variance explained by G×E was very low compared to the main genotype and environment components, which also explains why modeling a specific interaction covariance matrix did not give better results than modeling the main genotype and environment covariance matrices alone. In this

population, the main environment effect was better represented by the EC, and thus, prediction accuracies, when including an EC covariance matrix (W) or the EC in the PLS model, were higher than when using a G matrix or molecular markers alone.

Besides the ability of handling numerous and correlated predictors, an additional advantage of using PLS models is that we can detect which covariates are the most explanatory in our model by looking at the model coefficients (Wold et al. 2001; Mehmood et al. 2012). Previous studies have shown the benefits of PLS for identifying the set of EC that best explain G×E (Vargas et al. 1998; Vargas et al. 1999; Crossa et al. 1999). In these studies, the G×E component of the trait was used as a response and regressed to EC only. In our case, we decided to report the results of the regression of the trait means to both EC and markers, since regressing the G×E component to EC resulted in increased MSEP with an increasing number of components, and thus a poor model fit. For GY, minimum and average temperature, and effective precipitation during flowering time showed the highest positive coefficients for *indica* rice. In regions with a temperate climate, low temperatures during flowering can affect grain yield by inducing spikelet sterility (Yoshida 1981, Alvarado 2002). The probability of occurrence of temperatures under 15°C during January (when rice usually enters the flowering stage) in Eastern Uruguay is about 20%, and would be most detrimental for *indica* varieties, which are best adapted to tropical climates. In the *tropical japonica* population, the two EC that showed the highest (absolute value) coefficients for GY were wind speed during flowering, and maximum temperature during grain filling. Both wind speed and high temperatures during reproduction have been proven to negatively affect GY due to pollen dehydration and consequent spikelet sterility (Marchezan and da Silva 1993; Raju et al. 2013).

For the grain quality traits, EC related to humidity, solar radiation and sunshine duration during grain ripening were among the most important in both datasets. The positive coefficients for solar radiation, and the negative coefficients for humidity reflect the relative effects of these variables on milling quality, as previously reported (Siebenmorgen et al. 2012; Edwards et al. 2017). Many studies have reported negative effects of high temperatures on grain chalk and percent head rice (Tashiro and Wardlaw, 1991, Lyman et al. 2013). For example, for *japonica* cultivars, temperatures higher than 26°C can cause chalky grain appearance (Chen et al. 2016), but maximum daytime temperatures higher than 33°C cause dramatic changes in the distribution of head and broken rice, and increase the proportion of chalky grain (Ambardekar et al. 2011; Lyman et al. 2013). In Eastern Uruguay, maximum temperatures during February-March, the period in which rice kernels usually develop, rarely reach 32°C. In our own dataset, the average maximum temperatures were never higher than 30°C, so it is probable that in the absence of high stress-inducing temperatures in sub-tropical rice growing areas, other variables such as humidity and solar radiation are more important, as is reflected in our results.

3.5.2 QTL detection and interaction with environmental covariates

For this part of the analysis we used mixed-models to analyze QTL by EC interactions because of their flexibility, and the possibility of modeling genetic correlations between environments. We first performed an association mapping analysis for each of the four traits in each environment in both populations. In the case of PH, Rosas et al. (2017) performed a GWAS analysis on these same populations using the mean across environments and found a major effect QTL corresponding to the *sd-1* gene which was segregating in the *japonica* population, but fixed in the semi-dwarf

indica population (Rosas et al. 2017). When we performed a single environment scan we could not find any other QTL in either population, other than a major-effect QTL corresponding to the *sd-1* gene in *japonica.*

Of the 23 QTL we found for PHR and GC in both populations, 8 were coincident with QTL reported by Quero et al. (2018) in the same populations using the mean across environments. For PHR in *indica*, we found evidence of one genomic region, located on chromosome 2 that is affected by humidity, one of the main environmental factors that affect milling quality in rice (Cooper et al. 2008, Zhao and Fitzgerald 2013).

Two putative QTL in *tropical japonica* were co-located on chromosome 6: S6_27834772 for PHR and S6_27402260 for GC. These two QTL are in LD with qPHR.j.6.1 and qGC.j.6.2 previously found by Quero et al. (2018), and contain genes related to starch metabolism, such as *OsBEI* (LOC_Os06g51084). It is known that the expression of starch branching enzymes, like *OsBEI*, can be affected by temperature (Yamakawa et al. 2007; Sreenivasulu et al. 2014). According to our results, QTL S6_27402260 showed interaction with low temperature, precipitation and sunshine duration and solar radiation for GC. Other researchers have shown that periods of intense solar radiation and high humidity during the ripening stage can increase the incidence of chalky grains (Wakamatsu and Tanaka 2009, Zhao et al. 2016). But these reports do not constitute enough proof that there is a causal relationship between the expression of these QTL and the EC, because many EC are correlated in a complex way and not all EC were observed. In temperate climates, where day and night temperatures are never as high as in the tropics, other environmental factors such as humidity and solar radiation can affect milling quality in a negative way. These findings should be

confirmed by analyzing more lines in more environments to properly quantify QTL main and environment-specific effects.

The approach of mapping QTL by environment interaction used in this study requires a QTL to have a strong effect in a specific environment. This poses the limitation that QTL with smaller effects in individual environments but capable to explain larger proportions of the observed phenotypic G×E may be overlooked. In our datasets, the proportion of phenotypic variance explained by the G×Y component is low in the *tropical japonica* dataset (1.8%-7.96%, Table 3), but higher in the *indica* dataset (4.12%-20%). However, approaches for testing for genotype by year interaction at each SNP were performed and no significant QTL were found.

In this work we used PLS, multiplicative reaction norm and mixed models to analyze our data, predict genotypic performance for yield, height and milling quality traits, and detect QTL by EC interactions. In all these analyses we assumed that the relationships between molecular markers and EC were linear, which constitutes a major limitation since interactions between genes and environmental conditions may take many different forms. A next step would be to fit statistical models with more biological realism, using models that could accommodate non-linear and more complex responses over a more extensive number of environments. Crop growth models also hold promise as a way to integrate more complex biological knowledge into the prediction process of G×E (Bustos-Korts et al. 2015, Malosetti et al. 2016). Although rather small, our two datasets allowed us to extract some broad conclusions about the nature of G×E in the Uruguayan mega-environment. Additional research, including more environments and modeling non-linear relationships between genes and EC, will be of particular value to better understand and predict the nature of G×E for commercially relevant traits of rice grown in temperate regions. Results from PLS and

QTL by EC interactions suggest that in temperate and subtropical regions, humidity and solar radiation may have a stronger influence on milling quality traits than temperature, due to the fact that temperatures in these regions are never as high as in the tropics.

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CHAPTER 4:

STRATEGIES FOR CROSS SELECTION USING GENOMIC SELECTION FOR IMPROVING YIELD AND MILLING QUALITY TRAITS IN *tropical japonica* RICE

4.1 INTRODUCTION

Genomic prediction uses genome-wide molecular markers to predict the genotypic value of an individual for a trait of interest (Meuwissen et al. 2001). Marker effects are initially estimated from a training population that was previously genotyped and phenotyped, and these effects are then used to predict the performance of individuals in a test population that has been genotyped for the same markers. When these predictions are used as a selection strategy, it is called genomic selection (GS). GS has been extensively studied plant breeding, and several experimental studies have strongly indicated that it could be very useful in plant breeding (Asoro et al., 2013; Combs and Bernardo, 2013; Massman et al., 2013).

Selecting the best combinations between genotypes is key to accomplishing genetic gain in plant breeding. One of the main challenges for breeders focused on the development of inbred varieties, such as in rice, is to optimize the selection of parental lines for crossing in order to increase the selection response in subsequent cycles. The number of possible crosses is usually far greater than the number of lines the breeder can evaluate in the field.

Even when breeders try to use all the information available for selecting potential parents, many crosses are discarded in subsequent cycles, as they do not result in superior progeny (Heslot et al. 2015). Usually breeders focus on crosses among the highest performing breeding lines, trying to ensure both a high progeny mean, and a

large genetic variance (V_G) in the progeny population. Insufficient V_G in the progeny can be very detrimental to the progress of a breeding program, as can be seen from the breeder's equation (Falconer and Mackay, 1996). Response to selection (R) is dependent on selection intensity (i), trait heritability (h^2), and the phenotypic standard deviation of the population of selection candidates ($\sqrt{V_P}$).

The usefulness of a cross (Utz et al., 2001) is defined as $U = \mu + i\sigma_G h$, where μ and σ_G are the mean and the standard deviation of the genetic values of the lines derived from the cross, i is the selection intensity, and h is the square root of the heritability. The progeny mean of a cross is easily predictable from the mid-parent value (Bernardo, 2014). However, predicting the genetic variance of a progeny population is more difficult. Different methods have been proposed to predict genetic variance of a cross using either phenotype, pedigree or genetic distances (Souza and Sorrells, 1991; Utz et al. 2001; Melchinger et al. 1998), but none of these predictors provided good estimates of V_G (Mohammadi et al., 2015). Zhong and Jannink (2007) suggested estimating V_G from QTL effects estimates, assuming linked loci, and omitting h from the equation of usefulness, and they defined the superior progeny value as $s = \mu + i\sigma_G$. Their approach uses genetic additive effects estimated either from QTL mapping or genome-wide prediction. This approach was later validated by Mohammadi et al. (2015), Tiede et al. (2015), and Lado et al., (2017) by simulating progeny from all possible crosses in a set of parents, and calculating the mean and the V_G of each progeny.

Although grain yield is the primary focus for rice breeders, milling quality is also of great importance since both grain yield and milling quality jointly determine the economic value of rice from the field to the mill and in the market (Lyman et al., 2013). In many occasions, milling quality traits tend to be negatively correlated to paddy yield,

showing also a great influence of genotype-by-environment interaction (Lyman et al., 2013; Xu et al., 2015; Bao, 2018). For this reason, predicting and identifying crosses with the potential to deliver both high yield and high grain quality, and selecting the best progeny from these crosses, is of great importance for rice breeders.

The main objectives of this study were to 1) explore the use of genomic prediction to select the best combinations of parents for crossing, and 2) compare the predicted performance of the progeny of these crosses (calculated through genomic selection methods) with the empirical performance of these lines in the field.

For objective 1) we started from a training population (genotyped and phenotyped for yield and milling quality) from which 19 crosses were selected based on performance and genetic diversity preservation criteria (see Materials and Methods). From the simulated progeny of all possible cross combinations among these 19 F4 families, we assessed the impact of cross selection based only on mid-parent value or a combination of mid-parent value and progeny variance, and we also evaluated different parental selection criteria to deliver the parents best able to improve yield and particularly milling quality simultaneously. For objective 2), we used a total of 43 doubled-haploid (DH) families derived from random crosses among the 19 families, that were evaluated in the field in Uruguay for grain yield and milling quality.

4.2 MATERIALS AND METHODS

4.2.1 Training population

A *tropical japonica* population consisting of 309 elite breeding lines and two varieties (INIA Tacuari and EEA 404) belonging to the Uruguayan National Institute of Agricultural research (INIA), was used as training population in this study. Lines were evaluated during the growing seasons (October-March) in 2010-2011, 2011-2012, and

2012-2013 in replicated experiments. Adjusted means for each line were obtained using the following model:

$$y_{ijk} = \mu + \lambda_i + \beta_{j(i)} + G_k + \varepsilon_{ijk} \qquad [1]$$

where y_{ijk} is the response variable, μ is the overall mean, λ_i is a random variable associated with the *i*th trial with $\lambda_i \sim N(0,\sigma^2_\lambda)$, $\beta_{j(i)}$ is a random variable associated with the *j*th block nested within the *i*th trial with $\beta_{j(i)} \sim N(0,\sigma^2_\beta)$, G_k is the effect of the *k*th genotype, and ε_{ijk} is the residual error with $\varepsilon_{ijk} \sim N(0,\sigma^2_\varepsilon)$.

This population was evaluated for grain yield (GY), milling yield (MY), head rice percentage (PHR), and chalky grain percentage (GC). The phenotyping for these same traits of this training population was previously described in Chapters 2 and 3.

Best linear unbiased predictions (BLUPs) were obtained for each trait, using model [1] except that the genotypic effect was defined as random, assuming $G_k \sim N(0,\sigma^2_g)$. Pearson correlations among traits were calculated using BLUPs of phenotypic traits.

4.2.2 Crossing scheme for the training population

The main objectives of the crosses performed on the training population were to 1- Maintain genetic diversity, and 2- Improve milling quality and grain yield simultaneously by selecting those lines that showed good quality, and medium to high values for yield. Our strategy was to select genetically divergent lines for crosses using the information of the population genetic structure obtained from genetic diversity analyses. In addition, we tried to cross individuals with high BLUP values for one trait with individuals with high BLUPs for a different trait. Crosses were performed on field grown plants in February, 2014 at INIA Treinta y Tres Experimental Station, Treinta y

Tres, Uruguay (33°15'S, 54°25'W). At the time when crosses were scheduled, weather conditions in Treinta y Tres were not favorable for flowering and crossing, given that strong rain and thunder storms were registered during almost the entire period. This situation complicated all fieldwork, and the lack of sunshine during these days delayed plant flowering of many of the lines selected for crossing. This strongly affected the choice of crosses. Female and male plants were taken from the field to the lab, where females were manually emasculated and pollinated with pollen donors. Of the 26 crosses performed, 19 gave F1 seed and were sent to Cornell University in July 2014.

4.2.3 Crossing scheme for the F4 population

Twenty individuals from each of the 19 F1 families were planted in the greenhouse at Cornell University and advanced by single-seed descent until the F4 generation. All F4 lines were also genotyped by GBS at Cornell University. A total of 185 crosses were performed at random between the F4 families in the greenhouse, in order to obtain progeny from most of all possible cross combinations between families. A sample of F1 seed from all these crosses was then sent to the AgCenter-Rice Research Station at Louisiana State University (LSU) for Doubled Haploid (DH) line development.

4.2.4 Genotyping

Lines from both the training population and the F4 generation were genotyped using GBS in the Biotechnology Resource Center at Cornell University in 2014 and 2016 respectively. Single Nucleotide Polymorphism (SNP) calling was performed with the TASSEL version 3.0 GBS pipeline (Bradbury, 2017), and SNPs were aligned to the MSU Nipponbare reference genome version 7.0 using Bowtie version 2 (Langmead and

Salzberg, 2012). Both genotypic datasets were imputed using the FILLIN algorithm (Swarts et al., 2014). SNPs with a minor allele frequency <5% and >50% of missing data were filtered out. The final genotypic matrices contained 44,598 markers for the training population and 22,795 markers for the F4 population.

4.2.5 Population structure and principal component analysis for the training population

The Principal Component Analysis (PCA) was performed over the imputed numeric genotype marker matrix with alleles coded as -1, 0, and 1 (homozygous for the minor allele, heterozygous, and homozygous for the major allele, respectively). Population structure in the training population was analyzed with the software ADMIXTURE version 1.23 (Alexander et al., 2009). The number of populations (*k*) was selected according to two main criteria: first, the lowest cross-validation error across a range of *k* values (i.e., k=1-10); second, an *ad hoc* correspondence with pedigree information.

4.2.6 Cross simulation experiments

Cross simulation experiments were performed on both the training population and the 19 F4 families. The performance of each parent pairwise combination was calculated as follows: First, two different genomic prediction models (RR-BLUP and Bayesian LASSO) were evaluated for each trait in the parental populations (training population and F4) to obtain the marker effects. Parameters for the Bayes LASSO model were set according to Perez and de los Campos (2014), the iteration number was set at 12,000 and the first 300 iterations were discarded as burn-in. Random cross-validation was conducted by using 60% of the lines to train the models and predict the

remaining 40% of the lines. Each model was iterated 100 times and then the model with the highest prediction ability was used for subsequent analysis. All the parents in each of the two populations were used to estimate the marker effects and the parent's performance for each of the four traits: GY, MY, PHR, and GC. However, as there were no differences between the prediction accuracies in both models for any trait in either of the two populations, the RR-BLUP model was used to estimate the marker effects for all traits in both populations for the remaining analyses. Second, progeny of all possible pairwise cross combinations were simulated both in the training population and in the 19 F4 families, for GY, MY, PHR and GC, using the R package PopVar (Mohammadi et al., 2015). PopVar simulates recombinant-inbred lines (RILs) through the Rqtl package (Broman et al., 2003) by simulating recombination points along the chromosome by using independent crossovers. For each cross, 1000 RILs were simulated, and the genetic estimated breeding values (GEBVs) of the 1000 RILs were calculated from the estimated marker effects and the simulated RIL genotypes. Finally, the GEBVs of the 1000 RILs was used to estimate the progeny mean performance of each cross, while the variance of the predicted performance of the 1000 RILs was used to estimate the progeny variance performance of each cross. The mean of the top 10% of the progeny was estimated for each cross.

4.2.7 Parental selection schemes in the simulated progeny of the F4 population

Four schemes were considered to select parents most suitable to improve milling quality and grain yield simultaneously: 1- Select the top 10% of parent combinations that deliver the highest GY, from this group, select the top 50% of crosses that show the highest PHR, and then the top 50% for GC (S1), 2- Select the top 10% of crosses with the highest PHR, then the top 50% with highest GC, and from this group the 50% that

showed the highest values for GY (S2), 3- Select the top 10% of parent combinations for GC, then from these the top 50% for PHR, and finally select the 50% with the highest GY (S3), and 4- Use a multiplicative selection index (Elston, 1963), calculated for each cross *i* as:

$$I_i = (X_{i,GY} - k_{GY})(X_{i,MY} - k_{MY})(X_{i,PHR} - k_{PHR})(X'_{i,GC} + k_{GC})$$

where k_{GY}, k_{MY}, k_{PHR}, and k_{GC} are the minimum (and maximum, for GC) predicted values for each trait in the predicted progeny *i*, X_i is the mean of the trait for progeny i, and $X'_{i,GC} = -X_{i,GC}$ (S4). For each scheme the top 25 crosses were selected, and the mean and genetic variance of the selected progenies were calculated accordingly. The response to selection of each trait for each scheme was obtained by subtracting the mean in the training population from the mean of the selected progeny.

4.2.8 Evaluation of DH lines in the field

Seed from the successful DH lines developed in LSU, were planted in the field at INIA Treinta y Tres in December 2017. Up to 5 lines from each family were planted in a randomized complete block trial with three replications. Adjusted means per line for all four traits (GY, MY, PHR and GC) were calculated as:

$$y_{ijkl} = \mu + G_i + b_j + r_{k(j)} + c_{l(j)} + \varepsilon_{ijkl}$$

where y_{ijkl} is the response variable; μ is the intercept; G_i is the genotypic effect; b_j is the random block effect with $b_j \sim N(0,\sigma_b^2)$; $r_{k(j)}$ and $c_{l(j)}$ are row and column effects nested within blocks, with $r_{k(j)} \sim N(0,\sigma_r^2)$ and $c_{l(j)} \sim N(0,\sigma_c^2)$ respectively; and ε_{ijkl} is the residual.

4.3 RESULTS

4.3.1 Phenotypic analysis, trait correlations and population structure in the training population

Means and variances for all traits in the training population are summarized in Table 4.1. Among the milling quality traits, MY shows the lowest variance (3.07). Heritabilities and variance components for these traits in the same population were previously reported in Chapters 2 and 3.

Table 4.1: Mean and variances for four traits (Grain Yield, Milling Yield, Head Rice Percentage, and Chalky Grain) for a training population consisting of 311 *tropical japonica* rice breeding lines from the Uruguayan breeding program.

Trait	Mean	Variance
GY	9,361	453,469.5
MY	68.55	3.07
PHR	61.86	5.37
GC	11.26	10.87

Phenotypic and BLUP correlations among traits are shown in Table 4.2. Correlations values for milling quality traits were positive between PHR and MY, while GC was negatively correlated with both PHR and MY. GY was negatively correlated with MY and PHR, while positively correlated with GC.

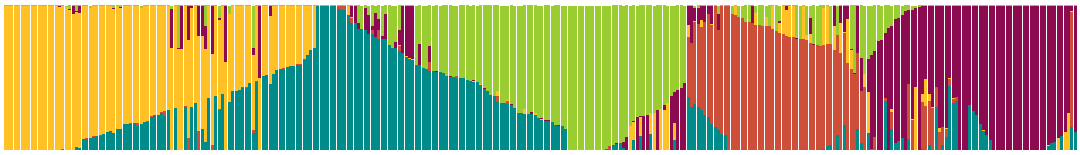
Table 4.2: Pearson coefficients of correlation between grain yield and milling quality traits in the training population. Phenotypic correlations are shown in the upper diagonal, and correlations between BLUP values in the lower diagonal.

Trait	GY	MY	PHR	GC
GY	-	-0.194***	-0.253***	0.328***
MY	-0.396***	-	0.599***	-0.423***
PHR	-0.407***	0.662***	-	-0.391***
GC	0.463***	-0.386***	-0.359***	-

***, significant at the 0.001 probability level

The clustering algorithm identified five groups in this population (Figure 4.1A), and reflected the family structure identified by standard pedigrees. This population can be defined as a multiparent cross where the lines were derived from 12 parents, and each of the five groups were comprised of half-sib families. The PCA analysis did not show evidence of strong population structure, being the proportion of variance explained by the first two eigenvectors equal to 11.2% (Figure 3.1B).

A



B

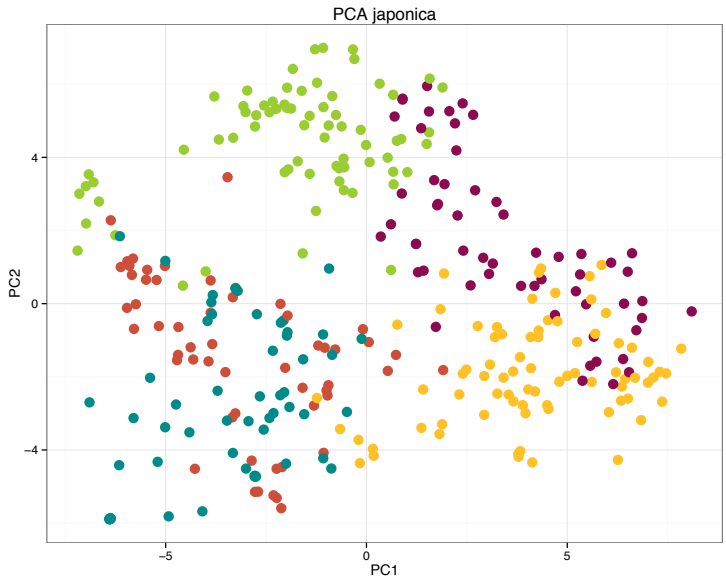


Figure 4.1: Genetic structure of the *tropical japonica* training population. (A) Population clustering diagram showing the five groups within the training population. (B) PCA analysis showing the two first principal components and the five groups found in the clustering analysis.

4.3.2 Genomic selection models on the training population

Prediction accuracies between predicted and observed breeding obtained with RR-BLUP and Bayesian LASSO models were very similar (Supplemental Table 4.1). For this reason, and because RR-BLUP models are more convenient in terms of computation time than Bayesian models, all further analyses were conducted using RR-BLUP model.

High correlations among predicted and observed breeding values were found for GY ($r_{(\hat{y},\bar{y})}$ = 0.54), MY ($r_{(\hat{y},\bar{y})}$ = 0.62), PHR ($r_{(\hat{y},\bar{y})}$ = 0.65), and GC ($r_{(\hat{y},\bar{y})}$ = 0.51) with the RR-BLUP model (Supplementary Figure 4.1). Marker effects for progeny prediction were calculated using this model.

4.3.3 Experimental crosses and progeny prediction in the training population

In order to maintain the genetic diversity of our breeding population we crossed genetically divergent individuals using the information of the population genetic structure in Figure 4.1 and the BLUPs for each line calculated in the phenotypic analysis. BLUP values and genetic subgroup membership for selected crosses are shown in Supplementary Figure 4.1, while Table 4.3 shows the phenotypic means of all the parents used in each cross, and whether that mean was one standard deviation above the overall mean in the training population (in green), below (in red), or within one standard deviation of the mean.

The predicted mean of the simulated progeny was perfectly correlated with the mid-parent value in all traits ($r > 0.999$ in all four traits). We found a triangular relationship between the mean and the variance of the predicted progeny in all traits (Figure 4.2). This is caused by the combination of two factors: 1- crosses between lines with similar trait means (i.e., high × high or low × low) yielding progeny with extreme mean values and low variance, and 2- crosses between lines with opposite extreme values resulting in progeny with intermediate means and high variance.

Table 4.3: Crosses performed in 2014 among lines from the training population of Uruguayan *tropical japonica* breeding lines. Phenotypic mean of each trait (GY, MY, PHR and GC) is shown for each parent (P1 and P2). Values highlighted in green are those that are higher than the total population mean plus one standard deviation, in red are those phenotypic means that are lower than the total mean minus one standard deviation, and in yellow the values that lie within plus or minus one standard deviation of the mean.

Cross name	P1	GY	MY	PHR	GC	P2	GY	MY	PHR	GC
JAP_1	L9063	10123	68.8	62.5	8.7	L9553	9548.0	68.6	63.6	7.9
JAP_2	L9430	9867.3	69.0	63.1	10.8	L9639	9455.0	69.0	65.6	7.6
JAP_3	L9312	9764.4	68.5	63.4	9.8	L9579	9756.2	68.1	63.3	7.0
JAP_4	L9054	10196	69.0	59.4	7.5	L9262	10065	68.5	65.3	9.9
JAP_5	L8770	9754.6	69.2	62.6	10.4	L9311	9673.0	69.2	61.9	10.3
JAP_6	L9563	10428	68.4	64.4	10.2	L9639	9455.0	69.0	65.6	7.6
JAP_7	L9430	9867.3	69.0	63.1	10.8	L9553	9548.0	68.6	63.6	7.9
JAP_8	L8968	9266.6	68.1	59.9	6.3	L9535	9851.6	68.2	64.7	9.6
JAP_9	L9430	9867.3	69.0	63.1	10.8	L9535	9851.6	68.2	64.7	9.6
JAP_10	L9363	10002	69.4	59.9	7.4	L9574	10585	67.0	63.0	11.4
JAP_11	L9363	10002	69.4	59.9	7.4	L9430	9867.3	69.0	63.1	10.8
JAP_12	L8817	10206	70.4	63.8	10.6	L9460	9753.0	68.3	61.6	7.7
JAP_13	L8770	9754.6	69.2	62.6	10.4	L8817	10206	70.4	63.8	10.6
JAP_14	L9262	10065	68.5	65.3	9.9	L9431	9818.7	68.2	61.0	10.1
JAP_15	L8802	9911.4	69.7	62.1	11.7	L9460	9753.0	68.3	61.6	7.7
JAP_16	L9553	9548.0	68.6	63.6	7.9	L9748	10429	67.4	63.4	11.1
JAP_17	L9363	10002	69.4	59.9	7.4	L9748	10429	67.4	63.4	11.1
JAP_18	L9261	9725.4	68.7	63.3	8.8	L9764	10058	67.9	62.4	12.4
JAP_19	L9617	10993	67.7	63.2	12.5	L9695	9144.0	68.7	64.3	7.8

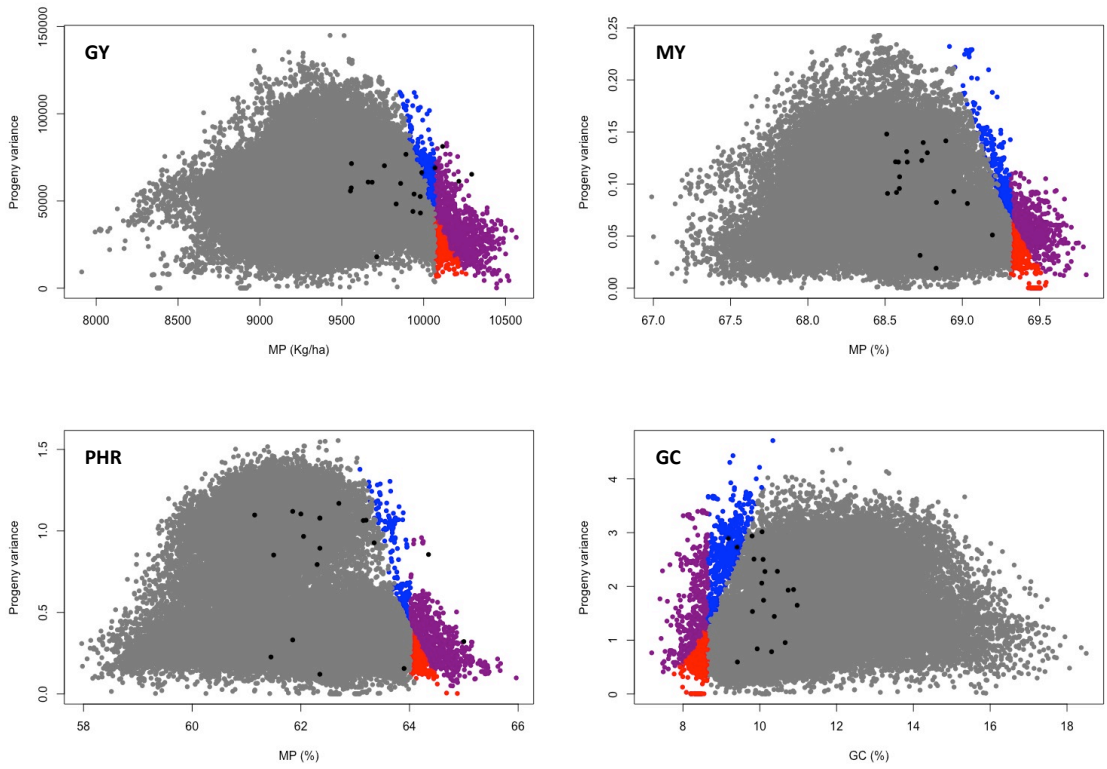


Figure 4.2: Expected mid-parent performance vs. predicted progeny variance from all pairwise biparental cross combinations in the training population for grain yield (GY), milling yield (MY), head rice percentage (PHR) and percentage of chalky grain (GC). Highlighted, the top 1000 crosses selected by mid-parent value (red), mean of the top 10% of the progeny (blue), the crosses in common between the two groups (purple), and the crosses conducted in 2014 (black).

When selecting crosses based on either mid-parent value or based on the mean of the top 10% of the progeny, we can observe that many of the selected crosses were the same for both selection methods (Figure 4.2, in purple). Among all traits, GY was the one that showed the highest number of crosses in common (858) between the overall mean and the mean of the top 10% value, followed by PHR (790), MY (692), and finally GC (594).

Among the crosses performed in 2014, three crosses (JAP_4, JAP_10, and JAP_17) were among the top 1000 crosses selected by both overall mean and the mean

of the top 10% value, and one (JAP_19) among the best crosses selected by the mean of the top 10% value for GY (Figure 4.2). For MY, none of the 2014 crosses were among the top 1000 crosses, while for PHR, one cross (JAP_6) was among the selected by both overall mean and the mean of the top 10% value, and for GC, two crosses (JAP_4 and JAP_8) were among the top 1000 crosses selected by the mean of the top 10% value (Figure 4.2).

Breeders often breed for multiple traits simultaneously. For the presented scenario, high values are desired for GY, MY and PHR, and low values are desired for GC. The correlations between BLUPs of GY and milling quality traits presented in Table 4.2, suggest that selecting for the best lines for GY will lead to lower values for MY and PHR, and higher values of GC, and likewise, selecting for best lines in terms of milling quality, will lead to selection of poorer performing lines for GY. Trait correlations changed from parental to progeny populations. For example, the average correlation between GC and GY in the simulated progeny is 0.39, but it ranges from -0.71 to 0.95 (Supplemental Figure 4.1), so even though a trait can show a strong unfavorable correlation with GY in the training population, it was still possible to see a favorable correlation in the progeny population derived from two parents selected from the training population. In Figure 4.3 we show the effect of correlated response on selection for each trait when selecting for the progeny mean of each milling quality trait. Despite the fact that the resulting correlation (r) between GY and either MY or PHR was negative in the progeny, while the average correlation between GY and GC was positive, there exists variability in GY values among crosses with similar means for MY, PHR, and GC (Figure 4.3). Also, correlations among traits for the crosses conducted tend to locate in those regions where the trait correlations are more favorable (Figure 4.3, in black).

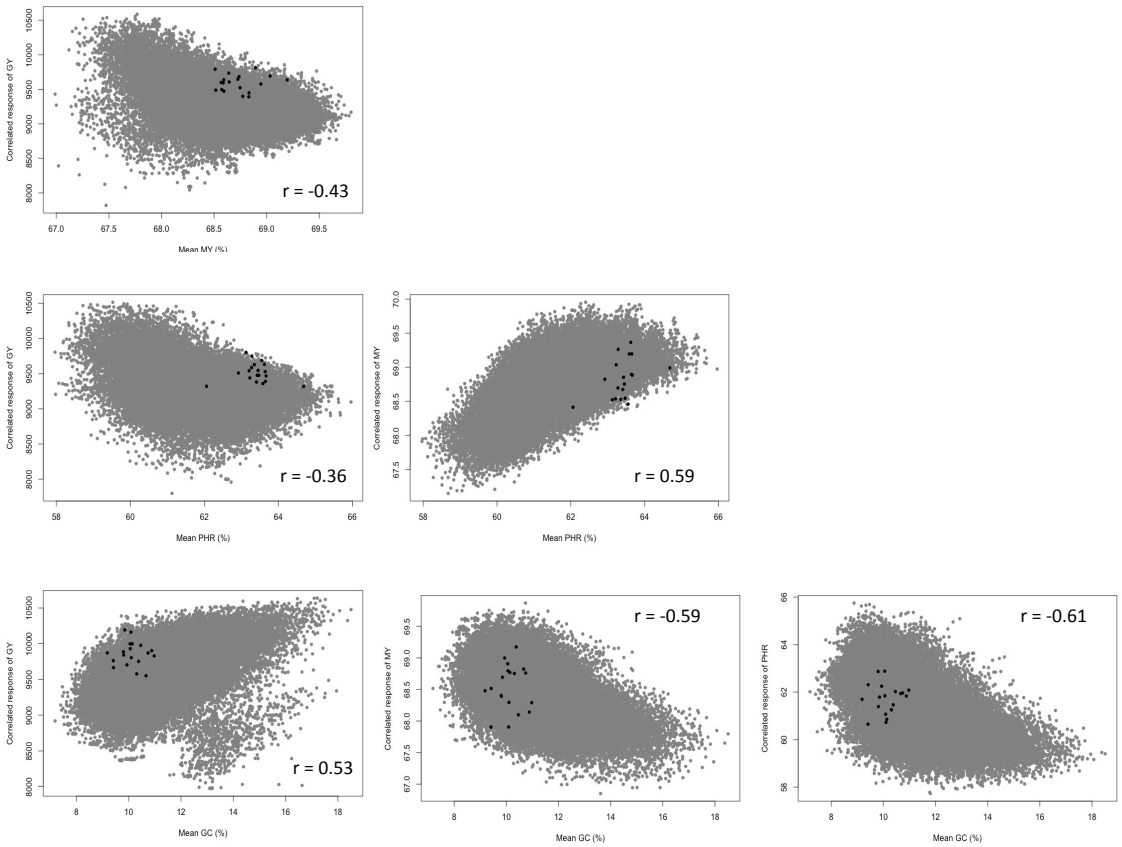


Figure 4.3: Relationship between the values of MY, PHR, and GC and average genotypic estimated breeding values of GY, MY and PHR for the corresponding lines for all pairwise crosses between 311 elite rice-breeding lines belonging to the training population. Values corresponding to the crosses performed in 2014 are shown in black.

4.3.4 Progeny prediction for crosses among the 19 F4 families

Figure 4.4 shows the expected mid-parent value and the predicted variance for each simulated cross for all pairwise combinations among the 19 F4 families. In this situation we also observed that many of the selected crosses and parents were the same when selecting on either mid-parent value or mean of the top 10% of the progeny (Figure 3.4A). Of the 100 best crosses based on these two selection methods, 69 were in common for GY, 54 for MY, 64 for PHR, and 50 for GC (Figure 4.4A, in purple).

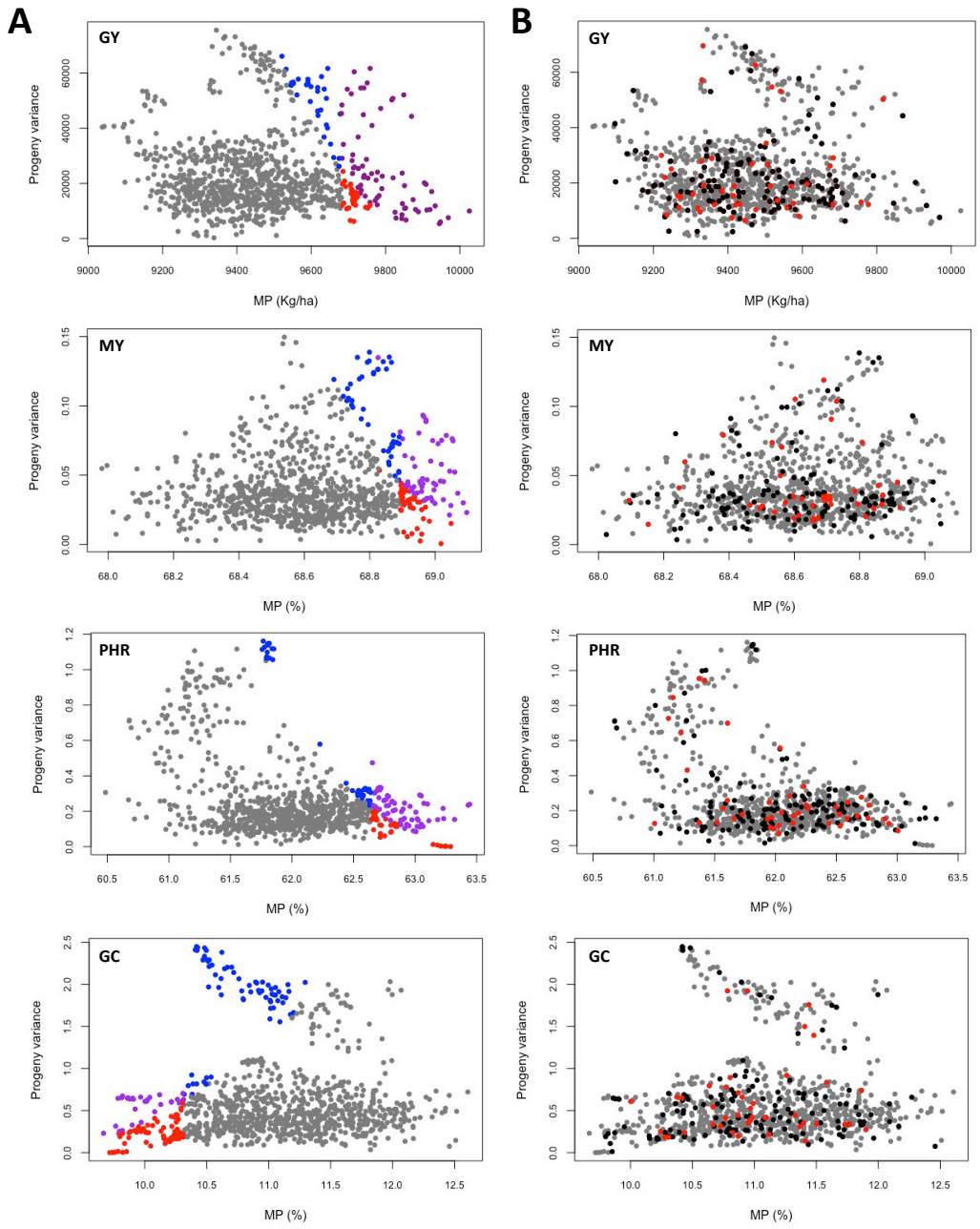


Figure 4.4: Expected mid-parent performance vs. predicted progeny variance from all pairwise biparental cross combinations among 19 F4 families for grain yield (GY), milling yield (MY), head rice percentage (PHR) and percentage of chalky grain (GC). A) Highlighted, the top 1000 crosses selected by mid-parent value (red), mean of the top 10% of the progeny (blue), the crosses in common between the two groups (purple). B) The random crosses conducted in the greenhouse are highlighted in black, and those that yielded DH lines are shown in red.

Figure 4.4B shows the predicted mean for all 185 cross combinations that were conducted at random in the greenhouse among the 19 families (in black), and of those, the ones for which we were able to regenerate plants after anther culture at LSU (in red). For all traits, the 185 crosses cover the whole range of means and variances.

Predicted correlated response to selection when selecting for milling quality traits is shown in Figure 4.5. There still exists an unfavorable relationship between GY and milling quality traits, but again, trait correlations changed between parental and progeny populations, providing new opportunities for selection. For instance, average correlations between GY and PHR across the 1000 simulated RILs, over all pairwise crosses is -0.17 and ranged between -0.78 to 0.95; and the average correlation between GY and GC was 0.30, ranging from -0.61 to 0.82 (Supplementary Figure 4.2).

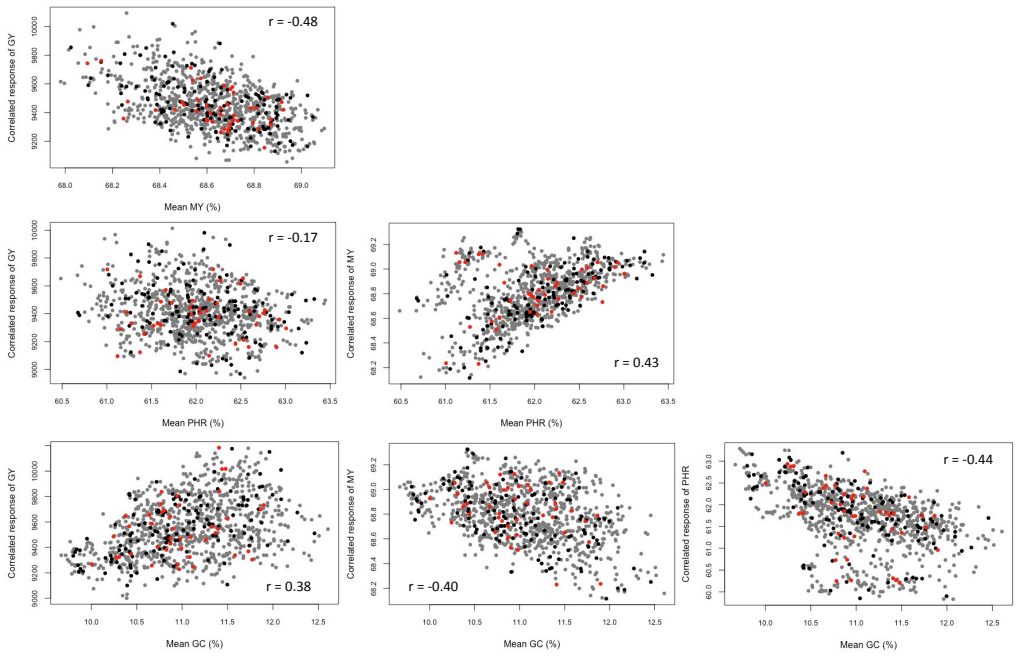


Figure 4.5: Relationship between midparent value of MY, PHR, and GC and average genotypic estimated breeding values of GY, MY and PHR for the corresponding lines for all pairwise crosses between 19 F4 families. Random crosses between families are shown in black, and DH families are shown in red.

Random crosses conducted between the F4 families, and those DH families that were further evaluated in the field are also shown in Figure 4.5; all crosses cover the whole range of means and correlated responses.

4.3.5 Selection schemes in the F4 population

In the four different selection schemes tried on the F4 population (S1-S4), GY, PHR and GC were assumed to be the target traits (Table 4.4). Scheme 1 generated the largest response for GY (493.05 $Kgha^{-1}$), but the responses for MY, PHR and GC were small (-0.03%, 0.19% and -0.12%, respectively). For scheme S2, the response for yield was the second largest (275.74 $Kgha^{-1}$), while the responses for MY and PHR were positive and the largest among all schemes (0.35% and 0.16%). Also, the response for GC in scheme 2 was the second largest among all selection schemes (-2.02%). In scheme 3, GC showed the largest negative favorable response (-2.18), but the response for GY was the smallest. For selection scheme S4, the response for PHR and GC were the second most favorable after S2. Among all traits, MY showed the smallest response to selection in all selection schemes, which could be related to the low variance for this trait shown in the training population.

Table 4.4: Selection response of the selected progenies from four parental selection schemes (S1 to S4). For comparison, the mean of the training population (TP) is also presented.

Trait	Selection response				
	S1	**S2**	**S3**	**S4**	**TP**
GY	493.05	275.74	4.846	234.3	9361.0
MY	-0.03	0.35	0.16	0.30	68.55
PHR	0.19	2.11	0.55	1.74	61.86
GC	-0.12	-2.02	-2.18	-1.47	11.26

Overall, scheme 2 was the one that gave the most favorable responses for all traits simultaneously, followed by S4.

4.3.6 Field evaluation of DH families

Out of the 185 families originated from random crosses among the 19 families, only 43 resulted in fully viable plants after the anther culture procedure. Up to five plants from each family were evaluated in a replicated trial in the field in Uruguay. Table 4.5 shows the means for each trait in the population.

Table 4.5: Means and standard deviations for four traits in the doubled-haploid population evaluated in the field.

Trait	Mean	SD
GY	4510.07	1480
MY	70.25	0.85
PHR	62.64	2.85
GC	3.62	3.31

Means for GY and GC in the DH population are lower than the mean in the training population, while the means for MY and PHR were higher (Table 4.5). Also, correlations between the BLUPs of GY and all three milling traits in this population were not significantly different than zero (Table 4.6), while the correlation between the BLUPs of PHR and MY was positive (0.3), and between PHR and GC was negative (-0.5). These two last correlations were both significant at a p-value of 0.001 (Table 4.6).

Table 4.6: Correlations between BLUPs of GY, MY, PHR and GC in the DH population evaluated in Uruguay.

Trait	GY	MY	PHR	GC
GY	-	ns	ns	ns
MY	0.178	-	***	ns
PHR	0.07	0.3	-	***
GC	0.123	-0.11	-0.5	-

***, significant at the 0.001 probability level
ns, non-significant

We also sought to test how well predictions obtained from genomic selection methods (using RR-BLUP models) for each trait individually, corresponded to empirical data based on field trials in Uruguay. Table 4.7 shows the ranking for GY of the top 10 DH families according to the predictions (left-side of the table) and how these 10 families ranked according to field data, and the ranking of the top 10 DH families according to the field data, and their position in the predictions ranking. For GY, only one DH family (DH_168) among the predicted top 10, was in the list of the top 10 families in the field (Table 4.7). Among the parents of the DH families of the predicted ranking, JAP_16 was the one that appeared most frequently in the top 10 crosses (4 times), however, it only appeared in one cross among the top 10 tested families.

For MY, four families (DH_110, DH_159, DH_183, and DH_65) among the top 10 predicted were also among the top 10 evaluated families in the field (Table 4.8). Only two of these four families had one parent in common (JAP_15). Among the field

tested lines, the parental line that appeared most frequently was JAP_6 (3 crosses) which appeared twice among the predicted crosses ranking (Table 4.8).

Table 4.7: Ranking of top 10 families for grain yield (GY) based on predictions (left side) and based on field evaluations (right side). P1 and P2 are the F4 parents of each family, MP is the mid-parent value based on the predicted values of the parents, the mean of the family according to field evaluation, and its corresponding position in the ranking of field evaluation for the prediction ranking, or its position in the ranking of predictions for the field evaluation.

Predicted						Field evaluated					
Cross	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in field**	**Cross**	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in pred**
DH_168	JAP_16	JAP_4	9816.9	5430.2	10	**DH_159**	JAP_7	JAP_13	9417.1	8498.9	24
DH_12	JAP_19	JAP_3	9777.6	1833.7	21	**DH_108**	JAP_17	JAP_15	9356.8	7307.5	29
DH_59	JAP_18	JAP_16	9757.9	5043.7	17	**DH_143**	JAP_10	JAP_13	9421.0	6980.7	23
DH_185	JAP_11	JAP_16	9683.3	1963.3	42	**DH_178**	JAP_2	JAP_4	9518.4	6399.1	14
DH_83	JAP_18	JAP_17	9682.6	4514.6	24	**DH_16**	JAP_9	JAP_6	9334.7	6382.7	31
DH_70	JAP_17	JAP_13	9674.9	5357.5	11	**DH_42**	JAP_14	JAP_10	9319.6	5799.5	36
DH_60	JAP_19	JAP_10	9613.3	5413.6	12	**DH_8**	JAP_8	JAP_14	9265.3	5552.1	40
DH_61	JAP_3	JAP_10	9590.2	4632.6	20	**DH_43**	JAP_14	JAP_7	9272.2	5515.6	38
DH_128	JAP_3	JAP_1	9576.4	3985.4	31	**DH_161**	JAP_11	JAP_19	9414.9	5449.8	25
DH_62	JAP_12	JAP_16	9570.4	4339.2	25	**DH_168**	JAP_16	JAP_4	9816.9	5430.2	1

Table 4.8: Ranking of top 10 families for milling yield (MY) based on predictions (left side) and based on field evaluations (right side). P1 and P2 are the F4 parents of each family, MP is the mid-parent value based on the predicted values of the parents, the mean of the family according to field evaluation, and its corresponding position in the ranking of field evaluation for the prediction ranking, or its position in the ranking of predictions for the field evaluation.

Predicted						Field evaluated					
Cross	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in field**	**Cross**	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in pred**
DH_110	JAP_5	JAP_15	69.03	71.368	5	**DH_159**	JAP_7	JAP_13	68.92	72.08	3
DH_68	JAP_12	JAP_15	68.92	69.85	31	**DH_40**	JAP_11	JAP_6	68.70	71.53	12
DH_159	JAP_11	JAP_19	68.92	72.077	1	**DH_183**	JAP_4	JAP_15	68.87	71.43	4
DH_183	JAP_4	JAP_15	68.87	71.43	3	**DH_28**	JAP_18	JAP_6	68.68	71.37	17
DH_16	JAP_9	JAP_6	68.87	70.224	19	**DH_110**	JAP_5	JAP_15	69.02	71.36	1
DH_65	JAP_7	JAP_6	68.82	71.26	7	**DH_128**	JAP_12	JAP_16	68.61	71.35	21
DH_77	JAP_12	JAP_13	68.79	70.026	26	**DH_65**	JAP_7	JAP_6	68.82	71.27	6
DH_167	JAP_15	JAP_1	68.74	69.532	37	**DH_15**	JAP_9	JAP_3	68.69	71.24	15
DH_143	JAP_10	JAP_13	68.72	70.896	11	**DH_60**	JAP_19	JAP_10	68.57	71.11	26
DH_8	JAP_8	JAP_14	68.71	69.981	29	**DH_90**	JAP_12	JAP_17	68.36	71.00	40

For PHR, four families among the top 10 predicted lines (DH_29, DH_42, DH_60, and DH_110) were among the top 10 evaluated lines (Table 4.9). Among these four families, only two (DH_42 and DH_60) shared one parent in common (JAP_10).

Finally, for GC, six families among the top 10 predicted (DH_8, DH_15, DH_19, DH_70, DH_156, and DH_161) were also among the top 10 performing families in the field (Table 4.10). Among these six families, JAP_3 was the only parental line that appeared in more than one cross.

Table 4.9: Ranking of top 10 families for percentage of head rice (PHR) based on predictions (left side) and based on field evaluations (right side). P1 and P2 are the F4 parents of each family, MP is the mid-parent value based on the predicted values of the parents, the mean of the family according to field evaluation, and its corresponding position in the ranking of field evaluation for the prediction ranking, or its position in the ranking of predictions for the field evaluation.

Predicted						Field evaluated					
Cross	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in field**	**Cross**	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in pred**
DH_16	JAP_9	JAP_6	62.93	62.91	25	**DH_60**	JAP_19	JAP_10	62.27	67.99	9
DH_15	JAP_9	JAP_3	62.77	63.71	15	**DH_29**	JAP_18	JAP_7	62.31	67.44	8
DH_161	JAP_11	JAP_19	62.76	63.50	17	**DH_143**	JAP_10	JAP_13	61.90	66.34	23
DH_8	JAP_8	JAP_14	62.67	63.44	19	**DH_156**	JAP_5	JAP_1	61.61	66.22	30
DH_42	JAP_14	JAP_10	62.62	65.50	7	**DH_110**	JAP_5	JAP_15	62.38	66.05	7
DH_65	JAP_7	JAP_6	62.53	64.39	12	**DH_70**	JAP_17	JAP_13	61.52	65.68	34
DH_110	JAP_5	JAP_15	62.38	66.05	5	**DH_42**	JAP_14	JAP_10	62.02	65.50	5
DH_29	JAP_18	JAP_7	62.31	67.44	2	**DH_62**	JAP_3	JAP_1	61.18	65.39	38
DH_60	JAP_19	JAP_10	62.27	67.99	1	**DH_97**	JAP_18	JAP_13	61.66	65.20	29
DH_159	JAP_7	JAP_13	62.26	62.05	28	**DH_90**	JAP_12	JAP_17	61.66	65.10	28

Table 4.10: Ranking of top 10 families for percentage chalky grain (GC) based on predictions (left side) and based on field evaluations (right side). P1 and P2 are the F4 parents of each family, MP is the mid-parent value based on the predicted values of the parents, the mean of the family according to field evaluation, and its corresponding position in the ranking of field evaluation for the prediction ranking, or its position in the ranking of predictions for the field evaluation.

Predicted						Field evaluated					
Cross	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in field**	**Cross**	**P1**	**P2**	**MP pred**	**Mean Field**	**Rank in pred**
DH_8	JAP_8	JAP_14	10.00	1.81	10	**DH_29**	JAP_18	JAP_7	11.33	0.28	4
DH_161	JAP_11	JAP_19	10.24	1.49	7	**DH_156**	JAP_5	JAP_1	10.64	0.74	8
DH_16	JAP_9	JAP_6	10.28	2.67	19	**DH_15**	JAP_9	JAP_3	10.67	1.09	10
DH_29	JAP_18	JAP_7	10.31	3.73	1	**DH_128**	JAP_12	JAP_16	11.08	1.14	27
DH_70	JAP_17	JAP_13	10.37	1.22	6	**DH_49**	JAP_5	JAP_10	10.87	1.19	19
DH_59	JAP_18	JAP_16	10.42	5.44	36	**DH_70**	JAP_17	JAP_13	10.37	1.22	5
DH_143	JAP_5	JAP_1	10.44	2.83	22	**DH_161**	JAP_11	JAP_19	10.24	1.49	2
DH_156	JAP_5	JAP_1	10.64	0.74	2	**DH_68**	JAP_12	JAP_15	11.37	1.51	32
DH_35	JAP_7	JAP_1	10.67	5.52	37	**DH_61**	JAP_3	JAP_10	10.77	1.58	12
DH_15	JAP_9	JAP_3	10.67	1.09	3	**DH_8**	JAP_8	JAP_14	10.00	1.81	1

4.4 DISCUSSION

4.4.1 Cross prediction

The mid-parent value is a commonly used predictor for cross prediction and parental selection. In our case, the predicted mean performance of the simulated progeny was perfectly correlated with the mid-parent value for all traits. This result is expected since in an additive model the expected value of the mean of the progeny calculated as the mid-parent value is the same as the mean of the RIL's performance. This observation was also found in maize for silking date and protein (Bernardo, 2014), barley for yield and deoxynivalenol (Mohammadi et al., 2015), and in wheat for grain yield, protein content, loaf volume and mixing time (Lado et al. 2017).

When both mid-parent GEBV and the mean of the top 10% of the population were used for cross prediction in the training and F4 populations, a large number of common crosses were found, mostly for GY. This result is consistent with previous studies (Zhong and Jannink, 2007; Mohammadi et al., 2015; and Lado et al., 2017), and indicates that the predicted mean progeny performance is the strongest driver for selecting superior crosses for GY. Zhong and Jannink (2007) previously showed that the utility of including estimates of genetic variance in cross prediction is greater when the variance of the progeny means is lower than the variance of the progenies standard deviation. However, for milling quality traits the percentage of selected crosses in common between the mid-parent value and the man of the top 10% of the predicted progeny was smaller. These results indicate that for milling quality traits, the influence of the variance could be a more relevant factor for cross selection. This is in accordance with results found by Lado et al. (2017) in wheat, where the percentage of crosses in common between mid-parent value and top 10% of the population was lower for grain quality compared to grain yield.

When looking at Figure 4.2, few of the 19 crosses between elite lines in the training population were among the top 1000 based on either the overall progeny mean or for the mean of the top 10% of the progeny. For MY, none of the 19 crosses were included in any of these categories. Given the trait correlations in this population, choosing the best crosses for a given trait, for example GY, may not be the best option since we could be selecting against milling quality. For this reason, the crosses selected originally were not among the top performing lines for any particular trait, but were good performing lines for all traits. Unfortunately, weather conditions in Uruguay during flowering season in 2014 delayed plant flowering and affected the choice of crosses. Nonetheless, some of the crosses performed showed a favorable trade-off among traits, as can be seen in the correlated response plots in Figure 4.3.

4.4.2 Parental selection for improving multiple traits

Simultaneous improvement of GY and milling quality in rice is a challenging due to the fact that these traits are often unfavorably correlated, as demonstrated in this study. However, individual genotypes may carry different favorable alleles, so genetic correlations among traits in the progeny can differ from correlations observed in the parental populations. Even when GY and MY and PHR were negatively correlated in the training population (or positively in the case of GY and GC), positive correlations (or negative) could be observed in some of the progeny populations (Supplemental Figures 4.1 and 4.2).

When multiple traits are targeted simultaneously in breeding, different selection strategies can be applied. In this work we compared three different types of independent culling selection (S1-S3), differing in the intensity of selection for each trait, and we also used a multiplicative index constructed from GY and three milling quality traits

(S4). The overall success for all traits in S1-S3 depended mainly on the intensity of selection applied to each trait. In S1, where the highest intensity was applied to GY, the response to selection obtained for milling quality was low for all three quality traits, particularly for GC. The opposite was true for S3, where GC was selected at higher intensity, resulting in the lowest response for GY. As a result, S2 appears to be the best strategy for multiple trait selection because it applies high selection intensity on PHR, which showed a weaker negative correlation with GY, and a stronger negative correlation with GC, which is more desirable. Using a multiplicative index as a selection strategy also proved to be a good strategy, although the response obtained for all target traits was lower than when S2 was used. Selection indices can be very useful when dealing with negatively correlated traits since they allow to retain individuals that can be outstanding for one trait and average for other.

4.4.3 Comparisons between predictions and field data

Random crosses were performed among the 19 F4 families with the objective of testing in the field in order to determine whether GS can accurately predict the best performing lines for four traits independently. In an attempt to speed-up the process of obtaining homozygous lines for field evaluation, we generated DH lines (via anther culture) from the F1's of all random crosses. Although it is generally assumed that *tropical japonica* genotypes are more responsive to callus induction and plant regeneration than *indica* genotypes, we could only recover viable plants from 43 crosses out of 185 (23%). Also, the number of plants recovered for each family was low, ranging from 1 to 8 individuals.

When looking at the field performance of these lines, we can see that GY was low compared to the predicted GY in the parents and the predictions obtained by GS.

This can be explained by late planting. Due to delayed arrival of the seed to Uruguay, they were planted in mid December, two months later than regular rice planting season in Uruguay. For this reason, plants typically lagged in development, going into the fall where low temperatures were registered during flowering and grain filling stages, and resulting in low yields. This helps explain why the ranking of the top 10 predicted lines according to GS had no lines in common with the ranking of top 10 lines according to field evaluations.

In contrast to GY, phenotypic values for MY and PHR were in the expected range. On the other hand, GC showed very low values across families, which was unexpected. PHR, MY and GC are measured on a sample of 100g of rice, so these traits were not affected by the low yield that resulted from late planting. However, late planting may induce grain breakage since grains tend to dry at a faster pace in the field, leading grains to fissure, crack and be chalky. Also, low minimum temperatures and low solar radiation can occur during late April-beginning of May (when this population was harvested), which would be expected to have a negative effect on chalk. Nonetheless, prediction accuracies for milling quality traits were about 40%-60% (Tables 4.8, 4.9 and 4.10), and GC showed the highest accuracy, with 6 families correctly predicted out of 10. These results suggest that GS could be a good strategy for improvement of milling quality in Uruguayan rice. Further confirmation based on more accurate phenotypic evaluations, with a higher number of families, and under the regular planting schedule should be performed in order to better understand the possible benefits of implementing GS in the Uruguayan breeding program.

The low number of plants regenerated after anther culture indicates that the use of DH lines may not be the best strategy for saving time in obtaining fixed lines with this germplasm. Even though *tropical japonica* rice tends to have a better response to

anther culture than *indica* rice, this response is highly genotype-dependent (He et al., 2006). Other approaches such as rapid-generation advance (RGA) in greenhouse facilities would be more appropriate as a strategy for shortening generation time; however, the economic investment required to support such facilities is often out of reach for small national breeding programs.

A second year of field evaluation of these lines is currently underway. DH families were planted in the field at the appropriate seeding time (beginning of October 2018), and the data will be ready for analysis at the end of the harvesting season (mid-March 2019). The progeny from all 185 crosses were advanced (in parallel with the DH lines) via single-seed descent in the greenhouse at Cornell University, and F4-derived lines will be sent to Uruguay for seed multiplication and further field evaluation. Results from these analyses may allow us to obtain more accurate estimations on the potential advantages of applying GS for yield and milling quality improvement.

In this study we estimated progeny means using genomic predicted mid-parent values, and found they were identical to the means of the simulated progeny. In this study, modeling the genetic variance to predict the best crosses had a small impact on prediction accuracies for complex traits like grain yield, but may have a higher influence in milling quality traits such as head rice percentage, milling yield and grain chalkiness. Use of a simulation approach for selecting suitable parents for crossing can help disrupt unfavorable correlations between traits, and make informed selection strategies using either independent culling or selection indices. Further validation of the strategies outlined in this chapter will provide a better foundation for future decision-making about the role that GS may play in the Uruguayan rice breeding program.

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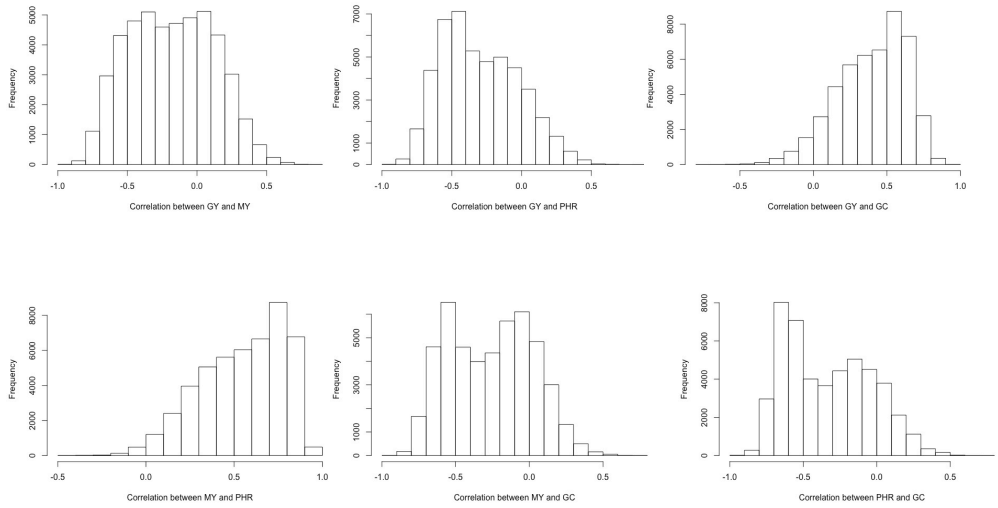
4.6 SUPPLEMENTARY MATERIAL

Supplementary Table 4.1: Prediction accuracies obtained for grain yield (GY), milling yield (MY), percentage of head tice (PHR), and percentage of chalky grain (GC) in the training population, with the rrBLUP and the BayesLASSO methods.

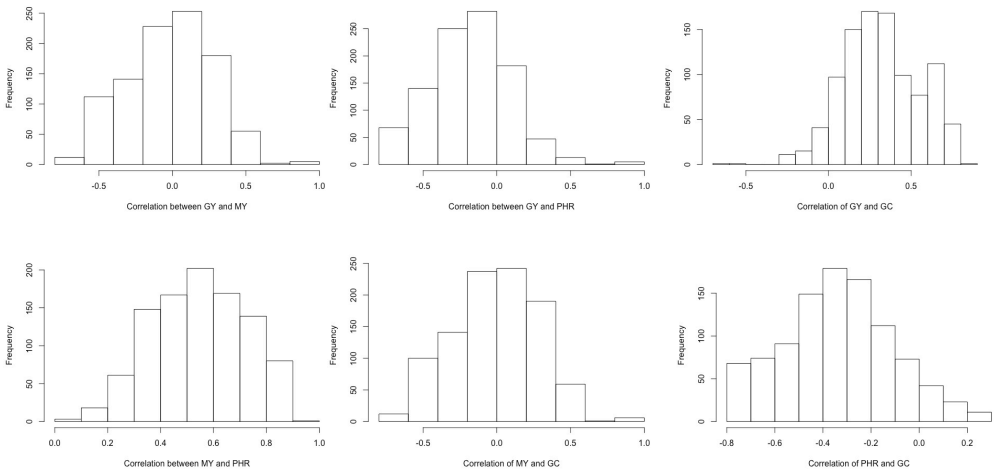
	rrBLUP		BayesLASSO	
	mean	sd	mean	sd
GY	0.54	0.03	0.53	0.02
MY	0.62	0.01	0.61	0.01
PHR	0.65	0.02	0.66	0.01
GC	0.51	0.02	0.51	0.02

Supplementary Table 4.2: Crosses performed in 2014 among lines from the training population of Uruguayan *tropical japonica* breeding lines. BLUP values of each trait (GY, MY, PHR and GC) and structure group membership are shown for each parent (P1 and P2).

Cross name	P1	Structure group	GY	MY	PHR	GC	P2	Structure group	GY	MY	PHR	GC
JAP_1	L9063		322.77	0.30	-1.76	-1.55	L9553		291.38	0.26	0.07	-3.07
JAP_2	L9430		733.94	1.22	0.37	0.17	L9639		341.80	1.12	2.32	-2.95
JAP_3	L9312		232.72	0.38	2.36	-0.86	L9579		462.84	-0.25	3.09	-1.45
JAP_4	L9054		703.51	1.71	-3.21	-1.90	L9262		285.61	0.15	2.93	-1.50
JAP_5	L8770		324.94	2.51	1.10	-1.03	L9311		98.40	1.51	0.29	-0.22
JAP_6	L9563		712.85	0.88	2.65	0.39	L9639		341.80	1.12	2.32	-2.95
JAP_7	L9430		733.94	1.22	0.37	0.17	L9553		291.38	0.26	0.07	-3.07
JAP_8	L8968		156.20	0.14	2.66	-3.41	L9535		253.17	0.08	1.81	-1.41
JAP_9	L9430		733.94	1.22	0.37	0.17	L9535		253.17	0.08	1.81	-1.41
JAP_10	L9363		30.78	2.25	0.32	-2.79	L9574		956.31	0.95	1.16	-1.36
JAP_11	L9363		30.78	2.25	0.32	-2.79	L9430		733.94	1.22	0.37	0.17
JAP_12	L8817		420.87	1.22	3.00	-0.05	L9460		240.66	-0.10	0.92	-2.30
JAP_13	L8770		324.94	2.51	1.10	-1.03	L8817		420.87	1.22	3.00	-0.05
JAP_14	L9262		285.61	0.15	2.93	-1.50	L9431		392.52	0.10	0.69	-1.40
JAP_15	L8802		159.67	1.68	0.11	0.80	L9460		240.66	-0.10	0.92	-2.30
JAP_16	L9553		291.38	0.26	0.07	-3.07	L9748		1052.70	-0.12	-0.55	0.19
JAP_17	L9363		30.78	2.25	0.32	-2.79	L9748		1052.70	-0.12	-0.55	0.19
JAP_18	L9261		289.27	0.37	1.64	0.00	L9764		1002.94	-0.04	0.40	0.11
JAP_19	L9617		948.77	0.19	1.32	0.44	L9695		87.94	0.01	2.73	-1.10



Supplementary Figure 4.1: Frequency distribution of correlations between grain yield (GY), milling yield (MY), percentage of head rice (PHR), and percentage of chalky grain (GC), in all pairwise cross combinations in the training population.



Supplementary Figure 4.2: Frequency distribution of correlations between grain yield (GY), milling yield (MY), percentage of head rice (PHR), and percentage of chalky grain (GC), in all pairwise cross combinations in the F4 population.

CHAPTER 5:

EXPLORING THE GENETIC BASIS OF ANTHER CULTURE RESPONSE IN DOUBLED HAPLOID *tropical japonica* RICE

5.1 INTRODUCTION

Creation of genetic variability is essential in any crop improvement program. Conventional recombinational breeding usually begins with hybridization between diverse parents followed by 6-9 cycles of selfing and 3-5 years of field evaluation before a pure breeding line is released as a new variety. Anther culture (AC) improves efficiency by generating homozygous lines directly from the F1; fertile DH lines can be phenotyped directly and provide an opportunity to evaluate genotypes with fixed gene combinations that would otherwise disappear after several generations of recombination using conventional breeding methods.

Anther culture consists of first, the initial development of calli and second, regeneration of green plants from embryogenic calli. Briefly, rice panicles are collected during the booting period. At this stage, the microspores are at the mid- to late-uninucleate stage. Spikelets are sterilized and anthers are dusted over the surface of callus-inducing medium and incubated in the dark. Then, the calli are transferred to petri dishes containing plant regeneration medium and incubated under artificial light for callus regeneration. Green plantlets are transferred to rooting medium to induce root formation, and plants with well-formed roots are then transferred to pots in a greenhouse (Mishra et al., 2013).

Although anther culture is widely used for practical breeding, its application is still limited by many factors, including low frequency of callus induction and plant regeneration, genotypic dependency of regeneration ability, and high frequency of

haploid plants (Medhabati et al. 2014, Tripathy, 2018). *Indica* rice varieties show the most limited response to current anther culture techniques in rice; they are given to early anther necrosis, poor callus proliferation and regeneration of albino plantlets (Chen et al., 1991). On the other hand, *japonica* varieties are generally easier to regenerate from anther culture than *indica* varieties, though their responsiveness varies widely even among *japonica* genotypes (He et al., 2006). Several genetic studies have been performed to improve regeneration ability from seed-derived calli in rice (Taguchi-Shiobara et al., 1996; Kwon et al., 2001). However plant regeneration ability is quantitatively inherited and greatly affected by the environment (Kwon et al., 2001; Schiantarelli et al. 2001; Taguchi-Shiobara et al. 2006; Li et al. 2013), which makes it difficult to reliably select responsive genotypes in efforts to improve regeneration ability.

In past decades various studies have tried to identify the genes or QTL associated with callus induction and plant regeneration in rice. Most of these studies have been performed on mapping populations from bi-parental crosses between *indica* and *japonica* varieties (Taguchi-Shiobara et al. 1997, Taguchi-Shiobara et al. 2006, Li et al. 2013). However, none of these studies have focused on exploring the genetic mechanisms underlying anther culture response within *japonica* germplasm.

The aim of this study was to identify QTLs for callus induction ability and plant regeneration within *tropical japonica* DH lines of interest to Uruguayan rice breeders using Genome Wide Association Studies (GWAS). We performed this analysis based on the evaluation of three traits: number of calli, number of plants regenerated, and number of panicles per regenerated plant.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials

A total of 191 *tropical japonica* genotypes belonging to 54 DH families from the Uruguayan National Rice Breeding Program were used as the GWAS population. All the individuals from this population come from random crosses between 19 original parents (Supplementary Table 5.1).

5.2.2 Anther culture and phenotyping

Anther culture procedures were conducted at the LSU AgCenter's Rice Research Station in Crowley, Louisiana. F1 seeds from the crosses shown in Supplementary Table 5.1 were grown in the field until the booting stage. The boots where then sterilized with 70% alcohol and cold pre-treated at 10 °C for 8 d. The spikelets were surface sterilized with 20% bleach and rinsed with de-ionized water. Anthers were inoculated into petri dishes with callus-induction medium and incubated in the dark at 25 °C for 3-4 weeks after inoculation. Then, calli were transferred onto regeneration medium and incubated under artificial light at 25 °C to promote callus regeneration. Green plantlets were transferred to rooting medium for root formation. Plants with well-formed roots were then transferred to pots in the greenhouse.

The phenotypic traits analyzed in this study were: number of calli plated per dish (CD), which was used as a measure of callus induction ability, number of plants regenerated per callus (PPC) as a measure of plant regeneration ability, and number of panicles per regenerated plant (PPP), as a measure of vigor of regenerated plants.

To comply with normality requisites, all correlations and GWAS analyses were performed on log10 transformed traits.

5.2.3 Genotyping

DNA was extracted using the CTAB method. DNA libraries were prepared using the Nextera Library and sequenced at the Biotechnology Resource Center at Cornell University. Single nucleotide polymorphisms (SNPs) were called and aligned to the MSU Nipponbare reference version 7.0 using BWA (Li and Durbin, 2009). Imputation of missing data was performed using BEAGLE 5.0 (Browning et al. 2018). Monomorphic SNPs, and SNPs with minor allele frequency < 5% were removed from the analysis. The final dataset contained 68,599 markers.

5.2.4 Association mapping study

Genome-wide association studies were performed with mixed models to correct for genetic relationships. We used the kinship model with:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where $\mathbf{y}$ is the vector of phenotypic values, $\mathbf{X}$ is the molecular marker score matrix, β is the vector of marker allelic effects, $\mathbf{Z}$ is an incidence matrix, $\mathbf{u}$ is the vector of polygenic background effects where $\mathbf{u} \sim N(0, \mathbf{K}\sigma_G^2)$ (with $\mathbf{K}$ being the kinship matrix and σ_G^2 is the genetic variance), and $\mathbf{e}$ is vector of residual errors. A GWAS analysis for each trait was performed with the R package *sommer* (Covarrubias, 2016). For QTL determination, the marker with the highest association was chosen as an anchor and a sliding window of 1Mb was used to identify all significant markers within that window. Given the big size of LD blocks expected for a DH population, it is unlikely to find an isolated significant SNP, but more likely to find groups of linked SNP showing the exact same p-value, as shown in Figure 5.2B. QTL were identified based on the occurrence of three or more significant SNPs (see Figure 5.2B) within a 1 Mb window. The threshold for declaring a significant marker-trait association was the false-discovery rate (FDR) (Benjamini and Hochberg, 1995) with an α level ≤ 0.05. Allelic

effects for each QTL were calculated as the difference between the average trait value for all lines that were homozygous for the major allele (AA) and the average trait value for all lines homozygous for the minor allele (BB) for a given SNP. The proportion of the total variance explained by a QTL was estimated by fitting a multi-QTL model with all significant SNPs from all genomic regions involved for a trait with the lme4 package (Bates et al., 2015) implemented in R statistical software (R Core Team, 2017).

5.3 RESULTS

5.3.1 Phenotypic variation for callus induction and plant regeneration traits

Phenotypic means and standard deviations for all traits are shown in Table 5.1 and distributions and correlations of transformed traits are shown in Figure 5.1.

Table 5.1: Means and standard deviations for the three traits analyzed in this study.

Trait	Mean	SD
CD	6.86	4.04
PPC	8.54	10.64
PPP	0.89	0.30

CD: Calli per dish, PPC: Plants per callus, PPP: Panicles per plant

There is a negative correlation between the number of calli plated and the number of plants regenerated (r = -0.5), and also a negative correlation between the number of plants regenerated and the number of panicles collected per plant (r = -0.4) (Figure 5.1).

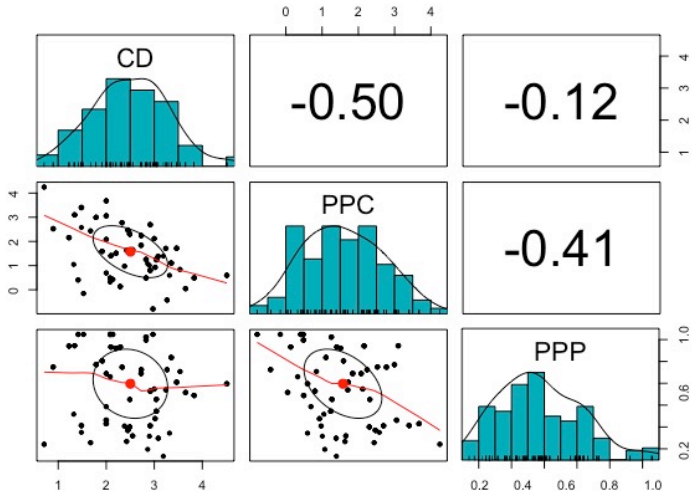


Figure 5.1: Trait distributions and correlations. CD: Calli per dish, PPC: Plants per callus, PPP: Panicles per plant

5.3.2 Quantitative trait loci identified by GWAS

We found a total of 21 putative QTL for the three traits (Figure 5.2, Table 5.2). Four QTL were identified for CD (located in chromosomes 2, 7, 11 and 12), 12 for PCC (chromosomes 2, 3, 5, 6, 7, 8, and 9), and five for PPP (chromosomes 2, 4 8 and 9).

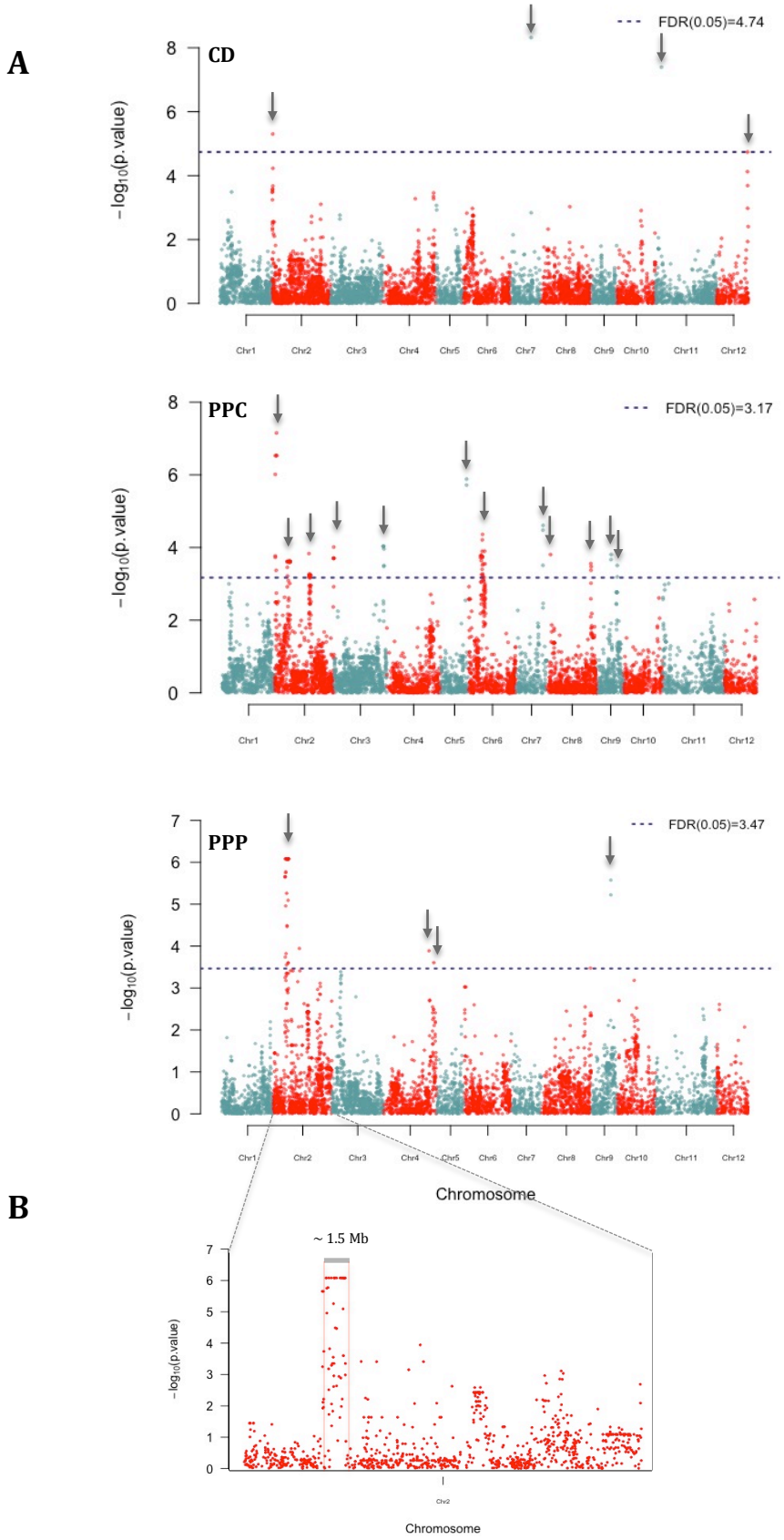


Figure 5.2: A- Manhattan plots for Calli per Dish (CD), Plants per callus (PPC), and Panicles per regenerated plant (PPP). Dashed lines show the 0.05 FDR thresholds for each trait. Arrows indicate QTL positions. B- Close-up view of chromosome 2 as an example, showing the extent of LD in this population.

Table 5.2: Putative QTL, score (-log10p), chromosome (Chr), most significant marker, position (bp) and allele effects for the significant associations found in a population of *tropical japonica* DH families.

Trait	Score	QTL	Chr	Marker	Position	Effect	PVE
CD	5.29	qCD-2	2	S2_215969	215969	-1.2	10.4
	8.3	qCD-7	7	S7_17424219	17424219	-7.94	26.5
	7.39	qCD-11	11	S11_7082543	7082543	-7.83	21.7
	4.73	qCD-12	12	S12_23791056	23791056	-5.34	9.3
PPC	7.15	qPPC-2.1	2	S2_716679	716679	6.38	24.3
	3.6	qPPC-2.2	2	S2_9555434	9555434	3.28	6.91
	3.83	qPPC-2.3	2	S2_18463053	18463053	-5.67	7.36
	4.01	qPPC-2.4	2	S2_35109189	35109189	5.68	7.7
	4.03	qPPC-3	3	S3_31761386	31761386	7.48	7.7
	5.9	qPPC-5	5	S5_25114691	25114691	-8.31	11.67
	4.36	qPPC-6	6	S6_10551939	10551939	4.60	9.48
	4.61	qPPC-7	7	S7_24114844	24114844	-12.3	9.0
	3.80	qPPC-8.1	8	S8_2679759	2679759	1.46	7.3
	3.56	qPPC-8.2	8	S8_26314338	26314338	-3.92	6.7
	4.9	qPPC-9.1	9	S9_11169765	11169765	-10.4	7.3
	3.8	qPPC-9.2	9	S9_14980347	14980347	-3.45	6.0
PPP	6.11	qPPP-2	2	S2_9555434	9555434	-0.21	18.68
	3.89	qPPP-4.1	4	S4_28836460	28836460	0.41	6.99
	3.60	qPPP-4.2	4	S4_31921216	31921216	-0.30	6.38
	3.48	qPPP-8	8	S8_28153686	28153686	0.135	6.10
	5.57	qPPP-9	9	S9_14980347	14980347	0.263	15.57

The genetic effects of the majority of QTL identified were relatively small (<20% of the phenotypic variance explained, PVE). CD was the only trait that showed QTLs with negative allelic effect, while the other two traits showed both negative and positive allele effects.

Three QTL, qCD-7, qCD-11, and qPPC-2.1 showed the largest effects (PVE = 26.5%, 21.7%, and 24.3%, respectively). Both qCD-7 and qCD-11 had the effect of decreasing the number of calli per dish by an average of ~ 8. On the other hand, qPPC-2.1 had the effect of increasing the number of regenerated plants by ~6 (Table 5.2).

QTLs qPPC-2.2 and qPPP-2 were co-located on chromosome 2, and they shared marker S2_9555434 as the most significant marker. For PPP this QTL had PVE ~19%, while for PPC PVE ~7%. Two other putative QTLs, qPPC-9.2 and qPPP-9 were co-located on chromosome 9, with PVE = 6% and 15.57%, respectively (Table 5.2).

5.3.3 Comparison of the putative QTL and reported QTLs related to rice anther culture response

A total of eight loci were located in same regions as previously reported QTLs related to callus induction and plant regeneration (Table 5.3). Three previously reported QTLs located on chromosomes 5, 7 and 9 corresponded to CD and PPC in our study; these QTLs were previously reported by Tian et al. (2013) to be related to callus induction frequency, and frequency of brown callus. Two loci associated with CD and PPC and located on chromosomes 2 and 12, were previously reported by Li et al. (2013) to be related to plant regeneration and callus proliferation and browning tendency. One locus associated with PPP in our study and located in chromosome 4, was previously reported by Zhao et al. (2008) to be related to frequency of regenerated plants. Finally, one putative QTL associated with PPC and located in chromosome 8, was previously reported by a GWAS study to be associated with callus induction-related traits based on evaluation of a set of 529 cultivated rice lines belonging to *indica*, *Aus*, and *tropical* and *temperate japonica* varieties. Interestingly, the specific QTL reported in Table 5.3 was found only among the *japonica* genotypes in that study (Zhang et al. 2018).

Table 5.3: Summary callus induction and plant regeneration associated QTL reported in the same genomic regions where callus induction and plant regeneration associated QTL were detected in this study.

Position (bp)	Chr	Trait in present study	Trait in reported study	QTL reported name	Mapping population and reference
35109189	2	PPC	CPA, CBT, RR	qCPA-2b, qCBT-2a, qRR-2	RIL indica × japonica cross (Li et al. 2013)
23791056	12	CD	NRS, RR	qNRS-12, qRR-12	
31921216	4	PPP	PRF	PRF	CSSL indica × japonica cross (Zhao et al. 2008)
25114691	5	PPC	CWI	qCWI5	RIL indica × indica cross (Tian et al. 2013)
17424219	7	CD	CIF	qCIF7.1	
24114844	7	PPC	CIF	qCIF7.2	
14980347	9	PPC, PPP	BCF	qBCF9	
26314338	8	PPC	T0	T0	529 rice cultivated accessions (Zhang et al. 2018)

PCC: Plants regenerated per callus, PPP: Panicles per regenerated plant, CD: callus per dish plated, CIF: Callus induction frequency, BCF: Frequency of Brown callus, CWI: Increase of callus weight, NRS: number of regenerated roots per callus, RR: Regeneration Rate, CPA: Callus Proliferation ability, CBT: Callus Browning Tendency, PRF: frequency of regenerated plants, T0: Time of the first callus appearance.

5.4 DISCUSSION

Anther culture response may be influenced by many factors, including the genotype of the donor line, the medium composition, the culture procedure, and the interactions between them (Henry et al. 1994; Lee et al. 2002; Bolibok and Rakoczy-Trojanowska 2006; Ge et al. 2006). Genotypic variations in callus induction and subsequent plant regeneration potential among different rice genotypes have been extensively studied by many researchers (He et al, 2006; Bagheri and Jelodar, 2008; Dash et al., 2014). In a study involving a wide range of cultivated *Oryza* genotypes, Dash et al. (2014) found that *O. glaberrima* responds more to callus induction and plantlet regeneration than *O. sativa* genotypes. Within *O. sativa*, *indica* genotypes were

recalcitrant to plant regeneration, while they found a broad variability of response to callus induction and plant regeneration among *japonica* genotypes (Dash et al. 2014).

Many studies have focused on finding QTL associated with callus induction and plant regeneration in rice (Kwon et al. 2000; Taguchi-Shiobara et al. 2006; Zhao et al. 2008; Li et al. 2013; Tian et al. 2013). However none of these studies focused on finding QTL within the *japonica* germplasm. In this study, we evaluated variation in callus induction ability, plant regeneration and viability of regenerated plants by assessing three traits (CD, PCC, PPP) in a population of 191 DH lines, and we found a total of 21 putative QTL related to the three traits analyzed. Most of the previous QTL mapping studies concerning anther culture response in rice were performed using sparse markers, such as RFLPs and SSRs (Taguchi-Shiobara et al. 1997; Kwon et al. 2000; Taguchi-Shiobara et al. 2006; Tian et al. 2013). The high resolution map based on SNP polymorphisms used in this study boosted the accuracy of QTL identification as it revealed more precise recombination breakpoints than was possible using sparsely populated molecular linkage maps.

Two pairs of QTL identified in this study, one on chromosome 2 (qPPC-2.2, qPPP-2.1) and one on chromosome 9 (qPPC-9.2, qPPC-9) were co-located in the same genomic region. For both groups the two pairs of traits involved were PPC and PPP, and in both cases, the traits were negatively correlated. Each DH line carried one favorable and one unfavorable allele at the loci, respectively, with no recombinants identified among the 192 lines. This could be suggesting either the existence of a single gene with pleiotropic effects or two tightly linked genes that control these traits.

A literature search identified previously reported QTL for callus induction, callus browning, and plant regeneration (Li et al., 2013; Tian et al., 2013) that overlapped with eight of the QTL identified in this study.

We also performed a literature search to identify candidate genes located within the QTL reported in this analysis. Genes encoding certain enzymes are essential for metabolism of differentiating cells, tissues and organs. Calli with higher levels of the peroxidase enzyme have been reported to display a greater regeneration potential than those with lower levels of the enzyme (Subhadra et al., 1998). Zhang et al. (2018) reported an auxin responsive gene, OsIAA10 (LOC_Os02g57250), along with stress response genes such as oxidoreductase and thioredoxin genes as candidate genes for response to callus induction. We discovered a high representation of peroxidase genes and other oxidative stress response genes in our QTL regions that matched those reported by Zhang et al. (2018) (data not shown).

DHs produced through anther culture of a cross involving diverse parents are genetically stable lines and each carries a different combination of alleles. This paves the way for increased selection response and allows identification of desirable genotypes. However, the success of the technique is limited due to low recovery of genotypes during the process and the low recombination frequency in these F1-derived lines. Until now, many studies have been performed to elucidate the genetic basis of the anther culture response, but the findings remain inconclusive. Phenotypic variation for three traits associated with anther culture response were evaluated in this study and combined with a high quality sequence-based genetic map, a total of 21QTLs were identified in a population of DH lines belonging to the *tropical japonica* subpopulation. Many of the QTL related to plant regeneration and number of panicles harvested per regenerated plant, were also found in previous studies focused on anther culture in rice. The results obtained from this study provide the foundation for fine mapping and eventual cloning of genes underlying major QTLs for anther culture in future experiments. The knowledge about the genes and pathways underlying plant

regeneration and the anther culture response would be helpful to better understand the molecular basis of anther culture response, and potentially to improve the ability of breeding lines to be fixed using the DH procedure.

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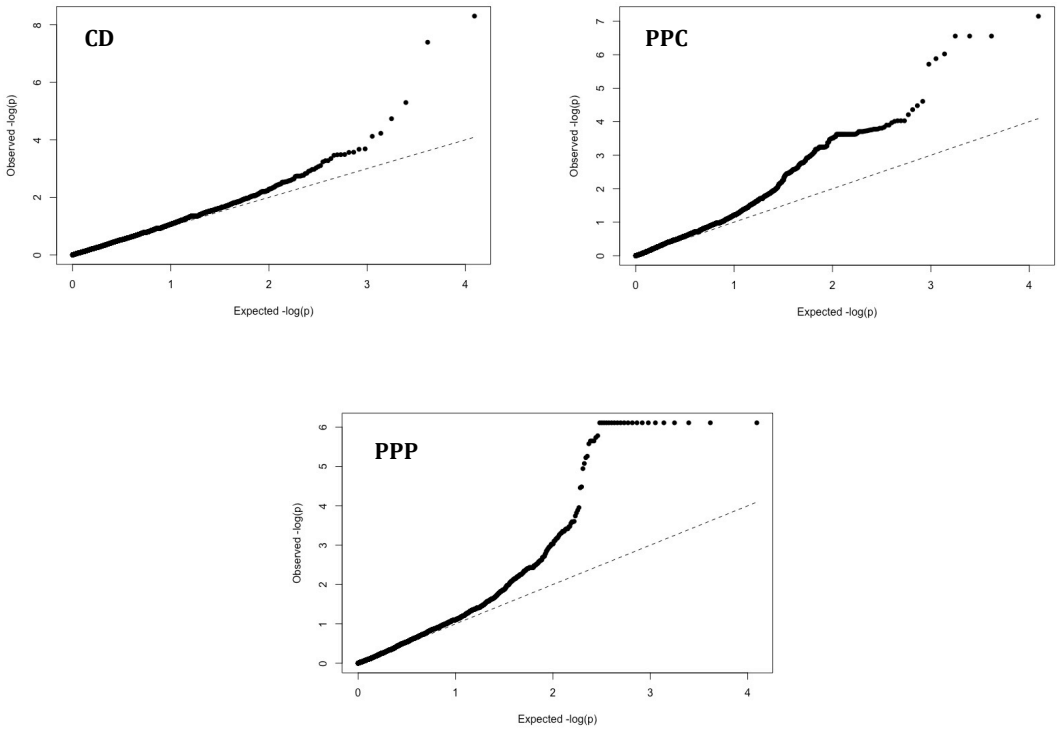
5.6 SUPPLEMENTARY MATERIAL

Supplementary Table 5.1: Family ID, parents involved in each cross (P1 and P2) and number of lines per family used in a GWAS study for anther culture response in *tropical japonica* rice.

Family ID	P1	P2	Number of lines
DH_8	JAP_8	JAP_14	5
DH_12	JAP_19	JAP_3	4
DH_15	JAP_9	JAP_3	5
DH_16	JAP_9	JAP_6	2
DH_19	JAP_9	JAP_17	5
DH_28	JAP_18	JAP_6	5
DH_29	JAP_18	JAP_7	2
DH_35	JAP_7	JAP_2	3
DH_38	JAP_9	JAP_18	2
DH_40	JAP_11	JAP_6	5
DH_42	JAP_14	JAP_10	4
DH_43	JAP_14	JAP_7	5
DH_44	JAP_18	JAP_9	5
DH_49	JAP_5	JAP_10	3
DH_52	JAP_7	JAP_6	1
DH_55	JAP_11	JAP_18	3
DH_59	JAP_18	JAP_16	5
DH_60	JAP_19	JAP_10	5
DH_61	JAP_3	JAP_10	5
DH_62	JAP_5	JAP_13	5
DH_65	JAP_3	JAP_1	3
DH_67	JAP_12	JAP_14	5
DH_68	JAP_12	JAP_15	5
DH_70	JAP_17	JAP_13	5
DH_75	JAP_11	JAP_10	5
DH_77	JAP_12	JAP_13	4
DH_79	JAP_16	JAP_10	2
DH_80	JAP_16	JAP_14	1
DH_83	JAP_18	JAP_17	3
DH_89	JAP_11	JAP_13	4
DH_90	JAP_12	JAP_17	4
DH_96	JAP_12	JAP_18	3
DH_97	JAP_18	JAP_13	5
DH_102	JAP_4	JAP_14	5
DH_108	JAP_17	JAP_15	5
DH_110	JAP_5	JAP_15	2
DH_111	JAP_5	JAP_18	1
DH_120	JAP_17	JAP_19	1
DH_128	JAP_12	JAP_16	5
DH_137	JAP_5	JAP_2	1
DH_141	JAP_9	JAP_15	2
DH_143	JAP_10	JAP_13	3
DH_154	JAP_4	JAP_11	5
DH_156	JAP_5	JAP_1	5
DH_156	JAP_5	JAP_1	4
DH_159	JAP_7	JAP_13	2
DH_161	JAP_11	JAP_19	2
DH_167	JAP_15	JAP_1	3
DH_168	JAP_16	JAP_4	3
DH_177	JAP_16	JAP_11	1
DH_178	JAP_2	JAP_4	5
DH_183	JAP_4	JAP_15	5
DH_184	JAP_4	JAP_1	5
DH_185	JAP_11	JAP_16	5



Supplementary Figure 5.1: QQ-plots for all 3 traits including number of calli per dish (CD), number of plants regenerated per callus (PPC), and number of panicles harvested per regenerated plant (PPP).