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Improving water use efficiency of irrigated rice: analysis of feasibility of plant breeding and crop management options

Ph.D. Thesis:

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Abstract

Rice production account for more than 50% of global irrigation water, thus, reducing water use is becoming a priority, mostly related to increase in surface air temperature and reduction of water availability due to the global warming. A solution would be to breed rice accessions adapted to aerobic water management system, which is characterized by periodic drying and re-flooding of rice field and allow reduction of water demand. However, since yield is penalized in most European temperate *japonica* rice when grown under aerobic conditions, it is important to maintain a sustainable level of yield while optimizing water use. To achieve this goal, genomic selection approaches were used to evaluate the feasibility of genome wide selection for the identification of rice breeding lines with tolerance to water scarcity and related genome wide-based selection tools. A training population of *japonica* rice composed of 283 rice accessions and a population of 97 F5-F7 progenies derived from 36 bi-tri-parental crosses between elite lines that belong to the training population were phenotyped in irrigated and aerobic conditions and subjected to genotyping by sequencing. The accuracies, as obtained from correlations between the Genome Estimated Breeding Values (GEBV) and True Breeding Values (TBVs) were evaluated for three agronomically relevant traits using cross validation in the training population panel and across generations using phenotypic and genotypic data of the progeny population. Moreover, also the effects on the accuracies of different levels of Minor allele frequency, linkage disequilibrium, prediction models and, for the evaluations across generations, of scenarios involving different set of the training panel, were evaluated. High levels of accuracies with both the procedures (cross validation and across generations) were achieved even for complex agronomic traits like panicle weight, flowering date and nitrogen index. Results permitted to assess the feasibility of genomic selection across generation in rice population and highlighted a group of progenies that can be exploited in the breeding for tolerance to water scarcity. Then, using phenotypic data obtained under two contrasted irrigation system, irrigated (I) and Aerobic (A), in a reference population (RP) and in a progeny population (PP) we tested two set of approaches for predicting response to aerobic system. The first approaches were based on response index and regression analysis, the seconds on explicit modelling of marker by environment interaction. Rank-correlation between the performances of the individual entries of the two population was high for flowering time and panicle weight traits, indicating rather limited level of GxE interactions. Accuracy of genomic predictions were much higher when GxE interactions were modelled explicitly. In the second part of the PhD Thesis, the economic performances of different water management systems, including the role

of availability of rice varieties adapted to each management system, were evaluated and a multi-objective model was implemented to explore economic and water saving at the farm level. Results showed that the advantages of the aerobic method adoption depend on production costs, irrigation water cost and efficiency of selected rice varieties in different rice ecosystems.

Contents

Chapter 1 : General introduction

The objective of the research activities reported in this monography was to explore the feasibility of improving the water use efficiency of the Italian irrigated rice cropping systems. The options explored included new water management systems needing less water, and the development of new rice varieties adapted to these new water management systems. In this introductive chapter, before further detailing the research objectives, we will review major biological characteristics of the rice plant and economical features of rice cropping systems and water management, as well as recent evolutions of rice breeding methods with emphasis on integration of genotype by environment interactions.

1.1 Rice plant and rice growing ecosystems

Rice is a monocotyledonous semi aquatic heliophile annual grass plant, which belongs to the *Poaceae* family, genus *Oryza*. The most recent taxonomic data consider that the *Oryza* genus has 22 species, of which two are cultivated: *Oryza sativa* originated from Asia and *Oryza glaberrima* originated from Africa. *O. sativa* was domesticated from the wild species *O. rufipogon* 8,000 to 10,000 years before present, independently in China and India. *Oryza sativa* comprises two major subspecies, *Indica* and *Japonica,* and at least three secondary groups, Aus, aromatics, and deep water (Glaszmann, 1987). The japonica subspecies is subdivided into a temperate component cultivated in lowland ecosystem in temperate regions, and a tropical component cultivated mainly in upland ecosystem.

Rice is a highly plastic crop and can be cultivated in various ecosystems:

- The rainfed lowland ecosystem: bunded fields with slight slopes; non-continuous flooding of variable depth and duration; submergence not exceeding 50 cm for more than 10 consecutive days; rice transplanted in puddled soil or direct seeded on puddle or plowed dry soil; alternating aerobic to anaerobic soil conditions of variable frequency and duration. This agro-system represents one quarter of the world’s rice growing areas.
- The upland ecosystem: sloping fields; rarely flooded, aerobic soil conditions; rice direct seeded on plowed dry soil or dibbled in wet, non-puddled soil. It represents around 13% of total rice growing area.
- The irrigated lowland ecosystem: Levelled and bunded fields with strict water control; rice transplanted or direct seeded in puddle soil; shallow flooded with anaerobic soil condition during crop growth. It covers over half of the world’s rice lands and provides about 75% of the world’s rice supply.

1.2 Rice production in the world and in Europe

Rice is grown in over a hundred countries, in all continents except Antarctica. It is the first staple food for around 3.5 billion people worldwide and approximately 480 million tons of milled rice are produced annually (World rice statistics, 2013). The Asian continent is the world's largest producer and consumer, China and India's production alone representing around 50% of the world production. Rice provides up to 50% of the dietary supply in Asia, as it is the main cereal consumed. The rice consumption is about 110 kg per capita annually in Asia (FAOSTAT, 2013). In Africa, mainly the sub-Saharian part, rice consumption per capita is steadily growing with a consumption per capita that has doubled since 1970 (50kg per capita in 2015). Caribbean countries and Latin America also reported an increase of rice consumption. In Europe, rice is the sixth cereal with a total of 4 million tons produced on 650,000 ha in 2013 (Faostat, 2014). Italy is the first European rice producer with 50% of the total production. The country produces over 1.4 million tons of paddy rice annually on more of 219,000 ha. The main rice producer regions are Piedmont and Lombardy. The secondary area of production includes Veneto (2% of country areas), Emilia Romagna (4%) and Sardinia (1%) and some very circumscribed areas in the central and southern Italy (Ente Nazionale risi, 2015). Rice consumption in Europe is around 3.5-5.5 kg per capita per year of milled rice in non-rice-growing countries of northern Europe and 6–18 kg in southern Europe (Maclean et al., 2002). However, in Europe, rice has a sociocultural and ecological role. Irrigated rice cultivation permit important ecosystem benefits such as the preservation of wetland habitats for some species (Miniotti et al., 2016).

1.3 Effect of climate change on rice production

Rice yields should increase by 60% by 2050 in order to respond to the growing demand of world's population (Tilman et al., 2002). However, due to increase of urban and industrial water demand and effect of climate change, rice production is threatened by water scarcity. In 2025, only for Asia continent, 2 million ha of irrigated dry-season rice areas and 13 million ha of irrigated wet-season rice areas may experience physical water scarcity (Tuong and Bouman, 2003).

Climate change is also a major challenge for rice production in Europe. Indeed, most European countries are experiencing a fast increase in air temperature and in temperature variability (Olesen et al., 2011). Between 1901 and 2005, a temperature increase of about0.9°C in annual mean temperature over the entire continent has been registered (Kjellström, 2004) primarily due to an increase in warm extremes (Klein Tank and Können, 2003). Mean precipitation has significantly decreased along the Mediterranean coasts (Klein Tank. et al., 2002). In Italy, the flow of the Po river, which irrigates a large part of Italian rice fields, has decreased by 20-25% in the last 30 years and has passed from historical values of 1800 m^3/s to the present values of 1400-1500 m^3, (Ciais et al., 2005). One of the consequences is the stagnation of cereal production and the increase of yield variability. One of the solution to this problem would be to reduce irrigation water allocated to rice cultivation.

1.4. Production cost and rice market

Rice production increased of about 130% during the Green Revolution period between 1960 and 1980. The major part of this increase was attribute to modernization of rice cultivation with the adoption of new farming technologies and the development of semi-dwarf and shorter cycle rice varieties, which permitted to (i) increase yield by using more chemical fertilizers and (ii) cultivate rice more than one time a year in the tropical regions (Uddin et al., 2016). However, rice import and export markets are quite volatile due to shortfall of production and the global rice trade that accounts for only 7% of total production. Rice market is governed by different types of policies. In Asia, many countries have strict policies and restriction in rice import and export to achieve domestic food security. Rice market depends also on the variety type and the degree of grain processing (parboiled, brown or white rice) and milling. In Europe, Common Agricultural Policy (CAP) of the European Union subsidizes rice cultivation in exchange of environment friendly agricultural practices. Despite the PAC policy, rice prices are still quite volatile while production costs follow a continuous increase (Ben Hassen et al., 2017). Cost of irrigation water is a non-negligible component of the production costs.

1.5 Methods to reduce water consumption

The most drastic option to reduce the quantity of water used for rice irrigation would be to expand the aerobic rice cultivation practices (Bouman et al., 2005). However such practices have many disadvantages. They reduce yield potential and increase the incidence of pests such as nematodes and weeds. Other agronomic practices at the level of the field management have been proposed such as field land levelling, tillage bund preparation (Bouman et al., 2007) .Less drastic options include mid-season drainage, dry seeding and delayed flooding, and intermittent irrigation(Kato et al., 2009; Dunn and Gaydon, 2011; Heenan and Thompson, 1984). Intermittent irrigation may lead to reduction in water requirement compared to continuous flooding by reducing seepage, percolation and evapotranspiration losses (Kato et al., 2009; Kato and Katsura, 2014). For instance, the alternate wetting and drying (AWD) irrigation system, where water is applied only few days after the disappearance of ponded water, reduces water consumption by 35% (Zhang et al., 2009). It has also been reported that such practices can increase the plant nutritional status, decrease toxic elements such as arsenic (Xu et al., 2008; Norton et al., 2012) and cadmium (Yang et al., 2009) and reduce the emission of greenhouse gases (Barker et al., 2010). Some studies reported that AWD can have no effect on grain yield (Yao et al., 2012) or a slight decrease in yield (Sudhir-Yadav et al., 2012). However the effect of AWD on yield depends on rice variety and the soil moisture degree at the end of each cycle of drying (Davies et al., 2011). Therefore, the development of rice varieties specifically adapted to AWD irrigation system is a necessary condition for the adoption of this system by farmers.

1.6 Methods for breeding new rice varieties

The most common breeding method for a self-pollinated crop such as rice is the pedigree method (Gallais, 2011). It starts with the crossing of two inbred lines, chosen to bring complementary traits, that leads to an homogeneous F1 generation. In the second generation F2, a segregation of all heterozygote loci is observed and the genetic variability is important. If the selection is based on the phenotype, breeder will target, at this generation, the most heritable traits. Progenies of the selected plants are sown again in ear-to-row to produce the next generation, and so on. From F3 to F6 generation, the best plants are selected based on intra (within the sown lines) and inter (between lines) family's performances. From F5 to F6, grain yield is evaluated and the best progenies (candidates) are multiplied. Then, from F9 to F10, the lines are tested for their registration and those who succeed in the test are then available to farmers. However, it is well known that the phenotypic response of the progenies is influenced by the interaction between their genotype and the breeding environment. Multiplying the phenotyping procedure in several environments can be expensive and time consuming. Since mid-90s, new breeding methods based on establishment of relationship between the phenotype and the genotype has been developed. They are supposed to prevent the abovementioned drawback of phenotype-based breeding and to accelerate the process of development of new varieties.

1.7 From Marker assisted selection to Genomic selection in rice

Rice, having the smallest genome of all cultivated cereals (430Mb), being diploid and self-pollinating, is considered as a model species among cereals for performing genetic studies.. Rice genome was the first crop species genome to be sequenced (Sequencing Project International Rice Genome, 2005).

Advances in genomics have been continuously providing rice breeders with tools and resources to facilitate the study of genotype and its relationship with phenotype. With a better understanding of the mechanisms and the genetics of trait of interest, breeders were able to use the more precise breeding approaches of marker-assisted selection. Today, marker- assisted selection (MAS) to improve breeding efficiency has become commonplace in rice breeding programs (Sandhu and Kumar, 2017). For instance, for many abiotic stresses, major QTLs/ genes were identified (Jena and Mackill, 2008; Ismail and Thomson, 2011), fin mapped and the genotype-phenotype information was used for marker assisted selection. The first generation of MAS was focusing on the transfer, into elite materiel, of the favorable allele of individual gene/QTL of large effect identified using experimental mapping populations. This was the case, for instance for submergence tolerance (Mackill et al., 2012), phosphorus uptake (Gamuyao et al., 2012), tolerance to salinity (Ismail et al., 2007) and tolerance to drought (Bernier et al., 2007; Venuprasad et al., 2009; Vikram et al., 2011).

The second generation of MAS consisted in “forward marker-assisted breeding” schemes, where QTLs for target traits are detected within the segregating progenies of elite lines crossed for their complementarities and then the marker–phenotype information guides the construction of the ideal genotype defined as the mosaic of favourable chromosomal segments from the two parents, which usually never occur in any Fn population of realistic size (Stam, 1995). Requiring several successive generations of crossing, such marker assisted recurrent selection (MARS) or genotype construction (Stam, 1995; Peleman and van der Voort, 2003; Moreau et al., 2004) was implemented for the improvement of drought tolerance (Kumar et al., 2014; Shamsudin et al., 2016).

The third generation of marker assisted selection, Genomic selection (GS), has arisen from the conjunction of new high-throughput marker technologies and new statistical methods that allow the analysis of the genetic architecture of complex traits in the framework of infinitesimal model effects, instead of the model of limited numbers of QTL of varying effects. It refers to methods that use genome-wide dense marker, mainly SNP, information for the prediction of genetic values with enough accuracy to allow selection on that prediction alone. It consists of two steps: (i) estimation of SNP effects, and (ii) prediction of genetic value based on SNP genotypes or genomic breeding value (GEBV) (Meuwissen et al., 2001a). It extends the use of markers to breed for highly polygenic traits, such as yield, drought tolerance and resource use efficiency (Jannink et al., 2010).

GS has been implemented in animal breeding, especially in dairy cattle, for the last decade, (Hayes et al., 2009; Dekkers, 2012) and possible applications in plant breeding have given rise to many studies using simulations or experimental data (Lorenz et al., 2011; Desta and Ortiz, 2014; Barabaschi et al., 2015). The effect of the statistical method on the accuracy of GEBV has been widely analyzed (Heslot et al., 2012). The statistical methods differ in the assumptions they make about the effects of markers and the variance of such effects across the genome. For example, penalized regression methods consider the effects as random and drawn from a normal distribution with equal variance for all markers that enter the final model (Li and Sillanpää, 2012). Bayesian methods, such as BayesA or BayesB proposed by Meuwissen et al., (2001), model the effect of each marker using a normal distribution with its own variance (BayesA), and, in addition, in the case of BayesB, the probability of a marker having an effect is also taken into consideration (Kärkkäinen and Sillanpää, 2012). Semi-parametric and non-parametric regressions for traits with complex genetic architecture include implicit non-additive components (De Los Campos et al., 2010). Several other methods were reported in the literature and the general conclusion is that there is no single best statistical method. Indeed, the accuracy of the different methods depends on other factors, such as the characteristics of the target trait, the density and distribution of the markers, the size and the structure of the Training population, and the degree of relatedness between training population and the candidate population (Pérez-Rodríguez et al., 2012).

The characteristics of the target trait reported to influence the accuracy of predictions include heritability, the number of QTLs, the distributions of their allelic effects and frequencies, and the relative magnitude of additive and non-additive genetic variance (Hayes et al., 2009; Jannink et al., 2010; Burstin et al., 2015). Regarding marker density, empirical data (Lorenzana and Bernardo, 2009; Lorenz et al., 2011; Poland et al., 2012; Heslot et al., 2013) have confirmed the theoretical stance that marker density should be high enough to ensure strong linkage disequilibrium (LD) with at least one marker for each QTL. Seeking cost effectiveness, several low-density genotyping options have been explored, such as the use of a panel of evenly spaced low-density markers (Habier et al., 2009); (ii) the selection of markers on the basis of their effects on the trait (Zhang et al., 2011); or (iii) the selection of markers using haplotype blocks (Cuyabano et al., 2015). The trade-off between the three options depends on the genetic architecture and the number of target traits in a breeding program (Ma et al., 2016). In addition to the genetic architecture of the trait, the effective population size, the size of TP, and the average LD between adjacent markers also have a significant effect on the performance of the low-density genotypic data (Calus et al., 2008; Tayeh et al., 2015).

The last set of factors that strongly influence the accuracy of predictions includes the size of the TP, its structure, and its relatedness with the CP. Reducing the size of the TP can negatively affect the estimation of marker effects, which in turn can reduce prediction accuracy (Heffner et al., 2011; Jarquín et al., 2014; Tayeh et al., 2015). For that reason, methods have been developed to optimize the composition of the TP (Rincent et al., 2012; Akdemir et al., 2015), by maximizing the expected reliability for a given set of individuals. The degree of relatedness between the TP and the CP also influences accuracy. Accounting for population structure through stratified sampling in the TP can significantly improve the accuracy of the predictions (Albrecht et al., 2011; Grenier et al., 2015; Isidro et al., 2015).

1.8 Genotype by environment interaction

The genotype by environment interaction is the variation of response of a given genotype to different environments/cropping conditions. In other word, the phenotype is a combination of genotype (G), environment (E) and genotype × environment interaction (G × E) effects. The presence of G × E usually complicates the process of breeding. When G x E interaction is significant, its nature, cause(s), and implications must be examined to improve a breeding/testing program. For instance, a strong genotype-by-location interaction for yield or any other trait implies the establishment of specific breeding programs for each location. If high yielding cultivars with stable performance across environments could be developed/identified, the need for location specific breeding programs would be reduced, and the breeding program would be more cost-effective. Consequently, multi-environment trials (METs) are widely used by plant breeders for evaluating the relative performance of genotypes over the target environments. Numerous methods have been developed to study and reveal the nature of G×E interaction, e.g., joint regression (Finlay and Wilkinson, 1963; ,Eberhart and Russel, 1966), additive main effects and multiplicative interaction (AMMI) (Gauch, 1992), and multivariate linear mixed-effect models framework (Crossa et al., 2004; Crossa et al., 2006; Burgueño et al., 2008). However, those models describe the GxE effect without an explicit modelling of the GxE interaction and account mainly for the average interaction effect across the genome (Lopez-Cruz et al., 2015). The recent availability of genotypic data has paved the way for the explicit modelling of GxE, taking into account of the contribution of different genomic region. This approach was first used to detect QTL by environment interactions (Moreau et al., 2004; (Boer et al., 2007; Malosetti et al., 2004; Malosetti et al., 2008) and was extended to genomic selection (GS) analysis. Burgueño et al., (2011) were the first to use multi-environment GS. Heslot et al., (2014), compared an extension of the factorial regression model in the framework of GBLUP. Lopez-Cruz et al. (2015) included marker environment effect in GBLUP to evaluate the interaction in three wheat populations genotyped and evaluated under simulated environmental conditions. Cuevas et al. (2016) included the M×E effect in the RKHS methodology with two kernel matrices that differed on the way the bandwidth was estimated. Thus, a large set of methods are now available for genomic prediction accounting for GxE interaction.

1.9 Objectives of the thesis work

The first objective of my PhD work was to evaluate the potential of genomic selection approach for the improvement of the rice water use efficiency in the framework of a pedigree breeding program, using genetic resources from the *japonica* group. This objective was declined into several sub-objectives:

1. Evaluate the accuracy of genomic prediction for different types of traits under the conventional irrigated rice cropping systems, using cross-validation within a diversity panel,
2. Evaluate the efficiency of calibrating genomic prediction models within a diversity panel to predict the performances of progenies derived from bi-parental crosses involving a member of the diversity panel,
3. Evaluate the potential of indirect selection to improve the response to a cropping system using phenotypic data from the alternative system that is irrigated system versus the aerobic system.

Evaluate different ways to model multi-environment phenotypes (implicit (two-steps) *versus* explicit (one-step) modelling of GxE).

The second objective of the Ph.D work was the understanding of the socioeconomic and environmental impact of rice water management, especially in the context of a shift from irrigated systems to aerobic systems in Italy. To this end, two sets of complementary research activity were undertaken:

- Analysis of the economic performances of different water management systems, including the role of availability of rice varieties adapted to each management system.
- Implementation of a multi-objective model to explore farm-level economic and water saving results of different water management options for rice cultivation in Italy, using real data obtained from field experiments.

Chapter 2: Rice diversity panel provides accurate genomic predictions for complex traits in the progenies of biparental crosses involving members of the panel

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2.1 Abstract

So far, potential applications of genomic prediction in plant improvement have mostly been explored using cross validation approaches. This is the first empirical study evaluating the accuracy of genomic prediction of the performances of progenies in a typical rice breeding program. Using a cross validation approach, we first analyzed the effects of marker selection and statistical methods on the accuracy of prediction of three traits of different heritability in a reference population (RP) of 284 inbred accessions. Next, we investigated the size and the degree of relatedness with the progeny population (PP) of sub-sets of the RP that maximize the accuracy of prediction of phenotype across generations, i.e. for 97 F5-F7 lines derived from bi-parental crosses between 31 accessions of the RP. The extent of linkage disequilibrium was high (r^2=0.2 at 0.80 Mb in RP and at 1.1 Mb in PP). Consequently, average marker density above 1 per 22 kb did not improve the accuracy of predictions in the RP. The accuracy of progeny prediction varied greatly depending on the composition of the training set, the trait, LD and minor allele frequency. The highest accuracy achieved for each trait exceeded 0.50 and was only slightly below the accuracy achieved by cross validation in the RP. Our results show that relatively high accuracy (0.41 to 0.54) can be achieved using only a rather small share of the RP, most related to the PP, as the training set. The practical implications of these results for rice breeding programs are discussed.

Keywords: Rice, genomic selection, progeny prediction, complex traits

2.2 Introduction

Genomic selection (GS) arose from the conjunction of new high-throughput marker technologies and new statistical methods (Meuwissen et al. 2001). GS allows analysis of the genetic architecture for complex traits in the framework of infinitesimal model effects. It consists in (i) building a model of genotype-phenotype relationships in a training population (TP) where the effects of all the markers (often large numbers) are estimated simultaneously, thus accounting also for linkage disequilibrium (LD) among markers, and (ii) using the model to predict the genomic estimate of breeding values (GEBV) of candidates in a breeding population (CP) (Meuwissenet al. 2001; Jannink et al. 2010). The effectiveness of GS depends on the degree of correlation between the predicted GEBV and the realized phenotype, i.e. the accuracy of prediction. Compared to conventional marker-assisted selection, whose efficiency is limited by the power of marker-trait association tests, GS is expected to be more efficient, especially for highly polygenic traits (Bernardo and Yu 2007).

GS has been implemented in animal breeding, especially in dairy cattle, for the last decade, (Hayes et al. 2009; Dekkers 2012), and possible applications in plant breeding have given rise to many studies using simulations or experimental data (Lorenz et al. 2011; Desta and Ortiz 2014; Barabaschi et al. 2016). The effect of the statistical method on the accuracy of GEBV has been widely analyzed (Heslot et al. 2012). The statistical methods differ in the assumptions they make about the effects of markers and the variance of such effects across the genome. For example, penalized regression methods consider the effects as random and drawn from a normal distribution with equal variance for all markers that enter the final model (Li and Sillanpää 2012). Bayesian methods, such as BayesA or BayesB proposed by Meuwisen et al. (2001) model the effect of each marker using a normal distribution with its own variance (BayesA), and, in addition, in the case of BayesB, the probability of a marker having an effect is also taken into consideration (Kärkkäinen et al. 2012). Semi-parametric and non-parametric regressions for traits with complex genetic architecture include implicit non-additive components (de los Campos et al. 2010). Several other methods are reported in the literature and the general conclusion is that there is no single best statistical method. Indeed, the accuracy of the different methods depends on other factors, such as the characteristics of the target trait, the density and distribution of the markers, the size and the structure of the TP, and the degree of relatedness between TP and CP (Pérez-Rodríguez et al. 2012).

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Rice (*Oryza sativa*) is the world's most important staple food and will continue to be so in the coming decades. Genetic improvement is one of the major pillars of sustainable adaptation of rice production to ongoing global changes (Atlin et al. 2017). GS is expected to accelerate genetic gain for traits such as yield potential and adaptation to constraints related to climate change and the efficient use of resources (water, nitrogen, etc.) (Ashikari 2017; Atlin et al. 2017). However, like in other crop species, few empirical studies have been conducted to evaluate the accuracy of genomic prediction for the purpose of making selection decisions in actual breeding programs, i.e. to predict the performances of progenies (Desta and Ortiz 2014). Indeed, studies on rice have mainly been based on cross validation within diversity panels (Table 1).

Table 1 Genomic selection studies conducted on rice

Plant materiel	Phenotypic data	Genotypic data	Statistical Methods	Range of accuracy of GEBV	Main conclusion	Reference
110 Asian cultivars	8 traits including days to flowering (FL),	3,071 SNPs	rrBLUP, ENet, GBLUP, RKHS, RF, Lasso, BL, EBL, wBSR	FL: 0.65-0.85	Reliability depended to a great extent on the traits targeted. Reliability was low when only a small number of cultivars were used for validation.	Onogi et al. 2015
Highly structured diversity panel of 413 accessions	8 traits including grain yield (GY), flowering date (FL) and plant height (PH),	36,901 SNPs (1 SNP per 10 Kb)	GBLUP	FL: 0.25 - 0.60 PH: 0.25 - 0.55 GY: 0.20 - 0.50	Maximizing the phenotypic variance captured by the training set is important for optimal performance. Stratified sampling of the training set ensures better accuracy than sampling based on the CDmean.	Isidro et al. 2015
	15 traits of rather high heritability, including, flowering time (FL), plant height (PH) and protein content	36,901 SNPs (1 SNP per 10 Kb)	GBLUP, GBLUP-CPS	FL: 0.44 - 0.66 PH: 0.50 - 0.75	Prediction accuracy was affected by the genomic relationship between TP and VP and by genomic heritability in the TP and VP.	Guo et al. 2014
369 elite breeding lines	6 traits including days to flowering (FL) and grain yield (GY)	73,147 SNPs	RR-BLUP, BL, RKHS, RF,	FL: 0.35 - 0.65 PH: 0.15 - 0.35 GY: 0.10 - 0.30	Using one marker every 0.2 cM is sufficient for genomic selection in this collection of rice breeding material. RR-BLUP was the most efficient statistical method for GY where no marked effect of QTLs was detected by GWAS.	Spindel et al. 2015
354 S3:4 lines	Days to flowering (FL), plant height (PH) and grain yield (GY)	8,336 SNPs 1 marker per 44.8 kb	RR-BLUP, GBLUP, Lasso, BL	FL: 0.20 - 0.30 PH: 0.50 - 0.60 GY: 0.20 – 0.31	Accuracy of GEBV is affected by (i) relatedness between TP and CP, (ii) trait heritability and interaction between traits and all the other factors studied (prediction models, LD, MAF, composition of the TP).	Grenier et al. 2015
115 lines of hybrids	8 traits including grain yield (GY), and plant height (PH),	2,395,866 SNPs,	GBLUP, GBLUP dominance effects	FL: - PH: 0.45 - 0.86 GY: 0.13 - 0.34	Model including the dominance effect provide more accurate prediction, particularly in multi-traits scenario for a low-heritability target trait, with highly correlated auxiliary traits.	Wang et al. 2017

Here we report the first empirical study to assess the performance of genomic prediction within and across generations in rice. It was undertaken in the framework of a rice breeding program conducted according to the most common rice breeding scheme, i.e. pedigree breeding within the progenies of bi-parental crosses, the parents being chosen within a working collection of inbred accessions. First, using a cross validation approach, we analyzed the effects of marker selection and statistical methods on prediction accuracy within the inbred accessions of a working collection of 284, individuals for three traits of different heritability (days to flowering, nitrogen index and panicle weight). Next, we investigated the characteristics (size and degree of relatedness with the candidate population, etc.) of sub-sets of the working collection that maximize the accuracy of prediction of phenotype across generations, i.e. for 97 inbred lines derived from bi-parental crosses between 31 accessions of the working collection.

2.3 Materials and methods

2.3.1 Plant material

The plant material comprised a diversity panel of 284 accessions and 97 advanced (F_5-F_7) inbreed lines. The diversity panel represents the working collection of the rice breeding program of Research Centre for Cereal and Industrial Crops (CREA), Vercelli, Italy. It is composed of 139 accessions of Italian origin and 145 accessions of diverse geographic origin (Supplementary Table 1), all belonging to the *japonica* subspecies of *O. sativa* and all adapted to cultivation in the irrigated rice ecosystem of temperate Mediterranean Europe (Faivre-Rampant *et al.* 2011; Biscarini et al. 2016). Hereafter we refer to this diversity panel as the "reference population" (RP). The 97 advanced lines were derived from 36 biparental crosses (including five backcrosses) involving 31 accessions of the diversity panel (Supplementary Table 1). The number of progenies per cross varied from 1 to 20 (Supplementary Table 2; Supplementary Figure 1). In the present study, these 97 lines constituted the "progeny population" (PP).

2.3.2 Field trials and phenotyping

Phenotyping of RP and PP took place at the CREA experimental station (45°19'24.00"N; 8°22'26.28"E; 134 m asl.), in an irrigated cropping system with standard crop management. The RP was phenotyped during the 2012 and 2013 rice cropping seasons under a complete randomization experimental design with three replicates per accession. The size of the individual plot was 1.70 m x 0.40 m, with three rows of 60 seeds each. The PP was phenotyped during the 2014 and 2015 rice cropping seasons under randomized complete block design with three replicates. The size of the individual plot was 1.20 m x 0.80 m, with six rows of 40 seeds each.

The target traits for both RP and PP were days to flowering (FL), panicle weight (PW), and the nitrogen balance index (NI). FL was recorded as the number of days after sowing when 50% of the plants in the plot were in flower. In the experiments related to RP, PW (g) was recorded by weighing a random sample of 50 panicles in the plot, and, in the experiments related to PP, by weighing 100 representative panicles. PW is thus a proxy for plot grain yield. NI, an indicator of the plant nitrogen status (Tremblay et al. 2012), was recorded using a Dualex ^{TM} instrument (Goulas et al. 2004), seven to 10 days after the flowering date, a period during which the nitrogen status of the plant is stable. In each plot, three measurements were made on the adaxial and the abaxial sides of a panicle leaf on three plants. The 18 measurements were then averaged to obtain a plot level NI.

2.3.3 Analysis of phenotypic data

Phenotypic data were analyzed using the proc mixed procedure of SAS 9.2 (SAS Institute, Cary NC, USA). The mixed model used for the RP was:

$$Y_{ijk} = \mu + g_i + y_j + (gy)_{ij} + e_{ijk} \qquad \text{(RP model)}$$

where Y_{ijk} is the observed phenotype of genotype i in year j and in plot k, μ, the overall mean, g_i, the genotype effect, y_j, the year effect, $(gy)_{ij}$, the interaction between genotype i and year j, and e_{ijk} the residual. Except for the overall mean, all the effects were considered random.

The mixed model used for the PP was:

$$Y_{ijk} = \mu + g_i + y_j + r(y)_{jk} + (gy)_{ij} + e_{ijk} \qquad \text{(PP model)}$$

where Y_{ijk}, μ, g_i, y_j, $(gy)_{ij}$ and e_{ijk} have the same meaning as in the RP model, and $r(y)_{jk}$, is the replicate within year effect. Like in the previous model, all the effects except μ were considered random.

A model-based diagnostic analysis was run for each field trial and each trait within the mixed model framework above, to detect potential outliers among the individual data points (plot level). This procedure resulted in the elimination of five data points (of PW) in the 2013 field trial involving the RP. The eliminated data were considered as missing in the following steps of data analysis.

Broad sense heritability of accession means, H^2, was calculated for each trait in each population using the formula of Holland et al, (2003) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gy}^2}{ny} + \frac{\sigma_e^2}{nr}}$$

where *ny* represents the mean number of years in which the accessions were tested and *nr*, the mean number of plots per accession across years. The means were calculated as harmonic means.

Finally, adjusted means of accessions were extracted for each trait to be used as phenotypes in the genomic prediction models.

2.3.4 Genotyping and genotypic data

The genotyping procedure is detailed in Biscarini et al. (2016). Briefly, genomic DNA was isolated from three-week-old leaves using the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) with a TECAN Freedom EVO150 liquid handling robot (TECAN Group Ltd, Männedorf, Switzerland). DNA digestion was performed using ApeKI restriction enzyme. Digested DNAs were ligated to 12 of 0.6 / adapter pairs (optimized to guarantee good quality libraries in rice), and the 96-plex library constructed according to the genotyping by sequencing (GBS) protocol. The libraries were loaded into a Genome Analyzer II (Illumina, Inc., San Diego, USA) for sequencing. The *Tassel* GBS pipeline v3.0 (Glaubitz et al. 2014) was used for filtering the raw data, sequence alignment to the rice reference genome (*Os-Nipponbare-Reference-IRGSP-1.0*), and for SNP calling. Missing SNP genotypes were then imputed using the FILLIN (Fast, Inbred Line Library ImputatioN) algorithm in the *Tassel* GBS pipeline v3.0, with default settings. When the FILLIN algorithm was unable to find haplotypes to satisfy any of the threshold requirements, the SNP locus was not imputed. After imputation, a total of 246,554 SNPs with a call rate greater than 80% were available. Filtering of this matrix for the rate of heterozygoty (threshold of 5%) and for a minor allele frequency (MAF, threshold of 2.5%) among the RP accessions and PP lines, considered together, led to a final working set of 43,686 SNP loci.

The genotypic data are available at http://tropgenedb.cirad.fr/tropgene/JSP/interface.jsp?module=RICE, (Choose Tab Studies) as GS-Ruse_CREA_GBSgenotype_RP&PP.

2.3.5 Genotypic characterization of RP and PP

The genetic structuring of the two populations was analyzed jointly using a distance based method. First, a matrix of 4,824 SNPs was extracted from the working genotypic dataset of 43,686 SNPs, by discarding loci that had imputed data and by imposing a minimum distance of 10 kb between two adjacent loci. Then an unweighted neighbor-joining tree based on a simple matching matrix was constructed using DarWin v6 (Perrier and Jacquemoud-Collet 2006).

Pairwise LD between SNP loci was calculated separately in RP and PP at the level of the individual chromosome, using the working genotypic dataset of 43,686 SNPs and the r^2 estimator proposed by Rogers and Huff (2009) for non-phased genotypic data.

2.3.5 Genomic prediction methods

Three statistical methods were tested: genomic best linear unbiased prediction (GBLUP), reproducing kernel Hilbert spaces regressions (RKHS) and BayesB (Meuwissen et al. 2001). The GBLUP method (VanRaden 2008) was implemented using the genomic matrix G = M*M' (M being the incidence matrix) and the Expectation-Maximization convergence algorithm. The RKHS method (Gianola and van Kaam 2008) was also used. Both methods were implemented using the KRMM package (https://sourceforge.net/projects/package-krmm/) described by Jacquin et al. (2016). The BayesB method was implemented using the BGLR statistical package (Perez and de Los Campos 2014).

2.3.6 Cross validation experiments

A total of 189 cross validation experiments were undertaken to investigate the effect of LD (7 threshold levels), MAF, (3 threshold levels), and the prediction methods (GBLUP, RKHS and BayesB) on the accuracy of genomic prediction of three phenotypic traits (FL, NI and PW), within the RP. Table 2 shows the size of the incidence matrix associated with the seven levels of LD thresholds and the three levels of MAF thresholds investigated, as well as the associated marker densities. The incidence matrices were constructed as follows. The SNP loci were first selected on the basis of the MAF threshold. Then r^2 was calculated between these loci and selection based on the r^2 threshold was performed for each SNP by counting the number of times its pairwise r^2 with other SNPs was above the threshold and discarding all SNPs that counted r^2 values above the threshold more than 30 times.

Table 2: Sizes of the incidence matrices used in the cross validation experiments in the reference population.

	Minor allele frequency (MAF)					
LD (r^2)	≥ 5%		≥ 10%		≥ 20%	
	N	D	N	D	N	D
≤ 0.25	3,322	8.7	1,927	5.0	1,173	3.1
≤ 0.36	5,365	14.0	3,450	9.0	2,270	5.9
≤ 0.49	8,324	21.7	5,738	14.9	4,013	10.5
≤ 0.64	12,099	31.5	8,744	22.8	6,095	15.9
≤ 0.81	16,923	44.1	12,652	34.2	8,917	23.2
≤ 0.98	28,164	73.3	23,119	60.2	16,750	43.6
≤ 1	32,066	83.5	26,845	69.9	20,104	52.4

N: Total number of SNPs; D: SNP density per Mb

The cross validation experiments used 189 (2/3) of the 284 accessions of the RP as the training set and the remaining 95 (1/3) accessions as the validation set. Each cross validation experiment was repeated 100 times, using 100 independent partitioning of the accessions into the training set and validation set. For each independent partitioning, the correlation between the predicted and the observed phenotype was calculated, so as to obtain 100 correlations for each cross validation experiment. The accuracy of each prediction experiment was computed as the mean value of the 100 correlations. The same 100 independent partitioning of the training and validation sets was used for all 189 cross validation experiments.

2.3.7 Genomic prediction across generations

Six scenarios, representing different degrees of relatedness between the training set and the progeny set and different sizes of the training set, were considered (Table 3). To this end, first, using pairwise Euclidian distances between each parental line and other accessions of the RP, the three closest accessions to each of the 31 parental accessions were identified. These accessions were then pooled to form the most related subset. Pooling led to a total of 58 accessions, because the closest accessions for some parents also happened to be the closest for other parents. Finally, this subset was combined, or not, with the parental lines and with the other accessions of RP to constitute the six training sets of the six prediction scenarios. For each scenario, the correlation between the predicted and the observed phenotypes of the 97 progeny lines was calculated, and represents the accuracy of the prediction experiment. In the case of scenario S6, in which the 31 accessions of the training set were randomly sampled 100 times from the RP excluding the parents, prediction accuracy was computed as the mean value of the 100 correlations between the predicted and the actual phenotypes of the 97 progeny lines. Comparisons between scenarios were, thus, based on progeny prediction accuracy (PPA) data for the non-replicated prediction experiments, and on the average PPA for the replicated experiments in scenario S6.

The six scenarios were implemented with seven incidence matrices corresponding to the seven thresholds of LD used in the cross validation experiments, a unique MAF threshold of ≥ 5%, and three prediction methods (GBLUP, RKHS and BayesB).

Table 3: Scenarios for genomic prediction across generations.

Scenario	Training set	Validation set
S1	31 parents	97 progeny
S2	58 related accessions	97 progeny
S3	31 parents + 58 related accessions	97 progeny
S4	31 parents + 252 accessions	97 progeny
S5	252 accessions, excluding the parents	97 progeny
S6	100 random sampling of 31 accessions excluding the parents	97 progeny

2.3.8 Analysis of sources of variation in genomic predictions

The mean correlations of all cross validation experiments were analyzed as dependent variables in an analysis of variance. A separate ANOVA was performed for the correlations of all the PPA and of the average PPA in the progeny prediction experiments. In each case, ANOVA was performed to partition the variance of accuracy into different sources, with all effects declared as fixed, and following two models: the first model comparing the effects each factor (phenotypic trait, LD, MAF and the prediction method in the cross validation experiments; phenotypic traits, LD, scenario and prediction method in the progeny prediction experiments), with no interaction; the second model accounting for all the principal effect as well as for all possible first-order interactions.

2.4 Results

2.4.1 Phenotypic diversity of the three traits investigated

The three traits investigated in the RP and PP populations exhibited a Gaussian distribution (Figure 1). For all three traits, the extent of phenotypic diversity was broader in the RP than in the PP. Moreover, the distribution of NI and PW in the PP remained among the lowest values for these traits, leading to lower mean values. The narrower phenotypic diversity of PP is probably linked to its narrower genetic diversity.

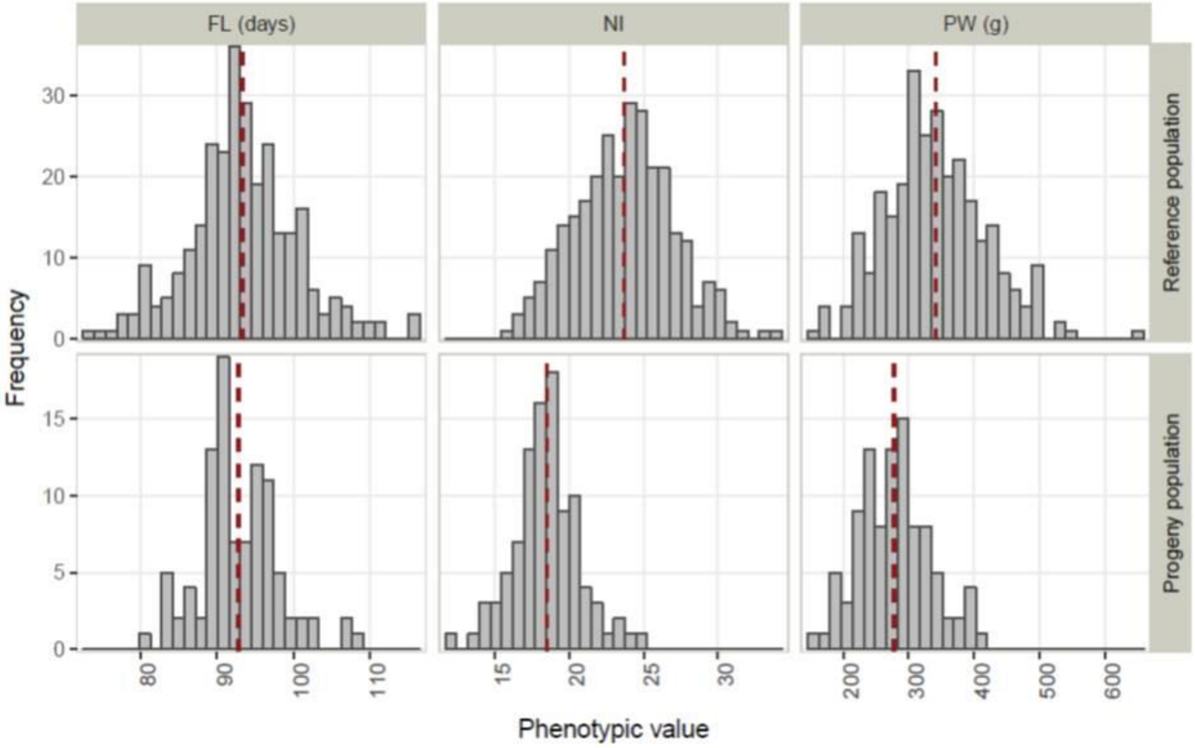

Figure 1: Distribution of phenotypic values for days to flowering (FL), Nitrogen balance index (NI) and 100 panicle weight (PW) in the reference and the progeny populations.

Separate ANOVA conducted in the RP and in the PP revealed a very highly significant effect of entry or genotype for the three traits (Table 4). The year effect was not significant whereas the effects of genotype by year interaction were significant.

H^2 was rather high for FL or PW, with $H^2>0.8$ and moderate for NI ($H^2=0.56$), in the RP. For the PP, H^2 was high for all traits ($H^2\geq0.8$). The precision of the estimates was reasonable, as the estimated standard error was well under 10% (i.e. <1% to 5%).

Table 4: Variance components of three phenotypic traits in the reference and progeny populations

Population	Factors	FL		NI		PW	
Reference population	Genotype	47.78	***	6.17	***	5,023.13	***
	Year	16.82	NS	2.96	NS	222.18	NS
	Year * Genotype	4.36	***	4.08	***	889.80	***
	Residual	5.95		16.74		2,378.04	
	H²	0.94		0.56		0.86	
Progeny population	Genotype	23.20	***	4.12	***	2,698.61	***
	Year	55.47	NS	4.99	NS	16.19	NS
	Year * Genotype	7.38	***	0.70	***	415.03	***
	Residual	2.27		3.72		554.11	
	H²	0.85		0.80		0.90	

FL: Days to flowering; NI: Nitrogen balance index; PW: 100 panicle weight; H²: Broad sense heritability; ***: significant at p=0.0001; NS: not significant.

2.4.2 Genotypic data and genetic diversity

The 43,686 SNP markers were unevenly distributed along the chromosomes. While the average marker density was 1 SNP per 8.8 kb, it ranged from 1 SNP every 5.1 kb on chromosome 11 to 1 SNP every 12.6 kb on chromosome 3 (Supplementary Table 3; Supplementary Figure 2). The distance between a pair of adjacent SNPs ranged from 0.001 to 644 kb, with a median of 1.20 kb. Almost 90%of the pairs of adjacent markers had a distance below 20 kb and 98.8% below 100 kb. Five hundred pairs of adjacent SNPs had distances > 100 kb, 114 above 200 kb and only one pair above 500 kb.

Even though the markers whose MAF was below 2.5% had been discarded, the distribution of MAF was still skewed toward low frequencies. The proportion of loci with a MAF < 10% was 38.5% in the RP and 30% in the PP. The two populations exhibited marked differences in MAF features: for 50% of loci the MAF in the PP was less than the one in the RP. Among the 32,066 SNPs with MAF > 5% in the RP, 9.7% had a MAF < 5% in the PP and 3.4% were monomorphic. The changes in MAF within the PP were even more pronounced for the smallest incidence matrix of 3,322 SNPs, with a MAF < 5% for 25.7% of the loci and 9.2% monomorphic loci.

The decay of LD along physical distance is presented in Figure 2 and Supplementary Table 4. For a between-marker distances of 0 to 25 kb, the r^2 value reached 0.62 and 0.66 in the RP and in the PP, respectively. In the RP, the r^2 value dropped to half its initial level at around 350 kb and reached 0.2 at 800 kb and 0.1 at 2.9 Mb. As expected, the decay of LD was slower in the PP, reaching an r^2 of 0.2 at 1.1 Mb and 0.1 at 3.9 Mb. Some differences in the speed of LD decay were observed between chromosomes, with the highest speed in chromosome 11 (r^2

=0.21) reached between 200 and 225 kb in the RP and an r² of 0.20 reached between 300 and 350 kb,, and the lowest in chromosome 5, with an r² of 0.2 at 1-1.5 Mb in both populations.

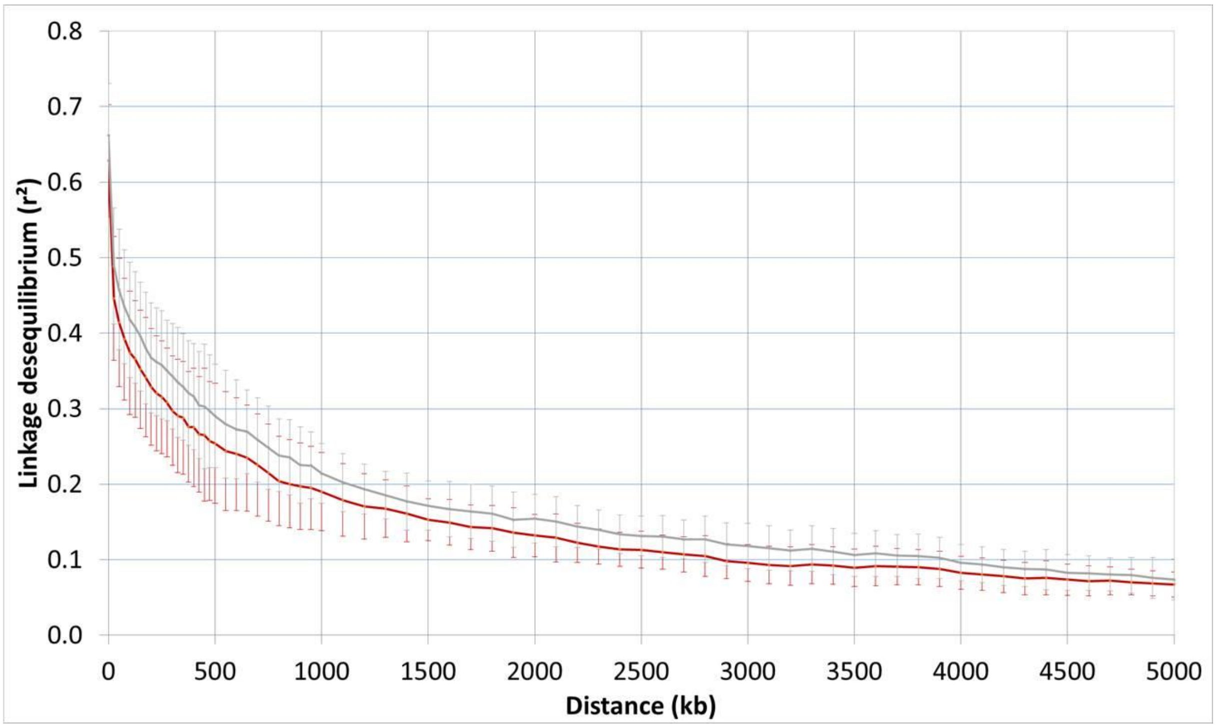

Figure 2: Patterns of decay in linkage disequilibrium in the reference population (red) and in the progeny population (grey). The curve represents the average r² among the 12 chromosomes and the bars the associated standard deviation

The genetic diversity analysis of the RP led to two major clusters corresponding to the well-known temperate *japonica* (217 accessions) and tropical *japonica* (67 accessions) sub-groups (Figure 3). The majority of the temperate *japonica* accessions are of European origin. The majority of tropical *japonica* accessions originate from the American continent. Interestingly, the average values for the three phenotypic traits investigated differed significantly in the two groups: 92 and 98 days for FL, 24.5 and 21.8 for NI and 354 and 305 g for PW in the temperate and the tropical *japonica* group, respectively. Among the 31 accessions involved in biparental crosses for the development of the PP lines, 24 belonged to the temperate *japonica* group and seven to the tropical *japonica* group. Including the PP in the diversity analysis did not modify the clustering into two groups, but only six progeny lines clustered with the tropical *japonica* group while out of the 97 lines, a total of 43 derived from 11 crosses involving a tropical *japonica* donor. The remaining 37 PP lines derived from crosses involving a tropical *japonica* donor clustered with the temperate *japonica* group (Figure 3; Supplementary Table 2).

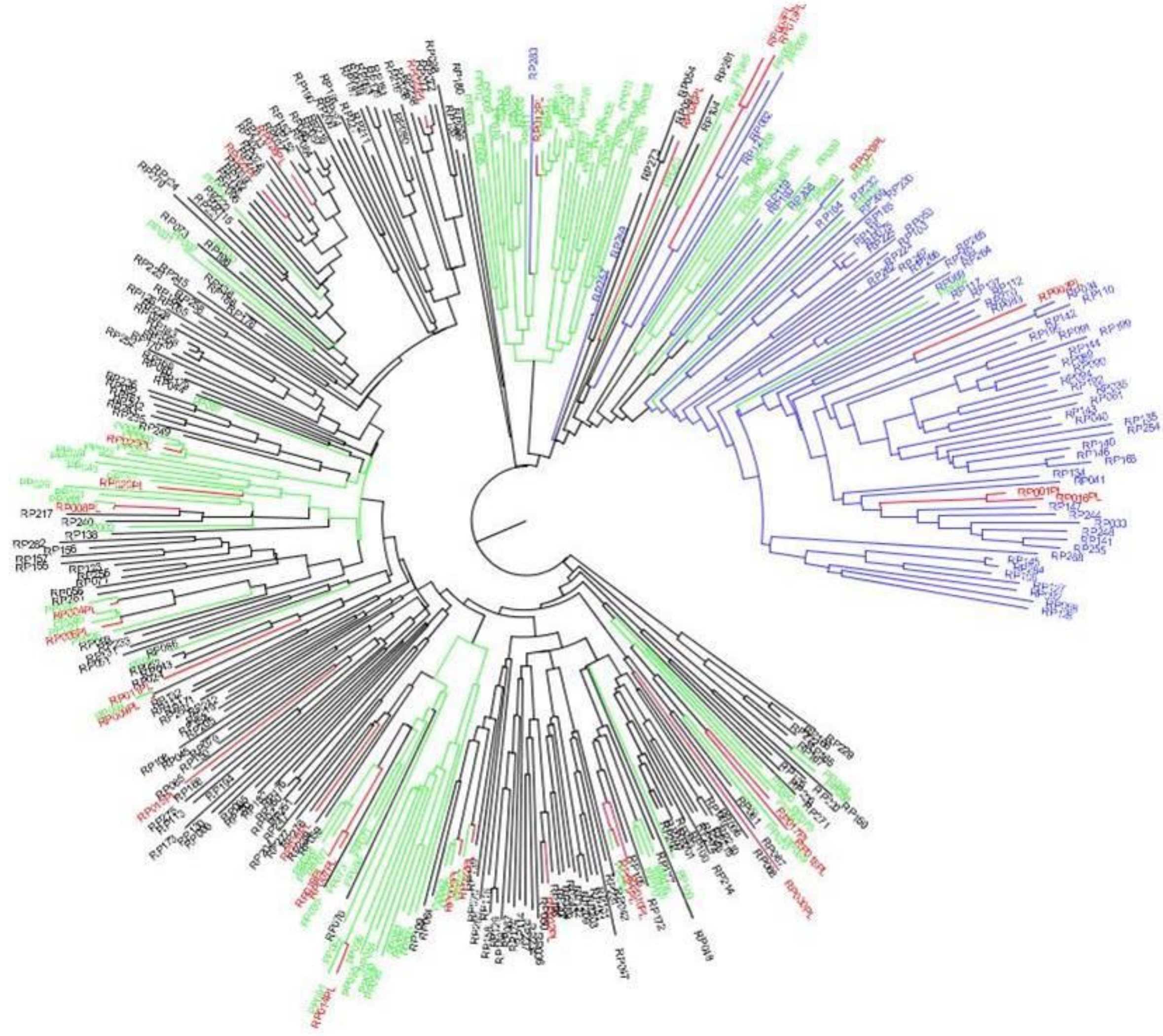

Figure 3: Unweighted neighbor-joining tree based on simple matching distances constructed from the genotype of 284 accessions of the reference population (RP) and 97 lines of the progeny population (PP), using 5,516 SNP markers. Red: parental lines (PL); Black and blue: RP accessions belonging to tropical *japonica* and temperate *japonica*, respectively.

2.4.3 Accuracy of genomic prediction in the diversity reference panel

The 189 cross validation experiments involving seven levels of LD, three levels of MAF, three prediction methods and the three phenotypic traits yielded average prediction accuracies (APA) ranging from 0.42 to 0.65 (Figure 4; Supplementary Table 5). Among the factors (trait, LD, MAF and method) whose possible effect on accuracy was investigated, all were found to be significant (Table 5). The overall APA was 0.63 for the FL trait, 0.50 for NI and 0.59 for PW. The LD threshold leading to the highest APA (0.60), considering the three traits, was $r^2 \leq 0.64$ and $r^2 \leq 0.81$. The LD threshold leading to the lowest APA (0.53 and 0.55) among the three traits was $r^2 \leq 0.25$ and $r^2 \leq 0.36$. The performance of the BayesB and RKHS methods was the same (0.58), that of GBLUP was 0.56. Finally, the MAF threshold leading to the overall highest

APA (0.58) was MAF ≥ 5%, all other MAF thresholds tested all led to the same lower APA (0.57). Although statistically significant, the difference in overall APA was small for these last factors, compared with the trait and LD factors.

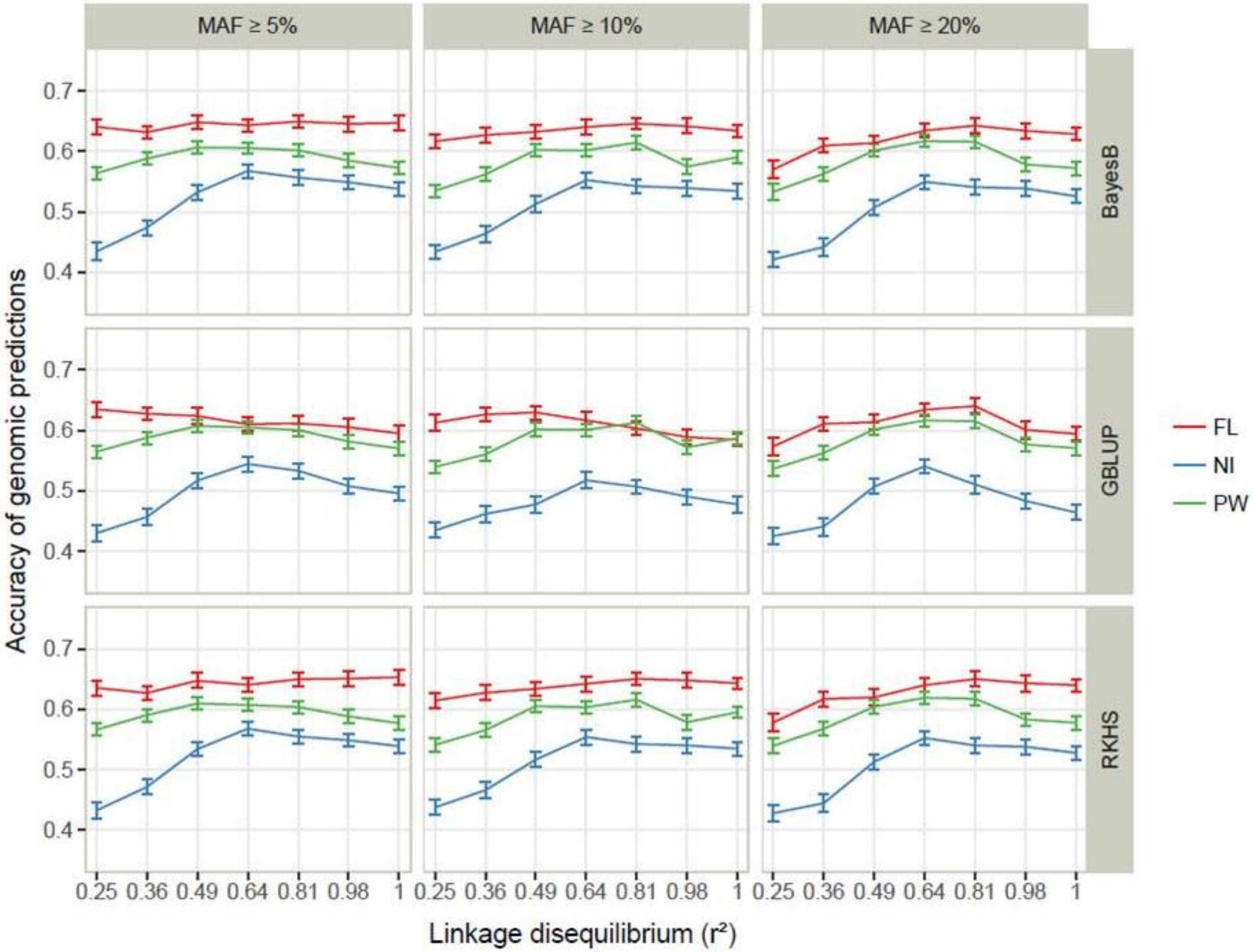

Figure 4: Accuracy of genomic prediction in cross validation experiments in the reference population for days to flowering (FL), nitrogen balance index (NI) and 100 panicle weight (PW), obtained with 3 statistical methods, BayesB, GBLUP and RKHS.

Among the first rank interactions between factors affecting APA, those involving LD proved to be the most significant (Table 5), suggesting that the effect of LD depends on the traits, method or MAF levels considered. Indeed, among the three phenotypic traits, NI showed the highest sensitivity to LD variation with a gain in APA of 0.12 (21.6%) between $r^2 = 0.25$ and $r^2 = 0.64$, i.e. the levels of LD giving the lowest and the highest APA, respectively. The gain in APA for FL and PW was only 4.6% and 10.5%, respectively. The LD also revealed an interaction with method. Among the three prediction methods, GBLUP was the most affected by the level of LD (Supplementary figure 3A). Indeed, for the higher levels of LD ($r^2 \geq 0.64$), RKHS and Bayes performed significantly better than GBLUP with an increase in accuracy of up to 0.04 for the highest levels of LD. Concerning the interaction between MAF and LD levels, the effect of MAF levels was significant only for the most stringent levels of LD ($r^2= 0.26$, Supplementary figure 3B).

Given these results, we decided to consider only one MAF threshold (≥ 5%) in the following steps of the study (progeny prediction) and to focus on analysis of the effect of LD and prediction method.

Table 5: ANOVA of factors affecting the accuracy of the 189 cross validation experiments in the reference population

Model	**Source** (2)	**DF**	**Type 3 SS**	**MS**	**F Value**	**ProbF**	**R²**	**CV**	**Root MSE**	**Mean**
Including only main effects of controlled factors	Model	12	0.6060	0.0505	133.82	<0.0001 ***	0.90	3.40	0.0194	0.57
	Error	176	0.0664	0.0004						
	Corrected total	188	0.6725							
	Trait	2	0.4897	0.2449	648.82	<0.0001 ***				
	LD	6	0.0986	0.0164	43.55	<0.0001 ***				
	Method	2	0.0126	0.0063	16.68	<0.0001 ***				
	MAF	2	0.0051	0.0026	6.76	0.0015 *				
Including main factors and first order interactions (1)	Model	60	0.6617	0.0110	131.56	<0.0001 ***	0.98	1.60	0.0092	0.57
	Error	128	0.0107	0.0001						
	Corrected total	188	0.6725							
	LD*Trait	12	0.0381	0.0032	37.89	<0.0001 ***				
	LD*Method	12	0.0068	0.0006	6.72	<0.0001 ***				
	LD*MAF	12	0.0048	0.0004	4.73	<0.0001 ***				
	Trait*Method	4	0.0046	0.0012	13.83	<0.0001 ***				
	Trait*MAF	4	0.0009	0.0002	2.78	0.0297 *				
	MAF*Method	4	0.0005	0.0001	1.44	0.2254 NS				

(1) But only tests of interest are given, i.e. the ones of first order interactions.

(2) Controlled factors: Trait with 3 levels (FL: Days to flowering; NI: Nitrogen balance index; PW: 100 panicle weight); Linkage disequilibrium (LD) with 7 levels (LD ≤ 0.25, LD ≤ 0.36, LD ≤ 0.49, LD ≤ 0.64, LD ≤ 0.81, LD ≤ 0.98, LD ≤ 1); Minor allele frequency (MAF) with 3 levels (MAF ≥ 5%, MAF ≥ 10%, MAF ≥ 20%); Prediction method (Method) with 3 levels (Bayes B, GBLUP, RKHS);

Four levels of significance (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001).

2.4.4 Accuracy of genomic prediction across generations

The 360 non-replicated experiments of genomic prediction of progenies' phenotype, involving the first five scenarios (S1 – S5) of the relationship between the training set and the progeny set, seven LD thresholds, and three prediction methods led to progeny prediction accuracies (PPA) ranging from 0.09 to 0.54. The 72 replicated prediction experiments in scenario S6 led to an average PPA ranging from 0.05 to 0.36. The following comparisons between scenarios are based on PPA data for the non-replicated prediction experiments and on the average-PPA for the replicated experiments of S6 (Figure 5; Supplementary Table 6).

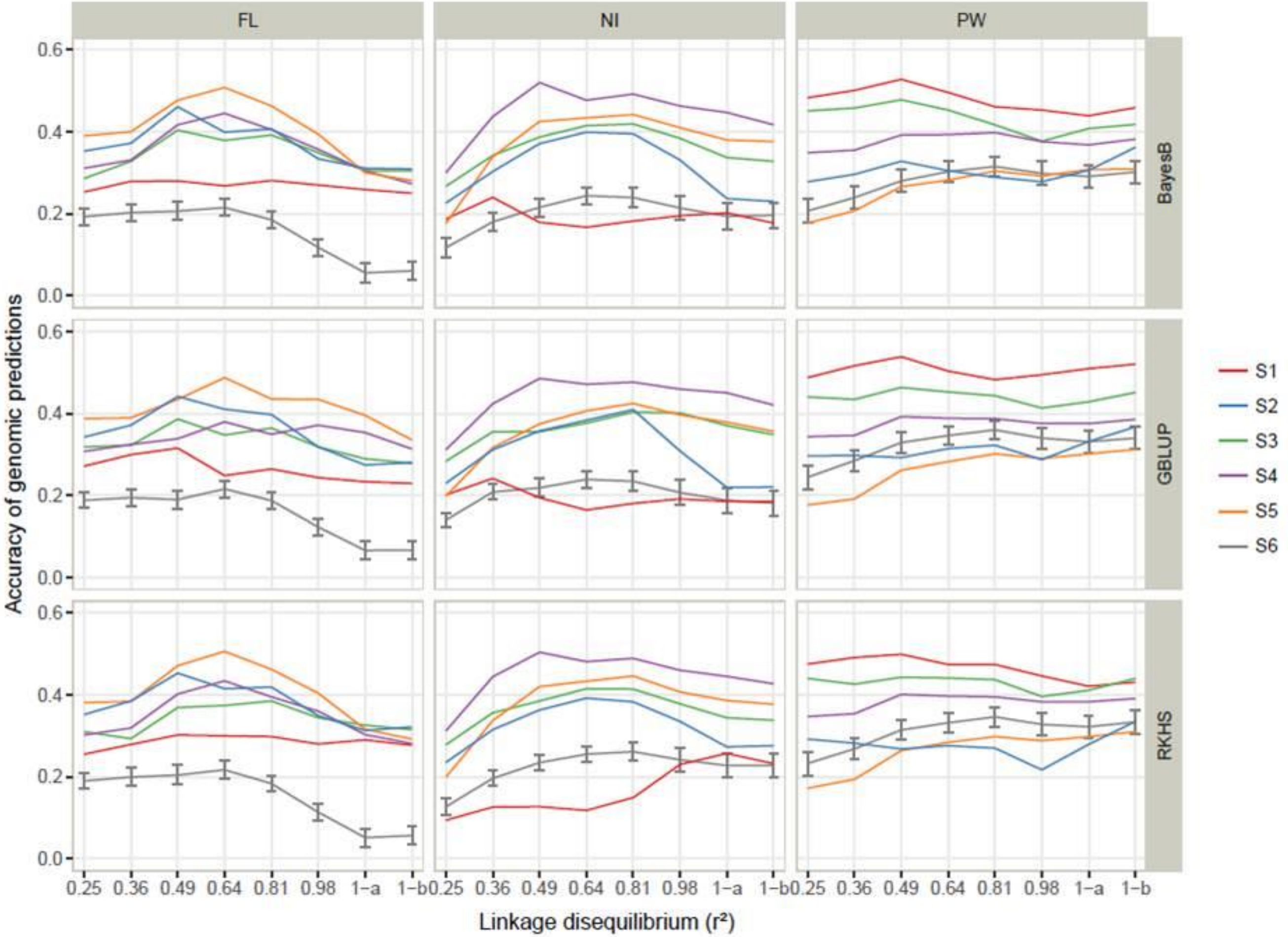

Figure 5: Accuracy of genomic prediction of progeny phenotype for days to flowering (FL), nitrogen balance index (NI) and 100 panicle weight (PW), obtained with 3 statistical methods, BayesB, GBLUP and RKHS. 1-a and 1-b, represent incidence matrices with no selection on r², but filtered with MAF > 5% and MAF > 2.5%, respectively

Among the factors whose potential effect on PPA was investigated, all except the prediction methods had a significant effect (Table 6). Significant effects were also observed for the interactions between scenario and trait, scenario and LD, and traits and LD.

The scenario effect had the greatest magnitude and, as expected, the trend of variations in PPA was related to the degree of relatedness between the training set and the progeny set. The highest mean PPA (0.40) was achieved with scenario S4 (training set = whole RP) and the lowest mean PPA was observed in scenario S6 (31 randomly sampled accessions excluding the parents). The second best mean PPA (0.38) was observed when the training set was composed of the 31 parents plus 52 accessions of RP most closely related to the 31 parents (S2). The second lowest mean PPA (0.31) was observed when the training set was composed of the 31 parents only (S1). It is also worth noting that a similar intermediate level of mean PPA (0.35) was achieved when the parents were not included in the training set (S2 and S5). Although significant, scenario x LD interaction did not lead to a major reversal of PPA. Conversely, the scenario x trait interaction led to much larger variations in PPA. For instance, the absence of the parental lines in the training set negatively affected the PPA of the WP (PPA of 0.30 and 0.27 for S2 and S5 respectively, versus a PPA of 0.48, 043 and 0.40 for S1, S3 and S4, respectively), and positively the PPA for FL (PPA of 0.37 and 0.41 for S2 and S5 respectively, versus a PPA of 0.27, 034 and 0.35 for S1, S3 and S4, respectively). Thus, in the case of FL, kinship between RP and PP does not appear to play a determining role in PPA. For NI, a mixed trend was observed with the highest PPA in scenario S4 (0.44) and the lowest in scenario S1 (0.18), the other scenarios leading to intermediate PPA (0.31 to 0.37).

The magnitude of variation in PPA in relation with LD was much narrower. The highest mean PPA (0.36) was achieved with LD thresholds of $r^2 \leq 0.49$ to $r^2 \leq 0.81$, when interactions with other factors were left aside. The PPA decreased smoothly with both lower and higher LD thresholds, and reached 0.28 for $r^2 \leq 0.25$, and 0.31 for $r^2 \leq 1$. The inclusion of additional markers under $r^2 \leq 1$, by lowering the MAF threshold to 2.5% neither deteriorated nor improved the PPA (Supplementary Table 6). Regarding the trait effect, the highest mean PPA (0.36) was observed for PW. FL and NI both had the same mean PPA (0.31).

Table 6: ANOVA on factors influencing the accuracy of 378 progeny prediction experiments

Model	**Source** (2)	**DF**	**Type 3 SS**	**MS**	**F Value**	**ProbF**	**R²**	**CV**	**Root MSE**	**Mean**
Including only main effects of controlled factors	Model	15	1.5513	0.1034	17.61	<0.0001 ***	0.42	23.10	129.00	0.33
	Error	362	2.1254	0.0059						
	Corrected total	377	3.6768							
	Scenario	5	1.1239	0.2248	38.28	<0.0001 ***				
	Trait	2	0.1094	0.0547	9.32	<0.0001 ***				
	LD	6	0.3005	0.0501	8.53	<0.0001 ***				
	Method	2	0.0000	0.0000	0.00	0.9965 NS				
Including main factors and first order interactions (1)	Model	93	3.4423	0.0370	44.83	<0.0001 ***	0.94	8.66	0.03	0.33
	Error	284	0.2345	0.0008						
	Corrected total	377	3.6768							
	Scenario*Trait	10	1.6133	0.1613	195.41	<0.0001 ***				
	Scenario*LD	30	0.1212	0.0040	4.89	<0.0001 ***				
	Trait* LD	12	0.1371	0.0114	13.84	<0.0001 ***				
	Trait*Method	4	0.0074	0.0019	2.25	0.0639 NS				
	Scenario*Method	10	0.0140	0.0014	1.70	0.0810 NS				
	LD *Method	12	0.0111	0.0009	1.12	0.3444 NS				

(1) Only tests of interest are given, i.e. the ones of first order interactions. (2) Controlled factors: Trait with three levels (FL: Days to flowering; NI: Nitrogen balance index; PW: 100 panicle weight); Linkage disequilibrium (LD) with 7 levels ($LD \leq 0.25$, $LD \leq 0.36$, $LD \leq 0.49$, $LD \leq 0.64$, $LD \leq 0.81$, $LD \leq 0.98$, $LD \leq 1$); Minor allele frequency (MAF) with 3 levels ($MAF \geq 5\%$, $MAF \geq 10\%$, $MAF \geq 20\%$); Prediction method (Method) with 3 levels (Bayes B, GBLUP, RKHS); Significance with 4 levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001).

2.5 Discussion

The main objectives of this work were to assess the performances of genomic prediction between two consecutive breeding cycles in rice and to investigate the effect of the size and the degree of relatedness of the training set compared with the candidate set, on the accuracy of predictions. In order to set a base line for prediction accuracy and to reduce the number of possible options to be tested regarding the size and the structure of the incidence matrix, we started our study by evaluating the accuracy of genomic prediction in the reference population using a cross validation approach.

2.5.1 Accuracy of genomic prediction in the reference population

The average genomic prediction accuracies within the reference population ranged from 0.51 for NI to 0.63 for FL, in line with their degree of broad sense heritability. The highest accuracies were 0.65 for FL, 0.57 for NI, and 0.62 for PW (a proxy of grain yield). While such levels of accuracy for phenotypic traits of high heritability are common in rice, this is not the case for grain yield (Table 1). Factors that might have contributed to the higher accuracy of genomic prediction we observed for PW include: (i) the very high broad sense heritability (0.86) we achieved in our field experiments during the two consecutive cropping seasons, while in the studies listed in Table 1, the heritability for grain yield was below 0.4; (ii) the rather narrow genetic diversity of our RP assembling temperate and tropical *japonica* adapted to the irrigated lowland ecosystem of Europe.

The accuracy of genomic prediction was affected in a complex way by interactions between LD, MAF and phenotypic traits but without questioning the well-established rule of balance between the number and the distribution of markers along the chromosome, and the LD within the population (Jannink et al. 2010). However, the GBS genotyping method resulted in heterogeneous marker distribution, with distances between adjacent marker varying from 1 base to more than 1 Mb. The pruning of SNP markers based on LD information enabled us to improve accuracy with non-redundant SNP matrices. In the present study, we used a simple procedure based on pairwise LD to eliminate the most redundant markers. Other procedures have been developed: selection of tag SNPs based on LD, diversity or hot spots of recombination (Carlton et al. 2004; Zhang et al. 2004; Halperin et al. 2005), weighing the contribution of each marker by a statistic called 'degree of tagging' that includes both pairwise LD and base-pair distance (Speed et al. 2012; Ramstein et al. 2016). The practical lesson one can draw is that the accuracy of prediction can be significantly improved by accounting for redundancy of information on markers.

2.5.2 Accuracy of genomic prediction of progeny performances

Our genomic prediction experiments on the line-value of F_5-F_7 progenies of bi-parental crosses, each involving two accessions belonging to the reference population, mimicked a rice breeding scheme in which the breeding cycle is shortened by rapid generation advancement (RGA) of the early generations, and where the phenotypic evaluation starts with the advanced F_5 or F_6 generation. RGA consists in the fixation of F_2 progenies through 2-3 generations of single seed descent per year in the greenhouse, until F_5 or F_6. However, our experiments diverged from this scheme by the very pronounced imbalance in the number of progenies per cross, which varied from 1 to 20. As our genomic progeny predictions are unprecedented in rice, we compared our results with the few existing similar studies in other crops (Hofheinz et al. 2012; Sallam et al. 2015; Michel et al. 2016; Gezan et al. 2017).

The accuracy of our progeny predictions varied greatly depending on the composition of the training set, trait, LD and MAF, but for each trait, the highest accuracy achieved was only slightly below the highest accuracy achieved in the cross validation experiments in the RP: 0.51 versus 0.65 for FL, 0.52 versus 0.57 for NI and 0.54 versus 0.62 for PW. Similar results were achieved by Hofheinz et al. (2012) using a reference set of 310 inbred sugar beet lines to predict the test cross value of 56 inbred progeny derived from eight crosses between six lines of the reference set. The average prediction accuracy of the sugar content trait was of 0.82 in the training set and of 0.79 for the test cross value of lines in the next breeding cycle. However, the high transferability of the effects estimates, to the next breeding cycle did not prove to be correct for the other trait considered, the standard molasses loss. For this trait, while the APA in the training set was 0.85, it fell to 0.40 in the progeny set. The authors concluded that to assess the accuracy of genome-based prediction with effects estimated in previous breeding cycles, cross validation within one cycle is not sufficient, and independent validation is required. Sallam et al. (2015), explored the accuracy of genomic prediction across generations using a training set of 168 barley lines and five sets of 96 progeny lines representative of the breeding lines developed in five consecutive years. The training set included the parents of the progeny sets. In most cases, they found a prediction accuracy of around 0.50 for grain yield for each of the five years, and concluded that with this level of accuracy, GS would be effective if it makes it possible to shorten the breeding cycle to half the length of the phenotypic selection cycle. Michel et al. (2016) analyzed data from five breeding cycles of a commercial winter wheat breeding program, and reported the APA in the breeding cycle to be slightly higher than the APA across two consecutive breeding cycles. The APA were rather low, 0.38 for grain yield and 0.16 for protein yield, the latter having much lower heritability. Likewise, Gezan et al. (2017), who used a panel representative of Florida University strawberry breeding program and sets of progenies derived from the circular mating of 31 members of the panel, reported higher accuracy in cross validation within training populations than across populations. Thus, the lower PPA we observed in progeny prediction compared to within RP prediction is in agreement with similar studies on other species.

Population parameters that affect the accuracy of progeny prediction include differences in LD and allele frequency between RP and PP, as well as the parental contributions to PP and the genetic distance, or number of generations, between the two populations (Daetwyler et al. 2010; Lorenz et al. 2012). Recombinations in breeding populations reduce LD between markers and QTLs over time while selection increases increased LD (Pfaffelhuber et al. 2008). In our case, the distance to reach the LD threshold of $r^2 = 0.2$ was, on average, 850 kb in the RP and 1,100 kb in the PP. These values are unexpectedly higher than the value for the temperate *japonica* group reported in the literature (Courtois et al. 2013), and suggest narrower genetic diversity of the RP compared to the whole *japonica* group. Selection thus appears to have notably affected the DL, while the number of recombinations, (only one cycle), was too small to significantly affect the LD between the markers and the QTL. The contrasted MAF between the RP and the PP observed for a large number of loci illustrates the effect of selection. Selection also strongly affected the parental contributions to the PP, which was extremely unbalanced, at both the RP population level and at the individual parent level. At the RP level, while the tropical *japonica* subgroup represented 24% of the accessions of RP, only 8% of PP lines clustered with the tropical *japonica* subgroup. At the level of individual crosses, voluntary or involuntary selection of progenies skewed their distribution toward the temperate *japonica* genetic background. Indeed, while 38% of the 36 bi-parental crosses involved a topical *japonica* accession of RP and produced more that 50% of the progeny lines of PP, only 15% of these progeny clustered with the tropical *japonica* subgroup. These unfavorable population parameters of our PP raised the question of how to choose the individuals that make up the training set to maximize the accuracy of progeny predictions.

2.5.3 Selection of the training set to optimize accuracy of progeny prediction

Several studies have shown that the accuracy of genomic predictions is highly influenced by the degree of relatedness between TP and CP (Pszczola *et al.* 2012; Rincent et al. 2012; Hayes et al. 2009b). As discussed above, in our study there was marked variation in the degree of relatedness between the individuals of the two populations. This large variation raised the question of the choice of the RP individuals to be included in the training set to maximize the accuracy of progeny predictions. The results of the six compositions of the training set scenarios we tested confirmed the complementary effects of relatedness between the training set and the PP, and the size of the training set. The lower mean PPA observed in scenario S1, compared to scenarios S3 and S4 shows that, in addition to relatedness between the training set and PP, the size of the training set also matters, and even distant accessions can positively contribute to prediction accuracy. The results of scenario S2 demonstrate that high APA can be achieved without the presence of the parental lines in the training set provided it is composed of individuals closely related to the parental lines. The highest APA observed in scenario S4 suggests there is still room for further optimization of the size and the composition of the training set, for instance, by weighing the contribution of each parental line to the composition of the pools of the most closely and most distantly related individuals in the RP, on the basis of their actual contribution (ratio of number of progeny to the total number of individuals in the PP) to the composition of the PP. The almost equal APA observed in S3 and S4 suggests that beyond a certain threshold of size of the training set composed of accessions closely related to the PP, inclusion of less closely related individuals does not improve the accuracy of predictions. These finding are in agreement with those of Pszczola et al. (2012), who showed that the relatedness between the reference individuals and between the candidates and the reference individuals has a strong effect on accuracy. However, it is noteworthy that the options for optimization of the training set we explored were all based on relatedness between the training set and the parental lines of the PP. Given the above mentioned effects of selection on PP, optimization methods that directly use information on relatedness between the individuals in the training set and the individual in the PP (Rincent et al. 2012; Isidro et al. 2015; Akdemir et al. 2015) might have led to better prediction accuracy.

When predicting GEBVs on progeny, the optimal size of the training set depends on the degree of relatedness (number of generations between the training set and the progeny set), the *Ne*, the length of the genetic map, and the architecture of the target trait (Jannink et al. 2010). Generally speaking, an increase in the size of the TP results in improved prediction accuracy, but in addition to size, the genetic structure of the TP and the relationship between this structure and the distribution of the target trait, also matter. For instance Technow et al. (2013) observed a 10% increase in prediction accuracy when they combined data from two heterotic groups of corn (flint and dent) to predict resistance to leaf blight in one of the groups. Conversely, Lorenz et al. (2012) observed no significant improvement in the prediction of resistance to fusarium head blight and its associated resistance to mycotoxins when they increased the size of the TP by combining different barley breeding populations. In the present study, the highest average accuracies were achieved with the largest training set for PW and NI traits that have complex genetic structure. Prediction accuracy was less responsive to the size of the training set for the FL trait, of oligo-genic determinism (Hori et al. 2015).

2.5.4 Practical implications for rice breeding programs

Pedigree breeding within the progenies of biparental crosses extracted from a working collection or reference population is the most common scheme for the improvement of complex traits in rice, like in many other autogamous crops (Bernardo 2014). We found that using phenotypic and genotypic data from the RP to train the prediction model made it possible to predict the performances of the first generation of advanced (F5-F7) progeny of a large set of biparental crosses. Accuracies of over 0.5 were obtained, even for complex traits such as grain yield, when the parameters that affect the accuracy were optimized. Thus, breeders can use this prediction approach in the framework of a pedigree breeding scheme associated with RGA of early generations (in off-season nurseries or controlled environments), a practice aimed at reducing the length of the breeding cycle and hence accelerating genetic gain per unit of time (O'Connor 2013). It can be also applied in breeding schemes that use the haplo-diploidization method for the rapid generation of homozygous lines from biparental crosses, at least in the japonica genetic group for which a high throughput haplo-diploidization method is available (Alemanno and Guiderdoni, 1994). As the advanced line selected in this way will then go through 2-3 cycles of phenotypic evaluation, the data collected will provide an opportunity to further refine the training model (Heffner et al. 2010).

We also found that (i) an average marker density above 1 per 22 kb (8,324 SNPs) did not improve the accuracy of prediction in either cross validation within the RP or in progeny prediction and (ii) relatively high accuracy could be achieved using only a rather small share of the RP, most related to PP, as the training set. Given the very uneven distribution of marker density along the chromosomes in our RP and PP, one would expect similar level of prediction accuracy with a much smaller number of evenly distributed markers, as already predicted in simulation studies (Habier et al. 2009; Lillehammer et al. 2013; Grattapaglia 2014). These finding attest to the feasibility of using the genomic selection approach in breeding programs with rather limited resources. The most efficient and affordable option would be rather dense genotyping of the RP accessions and much looser (a few hundred), but evenly distributed, genotyping of PP that can be densified through imputation, a method widely practiced in animal breeding (Marchini and Howie 2010).

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2.8 Supplementary Materiel:

Supplementary Table 1: The 284 accessions in the reference population and their main characteristics.

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP001	AIACE	Italy	long A	2003	J-Trop	89.17	24.83	224.93	1	
TP002	APOLLO	Italy	long B	2002	J-Trop	91.50	25.51	290.96	1	
TP003	ASIA	Italy	long B	2002	J-Trop	100.83	25.05	256.55	1	
TP004	AUGUSTO	Italy	long A	2002	J-Temp	86.67	23.05	325.11	1	
TP005	BALDO	Italy	long A	1977	J-Temp	93.33	23.38	493.42	1	
TP006	CARMEN	Italy	long A	2005	J-Temp	84.33	25.10	320.91	1	
TP007	CARNAROLI	Italy	long A	1983	J-Temp	100.50	25.86	453.83	1	
TP008	CENTAURO	Italy	round		J-Temp	87.00	26.76	253.37	1	
TP009	CRESO	Italy	long A		J-Temp	92.33	25.55	345.35	1	
TP010	DELFINO	Italy	long A	2001	J-Temp	89.00	26.28	328.77	1	
TP011	DIMITRA	Greece	long A		J-Temp	95.17	24.38	366.35	1	
TP012	EUROSIS	Italy	long B		J-Trop	91.33	24.34	345.08	1	
TP013	FRAGRANCE	Italy	long B		J-Trop	93.33	28.91	250.71	1	
TP014	GIANO	Italy	long B	2003	J-Temp	91.67	25.23	222.93	1	
TP015	GIGANTE VERCELLI	Italy	long A	1967	J-Temp	94.67	25.23	498.52	1	
TP016	GLADIO	Italy	long B	1998	J-Trop	88.17	23.46	256.84	1	
TP017	HANDAO 11	China	round		J-Temp	81.50	25.14	167.02	1	
TP018	HANDAO 297	China	round		J-Temp	94.50	27.04	382.14	1	
TP019	KARNAK	Italy	long A	2002	J-Temp	99.83	24.85	420.44	1	
TP020	KORAL	Italy	long A	1981	J-Temp	91.17	26.16	427.84	1	
TP021	LOTO	Italy	long A	1988	J-Temp	85.83	24.45	272.71	1	
TP022	LUXOR	Italy	long A	2008	J-Temp	97.33	25.66	427.48	1	
TP023	MARATELLI	Italy	medium	1919	J-Temp	91.33	24.80	416.15	1	
TP024	NEMBO	Italy	long A	1999	J-Temp	87.67	28.20	370.04		1

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP025	OPALE	Italy	long A	2008	J-Temp	89.00	26.50	449.75	1	
TP026	PECOS	USA	medium		J-Temp	99.50	22.29	411.32	1	
TP027	PERLA	Italy	round	1998	J-Temp	91.83	23.75	258.56	1	
TP028	SELENIO	Italy	round	1987	J-Temp	90.17	25.35	336.00	1	
TP029	SIS R215	Italy	long A	2002	J-Trop	90.67	24.02	325.39	1	
TP030	TEJO	Italy	long A	1999	J-Temp	92.67	24.16	311.97	1	
TP031	VIALONE NANO	Italy	medium	1967	J-Temp	91.83	25.11	395.09	1	
TP032	VOLANO	Italy	long A	1972	J-Temp	95.17	25.28	349.97	1	
TP033	A201	USA	long B		J-Trop	100.33	19.86	277.11		1
TP034	A301	USA	long B	1987	J-Trop	106.67	23.40	229.51		1
TP035	ADAIR	USA	long B	1993	J-Trop	101.67	26.66	327.86		
TP036	ADELAIDE CHIAPPELLI	Italy	long A		J-temp	85.54	24.51	265.95		
TP037	AGATA	Italy	round	2012	J-Temp	93.50	26.90	300.51		1
TP038	AGOSTANO	Italy	long A	1933	J-Temp	85.50	23.03	363.20		1
TP039	AKITAKOMACHI	Japon	round		J-Temp	92.83	21.30	234.48		1
TP040	ALAN	USA	long B		J-Trop	97.00	17.30	357.33		
TP041	ALEXANDROS	Greece	long B		J-Trop	100.83	19.85	256.60		
TP042	ALICE	Italy	long A	1996	J-Temp	89.83	24.28	388.02		
TP043	ALLORIO	Italy	long A	1915	J-Temp	88.33	22.88	373.31		1
TP044	ALPE	Italy	long A	1993	J-Temp	81.33	24.42	290.28		
TP045	ALPHA	Italy	long A	1979	J-Temp	88.17	22.59	301.94		
TP046	AMERICANO 1600	Italy	round	1904	J-Temp	92.67	21.52	341.21		1
TP047	ANSEATICO	Italy	long A	1972	J-Temp	96.83	25.59	351.35		
TP048	ANTARES				J-Temp	95.50	30.78	280.59		
TP049	ANTONI	Bulgary	long A		J-Temp	76.33	18.94	220.37		
TP050	ARBORIO	Italy	long A	1967	J-Temp	95.17	23.84	451.13		1

ID	Name	Origin	Class	Year of registration	Group	FL (days)	NI	PW (g)	Parental line of PP	Most related to PP parent
TP051	ARGO	Italy	medium	1978	J-Temp	94.17	20.67	427.20		
TP052	ARIETE	Italy	long A	1985	J-Temp	92.00	25.63	358.59		1
TP053	ARSENAL	Italy	long B		J-Trop	92.50	26.15	252.37		
TP054	ARTEMIDE	Italy	long B		J-Temp	95.00	20.34	257.23		
TP055	BAHIA	Spain	medium		J-Temp	95.33	29.15	432.60		
TP056	BAIXET	Spain	long A		J-Temp	89.67	27.72	303.40		
TP057	BALILLA	Italy	round	1967	J-Temp	96.00	28.00	368.00		1
TP058	BALZARETTI	Italy	medium		J-Temp	92.00	17.88	330.87		
TP059	BARAGGIA	Italy	round	1957	J-Temp	86.33	24.98	294.22		
TP060	BEIRAO	Portugal	long A		J-Temp	78.00	21.92	264.22		
TP061	BELLE PATNA	USA	long B		J-Trop	103.17	18.17	392.14		
TP062	BENGAL	USA	long A		J-Temp	104.67	22.09	368.63		
TP063	BERTONE	Italy	long A	1930	J-Temp	77.17	19.32	254.63		
TP064	BIANCA	Italy	long A	2002	J-Temp	95.33	24.37	450.70		
TP065	BOMBILLA	Spain	medium		J-Temp	92.50	20.12	287.26		
TP066	BOMBON	Spain	medium	1975	J-Temp	109.67	19.90	351.63		
TP067	BONNI	Italy	long A		J-Temp	84.50	17.03	380.53		
TP068	BRAZOS	USA	long A		J-Trop	98.00	20.91	289.72		
TP069	BURMA	Italy	long A		J-Trop	87.67	22.12	351.93		
TP070	CALENDAL	France	long A		J-Temp	94.50	20.03	487.83		
TP071	CALMOCHI 101	USA	medium		J-Temp	90.67	22.26	293.47		
TP072	CAMPINO	Portugal	medium		J-Temp	90.33	21.04	391.32		
TP073	CAPATAZ	Spain	long A		J-Temp	99.00	19.47	224.52		
TP074	CARINA	Bulgary	round		J-Temp	98.83	19.44	385.08		
TP075	CARIOCA	Italy	long B	1975	J-Trop	86.67	24.38	293.97		
TP076	CARNISE	Italy	long A		J-Temp	97.00	24.16	321.94		1

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP077	CARRICO	Portugal	round		J-Temp	86.50	22.93	397.08		1
TP078	CASTELMOCHI	Italy	round		J-Temp	91.33	25.79	259.90		1
TP079	CHIPKA	Bulgary	round		J-Temp	86.83	24.13	493.12		1
TP080	CIGALON	France	medium		J-Temp	81.00	28.13	217.32		
TP081	CINIA 40	Chili			J-Temp	97.17	20.67	318.53		1
TP082	CLOT	Spain	medium		J-Temp	89.50	27.58	326.33		
TP083	COCODRIE	USA	long B	2004	J-Trop	98.83	18.82	248.13		
TP084	COLINA	Spain	round		J-Temp	93.50	28.38	364.00		
TP085	CORBETTA	Italy	medium	1954	J-Temp	83.50	26.71	527.22		1
TP086	CRIPTO	Italy		1978	J-Temp	85.83	23.22	368.27		
TP087	CT36	Colombia	long B		J-Temp	100.67	30.36	307.60		
TP088	CT58	Colombia	long A		J-Temp	93.33	18.19	164.62		1
TP089	DELLROSE	USA	long A		J-Trop	107.33	21.41	308.97		
TP090	DELMONT				J-Trop	106.67	19.46	280.34		
TP091	DIXIEBELLE	USA	long A		J-Trop	109.83	18.91	223.14		
TP092	DOURADAO				J-Trop	96.83	21.60	371.64		
TP093	DRAGO	Italy	long A	1990	J-Temp	88.67	28.58	411.69		1
TP094	DREW	USA	long B		J-Trop	108.00	21.29	351.29		
TP095	DUCATO	Italy	round	2011	J-Temp	90.17	26.12	286.15		
TP096	ERCOLE	Italy	long A		J-Temp	92.83	24.95	417.66		
TP097	ERMES	Italy	long B		J-Temp	95.00	21.33	300.70		
TP098	ESCARLATE	Portugal	round		J-Temp	81.17	23.44	223.91		
TP099	ESTRELA	Portugal	long A		J-Temp	84.67	25.06	238.22		
TP100	EUROPA	Italy	long A	1974	J-Temp	99.17	34.07	404.48		
TP101	EUROSE	Italy	long A		J-Temp	92.17	26.71	374.51		
TP102	FAMILIA 181	Portugal	long A		J-Temp	96.67	21.88	280.08		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP103	FAST				J-Trop	89.22	26.56	237.00		
TP104	FIDJI	Philippines	long B	2001	J-temp	103.17	25.87	318.09		1
TP105	FLIPPER	Italy	long B	1997	J-Temp	89.17	26.34	306.59		1
TP106	FORTUNA	Italy	long A		J-Trop	110.67	16.48	311.94		
TP107	FRANCES	Spain	medium	2000	J-Temp	97.67	22.36	293.04		1
TP108	FULGENTE	Italy	medium		J-Temp	88.67	22.17	386.26		
TP109	GALILEO	Italy	long A	2002	J-Temp	89.33	23.18	385.86		1
TP110	GANGE	Italy	long B	1995	J-Trop	101.00	24.27	248.03		1
TP111	GARDE SADRI	Turkey	long A		J-Temp	93.50	27.53	391.43		
TP112	GIADA	Italy	long B		J-Trop	101.33	18.96	338.72		
TP113	GIOVANNI MARCHETTI	Italy	medium	1972	J-Temp	91.83	27.43	400.20		1
TP114	GITANO				J-Temp	86.83	29.05	313.06		
TP115	GIZA 177	Egypt	medium		J-Temp	96.50	22.32	308.15		
TP116	GLORIA				J-Temp	88.83	23.75	283.21		
TP117	GOOLARAH				J-Trop	115.67	17.88	200.95		
TP118	GRAAL	France	long B		J-Trop	85.83	24.99	299.24		
TP119	GRALDO	Italy	long B		J-Temp	91.17	18.99	312.26		
TP120	GREGGIO				J-Temp	92.00	21.02	383.21		
TP121	GREPPI	Italy	round	1908	J-Temp	104.83	23.49	384.83		1
TP122	GRITNA	Italy	long A		J-Temp	83.33	25.99	356.32		
TP123	GUADIAMAR	Spain	medium	1990	J-Temp	89.67	25.02	256.59		
TP124	GZ8367	Egypt			J-Temp	105.17	17.11	323.29		
TP125	HAREM	Portugal	long A		J-Temp	104.50	25.55	350.66		
TP126	HARRA	Australia	round		J-Temp	91.83	26.73	368.96		
TP127	HONDURAS	Spain	long A		J-Trop	115.29	17.73	231.18		
TP128	IAC32-52				J-Trop	109.17	16.66	479.07		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP129	IBO 380-33	Portugal	long A		J-Temp	88.17	24.67	258.98		
TP130	IBO 400	Portugal	long A		J-Temp	97.67	27.58	438.99		
TP131	ITALMOCHI	Italy	medium	1996	J-Temp	81.33	22.43	252.95		
TP132	ITALPATNA 48	Italy	long A		J-Temp	100.17	26.49	416.62		
TP133	ITALPATNAxMILYANG	Portugal	long A		J-Temp	93.00	31.03	306.20		
TP134	JACINTO	USA	long A		J-Trop	103.17	17.36	305.16		
TP135	JEFFERSON	USA	long A	1996	J-Trop	97.33	24.17	323.73		
TP136	JUBILIENI	Bulgary	round		J-Temp	80.33	28.31	319.80		
TP137	KING	Italy	long B		J-Trop	94.83	22.27	332.32		
TP138	KRISTALLINO				J-Temp	87.97	28.21	501.83		1
TP139	KULON	Russia	long A		J-Temp	82.50	25.21	367.43		1
TP140	L201	USA	long B		J-Trop	96.00	17.15	307.66		
TP141	L202	USA	long B		J-Trop	100.33	20.98	303.54		1
TP142	L204	USA	long B		J-Trop	93.67	24.51	350.21		
TP143	L205	USA	long B		J-Trop	98.33	19.05	328.90		
TP144	LACASSINE	USA	long B		J-Trop	106.83	24.10	279.00		
TP145	LADY WRIGHT	USA	medium		J-Trop	111.33	18.87	386.92		1
TP146	LAGRUE	USA	long A		J-Trop	100.83	22.51	356.82		
TP147	LAMONE	Italy	long B	1999	J-Trop	94.50	23.09	291.53		
TP148	LENCINO	Italy	round	1930	J-Temp	90.50	20.20	300.05		
TP149	LIDO	Italy	medium	1976	J-Temp	92.33	22.39	272.09		1
TP150	LOMELLINO	Italy	medium	1982	J-Temp	79.83	24.40	305.30		
TP151	LORD	Italy	long A	1988	J-Temp	94.67	25.47	353.47		
TP152	LUCERO	Italy	round		J-Temp	101.67	22.59	342.73		
TP153	LUNA	USA	medium		J-Temp	100.17	24.07	351.80		
TP154	LUSITO IRRADIADO	Portugal	long A		J-Temp	92.17	22.12	297.31		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP155	M202	USA	medium		J-Temp	91.83	25.08	335.35		
TP156	M203	USA	long A		J-Temp	95.00	23.75	376.41		1
TP157	M204	USA	long A		J-Temp	92.33	30.32	376.99		
TP158	M6	Italy	long A		J-Temp	90.67	26.69	330.40		
TP159	MAIORAL	Portugal	long A		J-Temp	99.33	21.82	348.08		
TP160	MANTOVA	Italy	long A	1933	J-Temp	93.00	25.84	401.44		
TP161	MARENY	Spain	long A		J-Temp	91.67	25.68	430.71		
TP162	MARTE	Italy	round	2000	J-Temp	93.67	30.26	332.84		
TP163	MAYBELLE	USA	long B		J-Trop	94.33	22.04	361.08		
TP164	MECO				J-Temp	92.33	29.70	415.18		
TP165	MEJANES	France	long B		J-Temp	86.00	22.12	304.99		
TP166	MELAS	Greece	long B		J-Temp	94.83	23.12	248.89		
TP167	MIARA	Italy	long B		J-Temp	82.33	25.90	177.63		
TP168	MILEV 21	Bulgary	round		J-Temp	89.33	22.04	447.78		
TP169	MOLO	Italy	long A		J-Trop	91.33	29.14	274.13		
TP170	MONTICELLI	Italy	medium	1967	J-Temp	92.67	22.82	446.11		
TP171	MUGA	Portugal	round		J-Temp	100.50	22.38	400.08		
TP172	MUSA				J-Temp	89.17	21.89	297.33		1
TP173	NANO	Italy	round		J-Temp	105.17	31.82	351.69		
TP174	NILO	Italy	long A		J-Temp	93.50	24.71	213.14		
TP175	NOVARA	Italy	medium	1933	J-Temp	83.33	19.46	305.00		
TP176	OLCENENGO	Italy	long A	1957	J-Temp	91.83	21.61	491.57		
TP177	ONICE				J-Temp	89.00	25.33	312.04		1
TP178	ORIGINARIO	Italy	round	1930	J-Temp	94.56	27.90	378.11		
TP179	ORIONE	Italy	long A		J-Temp	96.83	29.35	460.69		1
TP180	OSCARxSUWEON	Portugal	long A		J-Temp	95.67	21.88	416.00		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP181	OSTIGLIA	Italy	round	1923	J-Temp	88.00	19.36	289.14		
TP182	OTA	Portugal	long A		J-Temp	103.17	18.32	412.54		
TP183	P6	Italy	medium		J-Temp	89.67	21.12	328.05		
TP184	PADANO	Italy	long A		J-Temp	96.17	27.79	434.67		
TP185	PANDA	Italy		1988	J-Trop	93.00	24.73	382.88		
TP186	PEGONIL	Spain	medium		J-Temp	97.50	23.99	487.45		
TP187	PELDE	Australia			J-Temp	79.67	23.46	168.40		
TP188	PIEMONTE	Italy	long A	1983	J-Temp	93.50	29.06	467.27		
TP189	PIERINA MARCHETTI	Italy	long A		J-Temp	93.00	25.22	544.97		
TP190	PLOVDIV 22	Bulgary	long A		J-Temp	81.17	25.12	338.32		
TP191	PLOVDIV 24	Bulgary	round		J-Temp	90.19	30.26	388.40		
TP192	PLUS	Italy	long B		J-Trop	105.83	20.82	328.44		
TP193	PREVER	Italy	long B	1989	J-Temp	84.83	19.99	345.67		
TP194	PROMETEO	Italy	medium	1990	J-Temp	87.83	20.66	386.72		
TP195	PUNTAL	Spain	long B	1991	J-Trop	103.83	20.10	396.72		
TP196	RANGHINO	Italy	round		J-Temp	83.50	22.47	247.38		
TP197	RAZZA 77	Italy	medium		J-Temp	88.33	25.86	370.34		
TP198	REDI	Italy	long A	1967	J-Temp	96.00	23.77	421.35		1
TP199	REXMONT	USA	long B		J-Trop	105.50	22.48	308.55		
TP200	RIBE	Italy	long A	1967	J-Temp	94.67	30.04	339.84		1
TP201	RINALDO BERSANI	Italy	long A		J-Temp	96.67	22.62	399.64		
TP202	RINGO	Italy	long A	1972	J-Temp	97.50	27.12	471.18		
TP203	RIZZOTTO 51 1	Italy	long A		J-Temp	98.33	23.35	497.59		
TP204	ROBBIO SEL1	Italy	long A		J-Temp	94.33	22.33	342.00		
TP205	RODEO	Italy	long A	2002	J-Temp	79.33	24.32	265.32		
TP206	RODINA	Bulgary	round		J-Temp	92.50	18.92	479.80		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP207	ROMA	Italy	long A	1967	J-Temp	97.33	20.67	530.46		
TP208	RONALDO	Italy	long A		J-Temp	93.33	24.78	377.14		1
TP209	RONCAROLO				J-Temp	99.33	22.99	503.40		
TP210	RONCOLO	Italy	medium		J-Temp	94.50	26.65	460.89		
TP211	ROTUNDUS	Hungary	long A		J-Temp	80.17	22.65	225.00		
TP212	ROXANI	Greece	long A		J-Temp	101.67	21.90	426.48		
TP213	RPC 12	China	round		J-Temp	81.67	24.12	219.03		1
TP214	RUBI	Portugal			J-Temp	96.50	28.04	341.08		
TP215	RUBINO	Italy	round	1978	J-Temp	96.83	24.95	494.54		
TP216	RUSSO	Italy			J-Temp	73.50	20.13	197.95		
TP217	S101	USA	medium		J-Temp	88.83	24.83	313.95		1
TP218	SAEDINENIE	Bulgary	long A		J-Temp	80.50	22.85	420.40		
TP219	SAFARI	Portugal	long A		J-Temp	95.83	26.94	413.12		
TP220	SAGRES	Portugal	long A		J-Temp	102.00	20.94	340.56		
TP221	SAKHA 102	Egypt	medium		J-Temp	99.33	21.63	309.92		
TP222	SAKHA 103	Egypt	round		J-Temp	99.50	23.27	327.95		
TP223	SALOIO	Portugal	long B		J-Temp	88.33	22.83	263.52		
TP224	SALVO	Italy	long B	2008	J-Trop	92.17	20.28	308.80		1
TP225	SAMBA	Italy	long A		J-Trop	89.17	26.73	325.36		
TP226	SANDOCA	Portugal	long B		J-Temp	101.00	22.30	350.28		
TP227	SANDORA	Hungary	long A		J-Temp	74.33	21.13	162.66		
TP228	SANTANDREA	Italy	long A	1974	J-Temp	91.17	21.82	426.91		
TP229	SANTERNO	Italy	long B	1998	J-Temp	99.17	18.59	291.84		
TP230	SATURNO	Italy	long B		J-Trop	89.67	26.38	246.46		1
TP231	SAVIO	Italy	long A	1995	J-Temp	88.50	26.84	289.26		
TP232	SCUDO	Italy	long B		J-Trop	93.33	19.48	333.98		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP233	SELN 244A620	Australia	medium		J-Temp	96.00	23.99	328.78		
TP234	SENATORE NOVELLI	Italy	long A		J-Temp	92.33	19.44	315.21		1
TP235	SENIA	Spain	medium	1986	J-Temp	94.83	26.50	368.78		1
TP236	SEQUIAL	Spain	medium		J-Temp	93.17	26.82	307.78		
TP237	SESIA	Italy	long A		J-Temp	93.17	25.64	305.01		
TP238	SESIAMOCHI	Italy	long A		J-Temp	90.00	24.30	439.40		1
TP239	SETANTUNO	Portugal	round		J-Temp	95.00	23.07	652.79		1
TP240	SFERA				J-Temp	89.50	24.26	225.78		1
TP241	SHSS 381	Spain	long A		J-Temp	96.00	26.77	432.23		1
TP242	SHSS 53	Spain	long A		J-Temp	96.17	26.90	363.70		
TP243	SILLA	Italy	long A	1973	J-Temp	83.83	24.67	338.02		
TP244	SIRIO	Italy	long A		J-Trop	85.17	26.43	336.75		1
TP245	SLAVA	Bulgary	medium		J-Temp	91.50	23.22	302.95		
TP246	SMERALDO	Italy	long A	1982	J-Temp	89.33	29.77	400.42		
TP247	SOURE	Portugal	long A		J-Temp	94.50	20.17	303.65		
TP248	SPRINT	Italy	long B	2002	J-Trop	88.33	25.62	270.30		
TP249	SR 113	Spain	long A		J-Temp	92.50	23.59	385.38		1
TP250	STRELLA	Italy	long A	1981	J-Temp	91.83	24.38	338.97		
TP251	SUPER	Portugal			J-Temp	92.67	23.99	352.16		
TP252	T757	India			J-Temp	91.17	27.14	423.91		
TP253	TAICHUNG 65	Thailand			J-Temp	94.00	27.95	341.79		
TP254	TEXMONT	USA	long A		J-Trop	98.33	26.89	311.49		
TP255	THAIBONNET	Italy	long B	1992	J-Trop	101.83	17.91	275.32		1
TP256	THAIPERLA				J-Temp	92.00	25.94	370.25		1
TP257	TITANIO	Italy			J-Temp	77.33	26.36	360.47		1
TP258	TOPAZIO	Italy	medium		J-Temp	80.83	22.74	343.45		

ID	**Name**	**Origin**	**Class**	**Year of registration**	**Group**	**FL (days)**	**NI**	**PW (g)**	Parental line of PP	Most related to PP parent
TP259	TORIO	Portugal	long A		J-Temp	91.33	23.95	417.37		
TP260	ULISSE	Italy	long A		J-Temp	93.17	30.12	360.36		
TP261	ULLAL	Spain	round	1998	J-Temp	91.17	33.12	379.08		
TP262	UPLA 32	Argentina	long B		J-Trop	96.50	22.36	218.18		
TP263	UPLA 63	Argentina	long B		J-Trop	101.83	16.86	295.25		
TP264	UPLA 64	Argentina	long B		J-Trop	94.33	19.53	278.03		
TP265	UPLA 66	Argentina	long B		J-Trop	93.67	20.42	277.94		
TP266	UPLA 68	Argentina	long B		J-Trop	102.00	18.91	304.48		
TP267	UPLA 75	Argentina	long B		J-Trop	101.67	21.23	286.16		
TP268	UPLA 77	Argentina	long B		J-Trop	98.33	22.77	244.43		
TP269	UPLA 80	Argentina	long B		J-Trop	94.50	22.13	348.41		
TP270	UPLA 91	Argentina	long B		J-Trop	101.05	21.41	320.25		
TP271	VALTEJO	Portugal	round		J-Temp	96.50	21.62	384.50		
TP272	VELA	Italy	long A		J-Temp	96.33	21.31	255.84		
TP273	VENERE	Italy	long B	1997	J-Temp	84.67	20.32	209.37		
TP274	VENERIA	Italy	long A	1978	J-Temp	92.17	25.14	456.15		1
TP275	VIALE	Italy	long A		J-Temp	90.33	26.47	316.98		
TP276	VIALONE 190	Italy	medium		J-Temp	91.17	21.37	343.68		1
TP277	VIALONE NERO				J-Temp	97.50	18.56	422.61		1
TP278	VICTORIA	Argentina	round		J-Temp	89.33	19.40	268.07		1
TP279	VIRGO				J-Temp	88.00	27.42	465.88		
TP280	VULCANO				J-Temp	97.50	23.05	499.76		
TP281	XIANGHU2				J-temp	93.50	19.82	277.02		
TP282	YRM 6 2	Australia	medium		J-Temp	94.33	27.33	412.06		
TP283	ZENA	Italy	long B	1994	J-Trop	91.33	20.84	343.71		1
TP284	ZENITH	USA	medium		J-Trop	116.00	15.59	324.08		

Supplementary Table 2: The 97 F5-F7 lines in the progeny population and their phenotype for days to flowering (FL), nitrogen balance index (NI) and 100 panicle weight (PW).

Genotype	Crosses	FL (days)	NI	PW (g)
PF043	Aiace / Perla	97.83	15.42	184.71
PF048	Apollo / Volano	96.50	15.64	272.91
PF031	Apollo/Selenio//Apollo	92.33	17.14	216.46
PF081	Apollo/Selenio//Apollo	94.00	16.76	255.47
PF057	Asia / 2*Selenio	94.83	20.34	316.05
PF058	Asia / 2*Selenio	94.83	19.00	195.4
PF059	Asia / 2*Selenio	107.00	20.86	210.07
PF085	Asia / Centauro	97.00	17.23	182.2
PF087	Asia / Centauro	96.33	17.11	243.27
PF030	Augusto / Gigante Vercelli	80.17	22.66	281.54
PF069	Augusto / Handao 297	94.67	20.38	173.15
PF070	Augusto / Handao 297	95.33	19.95	227.75
PF042	Baldo / Handao 297	91.50	19.29	398.7
PF052	Baldo / Handao 297	85.50	22.22	303.79
PF053	Baldo / Handao 297	90.67	18.91	285.46
PF055	Baldo / Handao 297	90.17	17.72	284.33
PF019	Baldo / Opale	90.67	17.58	292.24
PF020	Baldo / Opale	91.67	18.93	380.09
PF021	Baldo / Opale	96.17	17.77	328.84
PF022	Baldo / Opale	90.33	18.78	274.58
PF023	Baldo / Opale	83.83	17.45	278.17
PF024	Baldo / Opale	90.83	18.55	286.5
PF015	Carmen / Creso	95.00	18.17	323.01
PF016	Carmen / Creso	93.50	17.71	301.36
PF017	Carmen / Creso	95.50	16.32	325.19
PF026	Carmen / Loto	89.26	17.28	276.74
PF027	Carmen / Loto	88.83	19.63	182.44
PF068	Centauro / Dimitra	89.00	20.94	259.01
PF051	Centauro / Koral	83.33	21.77	289.97
PF071	Centauro / Koral	100.17	23.66	286.79
PF072	Centauro / Koral	86.33	20.91	254.45
PF073	Centauro / Koral	99.83	23.96	280.09
PF047	Creso / Apollo	91.50	18.25	259.26
PF001	Delfino / 2*Centauro	88.67	18.84	248.21
PF002	Delfino / 2*Centauro	92.00	19.18	256.57
PF067	Delfino / Centauro	95.00	21.56	342.31
PF100	Delfino / Selenio	84.00	19.12	196.06
PF084	Eurosis / Gladio	88.67	15.75	233.59
PF004	Eurosis / Handao 11	88.00	18.17	303.74

Genotype	**Crosses**	**FL (days)**	**NI**	**PW (g)**
PF005	Eurosis / Handao 11	86.00	12.21	228.03
PF006	Eurosis / Handao 11	90.93	18.91	320.01
PF007	Eurosis / Handao 11	97.67	19.17	293.16
PF008	Eurosis / Handao 11	102.50	18.19	308.41
PF009	Eurosis / Handao 11	96.83	15.53	245.35
PF010	Eurosis / Handao 11	102.50	14.59	240.41
PF011	Eurosis / Handao 11	95.00	17.18	299.83
PF012	Eurosis / Handao 11	92.83	17.37	363.48
PF013	Eurosis / Handao 11	101.50	17.72	298.86
PF028	Eurosis / Handao 11	83.67	18.31	240.69
PF032	Eurosis / Handao 11	108.83	19.14	256.69
PF033	Eurosis / Handao 11	90.50	25.13	247.38
PF034	Eurosis / Handao 11	96.33	16.38	240.44
PF035	Eurosis / Handao 11	88.83	18.90	287.98
PF036	Eurosis / Handao 11	92.83	20.43	248.68
PF037	Eurosis / Handao 11	91.00	20.33	241.5
PF038	Eurosis / Handao 11	91.33	14.71	266.5
PF039	Eurosis / Handao 11	98.00	15.85	304.32
PF046	Eurosis / Handao 11	87.67	15.57	283.06
PF082	Fragrance / Karnak	107.50	21.35	219.11
PF091	Giano / Loto	89.50	18.37	155.66
PF025	Giano / Vialone Nano	90.83	17.84	391.12
PF054	Giano / Vialone Nano	86.33	15.41	222.66
PF092	Giano / Vialone Nano	89.83	18.95	395.66
PF093	Giano / Vialone Nano	90.50	19.66	397.67
PF094	Giano / Vialone Nano	94.17	18.59	268.45
PF095	Giano / Vialone Nano	93.33	20.08	210.72
PF096	Giano / Vialone Nano	90.17	16.89	243.39
PF097	Giano / Vialone Nano	91.33	19.50	267.96
PF098	Giano / Vialone Nano	94.17	20.64	219.17
PF060	Gladio / Eurosis /Gladio	90.00	16.62	384.51
PF061	Gladio / Opale	96.50	16.63	276.44
PF062	Gladio / Opale	89.33	17.80	417.21
PF063	Gladio / Opale	88.83	14.40	319.45
PF064	Gladio / Opale	98.17	14.49	219.05
PF065	Gladio / Opale	92.67	17.17	341.53
PF066	Gladio / Opale	97.17	18.15	292.72
PF049	Handao 297 / Luxor	89.33	18.73	322.09
PF050	Handao 297 / Luxor	98.17	20.34	275.73
PF040	Karnak / 2*Giano	94.67	18.17	290.98
PF041	Karnak / 2*Giano	96.00	20.00	304.77
PF076	Karnak / Delfino	95.67	18.12	354.78

Genotype	**Crosses**	**FL (days)**	**NI**	**PW (g)**
PF077	Karnak / Delfino	95.33	19.48	349.45
PF078	Karnak / Delfino	95.67	18.57	337.78
PF075	Karnak / Giano	96.33	17.23	292.19
PF088	Karnak / Giano	96.50	17.36	207.7
PF056	Loto / Karnak	93.33	18.88	288.34
PF044	Maratelli / Carmen	84.17	20.52	269.72
PF045	Maratelli / Carmen	85.67	20.44	273.15
PF083	Pecos / Delfino	89.50	17.10	322.59
PF014	Pecos / Gladio	92.00	19.43	221.21
PF099	Pecos / Gladio	101.83	18.14	234.23
PF089	SIS R215 / Carnaroli	91.17	18.32	239.18
PF090	SIS R215 / Carnaroli	87.17	19.45	235.35
PF079	SIS R215 / Loto	90.67	19.08	337.12
PF080	SIS R215 / Loto	91.42	16.22	305.25
PF029	Tejo / Aiace	90.50	19.30	219.68
PF074	Tejo / Centauro	93.83	23.48	330.89

Supplementary Table 3: Variability of marker density and frequency of minor allele (MAF) along the 12 chromosomes in the reference and the progeny populations.

	Chr	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	Chr7	Chr8	Chr9	Chr10	Chr11	Chr12	All
	Size ()	43,154,269	35,770,733	36,303,800	35,495,001	29,863,328	31,092,718	29,650,697	28,424,033	22,791,537	23,065,658	29,015,845	27377100	27,410,737
Distribution of distance (bp) between two adjacent SNP loci	**Number**	5183	3719	2953	3553	2576	3116	3199	3937	1947	4378	6128	2997	43,686
	density	120	86	68	82	60	72	74	91	45	101	142	69	1012
	Minimum	1	1	1	1	1	1	3	1	2	1	1	1	1
	1st Quartile	103	71	74	46	79	63.5	60	57	67	43	36	46	46
	Median	1834	1651.5	1,756	1,096	1,919	1601	1,329	1,371	2,049	531	299	851	1,486
	3rd Quartile	7591	9367	10720	8428	9186	9243	7951	7696	11592	5328	4255	8159	9274
	Maximum	432999	403965	347,318	643,880	458,043	9242.5	399,519	289,104	344,748	195,190	273,928	340246	643,880

Distribution of the minor allele frequency (MAF %)	**Reference population**	**Minimum**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
		1st Quartile	3.9	3.9	4.2	6.4	7.1	5.0	6.3	4.2	4.6	5.6	3.6	10.2	4.6
		Median	12.7	10.2	10.6	26.1	31.5	17.7	25.4	17.0	20.8	20.4	12.4	24.4	17.7
		3rd Quartile	24.7	31.1	26.5	42.0	43.5	33.1	37.0	34.6	33.5	35.3	28.5	39.0	34.6
		Maximum	50.0	50.0	49.8	50.0	50.0	49.8	50.0	49.8	50.0	50.0	50.0	49.3	50.0
	Progeny population	**Minimum**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		1st Quartile	8.0	3.1	6.1	7.1	11.2	6.1	7.1	4.1	5.1	6.1	8.2	12.2	7.1
		Median	14.3	14.3	13.3	18.4	30.6	15.3	18.4	18.4	20.4	15.8	16.3	27.6	16.3
		3rd Quartile	23.5	25.5	26.5	35.7	41.8	30.6	31.6	38.8	30.6	31.6	26.5	38.8	31.6
		Maximum	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0

Supplementary Table 4: Variability of decay of pairwise linkage disequilibrium with distance between markers among the 12 chromosomes in the reference and the progeny populations

Reference population.

Chr	**0-25**	**25-50**	**50-75**	**75-100**	**100-125**	**125-150**	**150-175**	**175-200**	**200-225**	**225-250**	**250-300**	**300-350**	**350-400**	**400-450**	**450-500**	**500-600**	**600-700**	**700-800**	**800-900**	**900-1000**
1	0.614	0.446	0.422	0.395	0.376	0.372	0.356	0.334	0.318	0.320	0.315	0.295	0.285	0.270	0.252	0.243	0.235	0.222	0.208	0.199
2	0.613	0.444	0.391	0.373	0.357	0.331	0.328	0.329	0.312	0.282	0.280	0.252	0.225	0.205	0.278	0.173	0.172	0.150	0.146	0.143
3	0.693	0.505	0.493	0.459	0.432	0.443	0.402	0.391	0.394	0.374	0.349	0.345	0.304	0.304	0.416	0.294	0.266	0.241	0.199	0.176
4	0.613	0.402	0.372	0.366	0.316	0.335	0.312	0.299	0.292	0.289	0.291	0.282	0.279	0.246	0.251	0.211	0.216	0.207	0.174	0.167
5	0.763	0.631	0.600	0.580	0.559	0.542	0.537	0.517	0.499	0.474	0.464	0.449	0.426	0.420	0.347	0.420	0.396	0.365	0.302	0.275
6	0.646	0.448	0.409	0.374	0.340	0.327	0.324	0.302	0.287	0.274	0.257	0.237	0.212	0.190	0.191	0.179	0.166	0.171	0.138	0.134
7	0.685	0.504	0.479	0.446	0.444	0.420	0.416	0.422	0.403	0.426	0.417	0.399	0.394	0.392	0.394	0.364	0.336	0.306	0.287	0.269
8	0.607	0.400	0.361	0.347	0.326	0.314	0.291	0.270	0.266	0.257	0.258	0.238	0.221	0.213	0.208	0.206	0.196	0.178	0.178	0.175
9	0.675	0.449	0.423	0.393	0.399	0.365	0.343	0.345	0.333	0.335	0.307	0.277	0.292	0.264	0.246	0.231	0.221	0.201	0.179	0.169
10	0.526	0.367	0.351	0.334	0.316	0.329	0.304	0.292	0.266	0.275	0.264	0.260	0.258	0.250	0.228	0.222	0.210	0.201	0.185	0.177
11	0.482	0.296	0.256	0.243	0.235	0.233	0.224	0.215	0.209	0.198	0.199	0.184	0.171	0.174	0.161	0.159	0.151	0.138	0.127	0.116
12	0.621	0.457	0.414	0.396	0.386	0.381	0.389	0.382	0.365	0.343	0.339	0.307	0.317	0.319	0.289	0.281	0.282	0.264	0.244	0.234
Average	0.628	0.446	0.414	0.392	0.374	0.366	0.352	0.342	0.329	0.320	0.312	0.294	0.282	0.271	0.272	0.249	0.237	0.220	0.197	0.186
Stdev	0.075	0.082	0.085	0.081	0.081	0.077	0.079	0.079	0.077	0.076	0.073	0.074	0.074	0.077	0.079	0.079	0.072	0.066	0.056	0.050

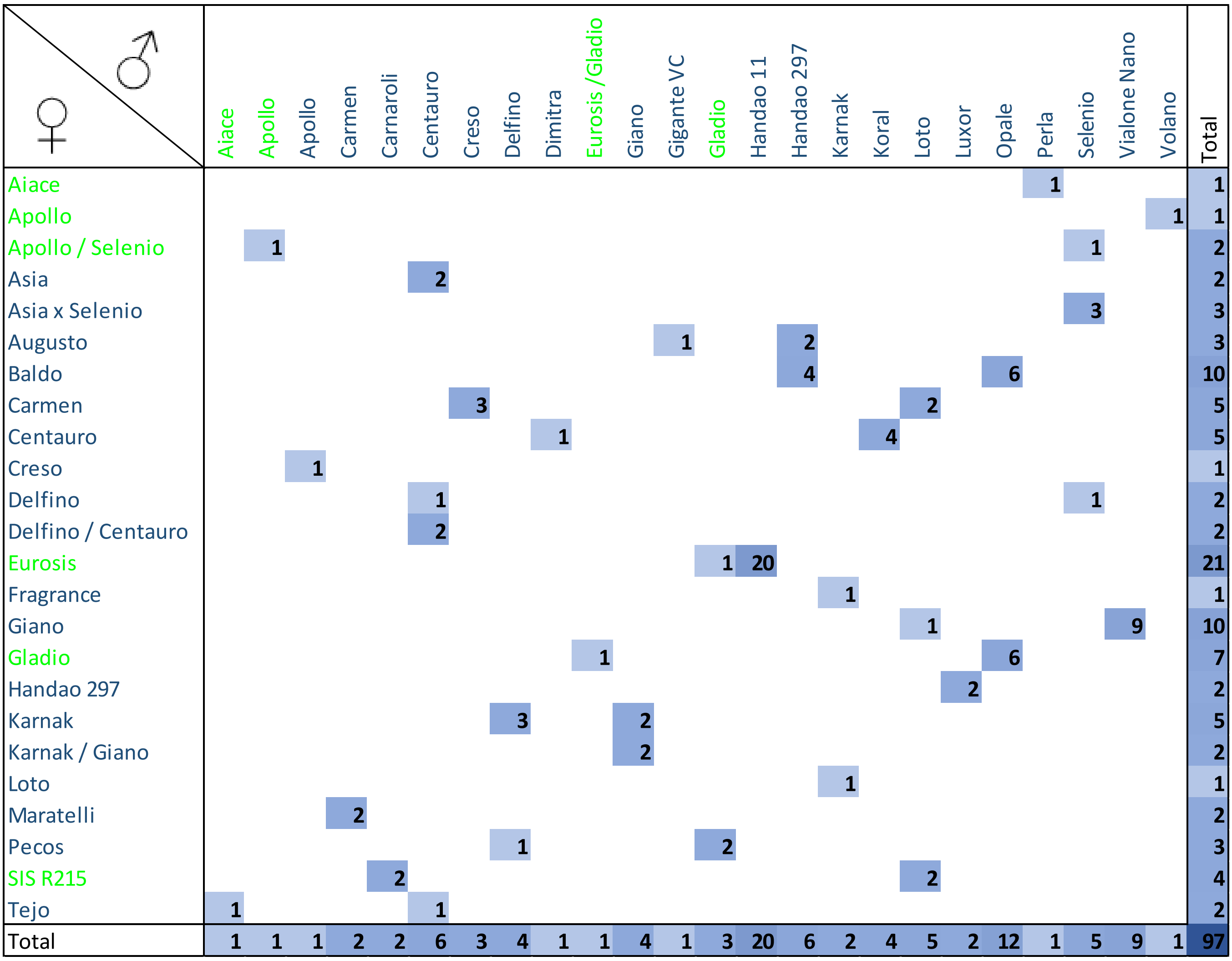
Progeny population

Chr	**"0-25"**	**25-50**	**50-75**	**75-100**	**100-125**	**125-150**	**150-175**	**175-200**	**200-225**	**225-250**	**250-300**	**300-350**	**350-400**	**400-450**	**450-500**	**500-600**	**600-700**	**700-800**	**800-900**	**900-1000**
1	0.649	0.498	0.478	0.453	0.435	0.427	0.411	0.384	0.373	0.370	0.369	0.349	0.336	0.320	0.303	0.286	0.271	0.250	0.241	0.229
2	0.671	0.520	0.468	0.458	0.435	0.410	0.403	0.388	0.361	0.346	0.352	0.344	0.297	0.278	0.273	0.244	0.245	0.243	0.240	0.218
3	0.722	0.549	0.545	0.514	0.487	0.494	0.469	0.462	0.455	0.443	0.416	0.402	0.373	0.366	0.357	0.357	0.333	0.313	0.284	0.260
4	0.655	0.449	0.421	0.406	0.360	0.366	0.347	0.332	0.329	0.330	0.329	0.322	0.315	0.282	0.267	0.252	0.261	0.252	0.222	0.200
5	0.771	0.629	0.590	0.572	0.551	0.536	0.520	0.503	0.490	0.467	0.472	0.458	0.433	0.416	0.424	0.408	0.372	0.344	0.298	0.272
6	0.679	0.495	0.458	0.423	0.397	0.385	0.392	0.358	0.342	0.350	0.326	0.301	0.278	0.264	0.248	0.238	0.214	0.203	0.179	0.165
7	0.723	0.554	0.524	0.487	0.496	0.470	0.460	0.466	0.453	0.466	0.447	0.446	0.435	0.429	0.450	0.391	0.342	0.312	0.311	0.317
8	0.650	0.448	0.418	0.399	0.379	0.364	0.350	0.321	0.323	0.314	0.309	0.282	0.262	0.251	0.239	0.244	0.234	0.215	0.212	0.220
9	0.697	0.498	0.467	0.428	0.432	0.413	0.400	0.378	0.357	0.358	0.356	0.329	0.331	0.298	0.274	0.260	0.260	0.244	0.227	0.212
10	0.570	0.416	0.395	0.382	0.363	0.377	0.347	0.334	0.308	0.314	0.299	0.298	0.300	0.291	0.273	0.260	0.249	0.232	0.222	0.214
11	0.513	0.323	0.277	0.265	0.254	0.244	0.238	0.232	0.226	0.214	0.217	0.201	0.188	0.183	0.173	0.169	0.158	0.145	0.142	0.140
12	0.643	0.486	0.454	0.431	0.422	0.408	0.408	0.404	0.394	0.371	0.358	0.335	0.348	0.347	0.319	0.311	0.314	0.289	0.265	0.256
Average	0.662	0.489	0.458	0.435	0.417	0.408	0.395	0.380	0.368	0.362	0.354	0.339	0.325	0.310	0.300	0.285	0.271	0.254	0.237	0.225
Stdev	0.069	0.077	0.080	0.075	0.077	0.074	0.072	0.074	0.073	0.072	0.069	0.071	0.070	0.070	0.078	0.070	0.060	0.055	0.048	0.047

Supplementary Table 5: Average accuracy of genomic prediction in cross validation experiments within the reference population for days to flowering (FL), nitrogen balance index (NI) and 100 panicle weight (PW), obtained with 3 statistical methods, BayesB, GBLUP and RKHS.

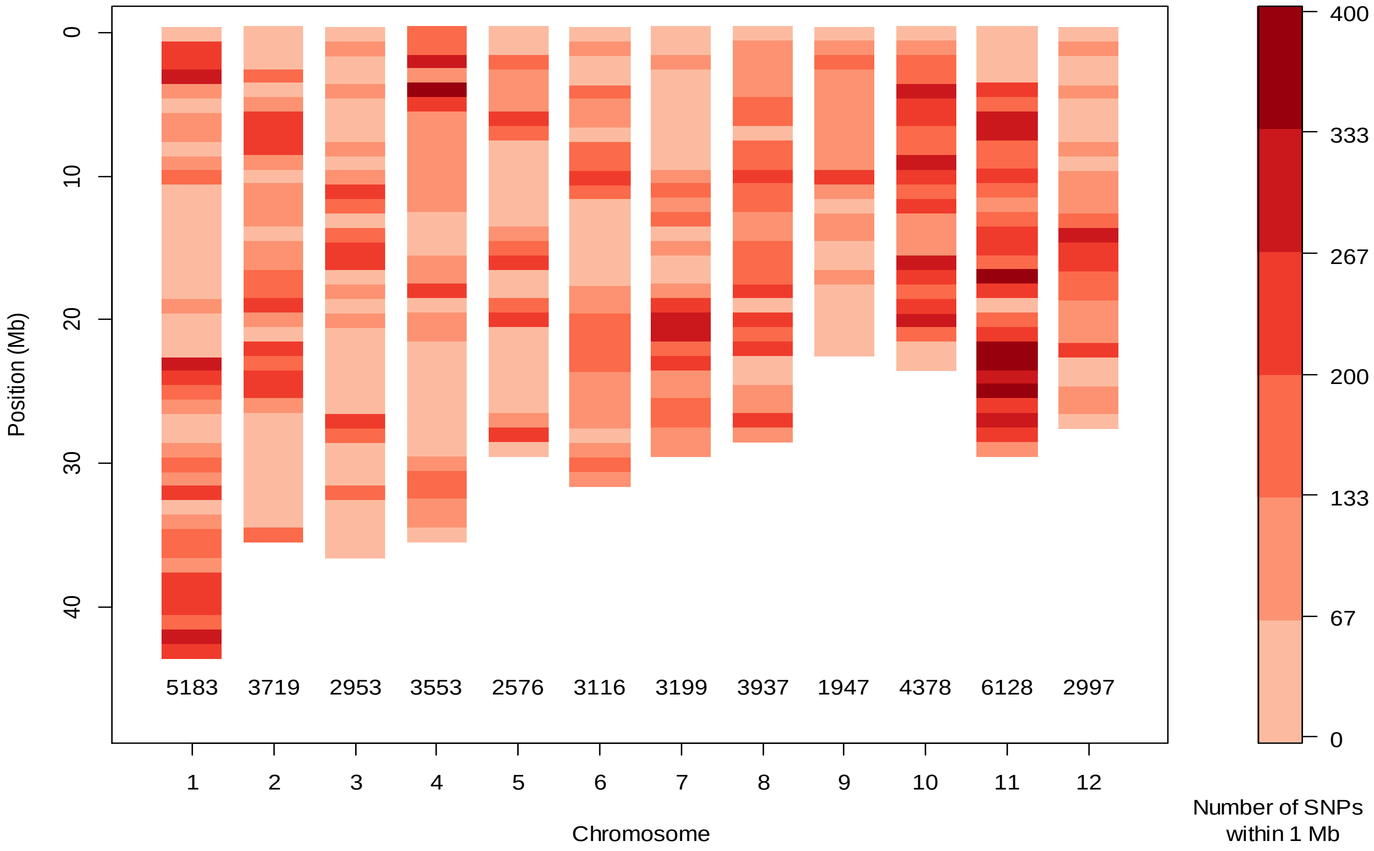
			FL			NI			PW		
Scenario	r^2	MAF (%)	BayesB	GBLUP	RKHS	BayesB	GBLUP	RKHS	BayesB	GBLUP	RKHS
S1	0.25	5	0.25	0.27	0.26	0.19	0.20	0.09	0.48	0.49	0.48
	0.36	5	0.28	0.30	0.28	0.24	0.24	0.13	0.50	0.52	0.49
	0.49	5	0.28	0.32	0.30	0.18	0.19	0.13	0.53	0.54	0.50
	0.64	5	0.27	0.25	0.30	0.17	0.16	0.12	0.50	0.50	0.47
	0.81	5	0.28	0.26	0.30	0.18	0.18	0.15	0.46	0.48	0.47
	0.98	5	0.27	0.24	0.28	0.20	0.19	0.23	0.45	0.49	0.45
	1a	5	0.26	0.23	0.29	0.20	0.19	0.26	0.44	0.51	0.42
	1b	2.5	0.25	0.23	0.28	0.18	0.18	0.23	0.46	0.52	0.43
S2	0.25	5	0.35	0.34	0.35	0.23	0.23	0.24	0.28	0.30	0.29
	0.36	5	0.37	0.37	0.39	0.30	0.31	0.32	0.30	0.30	0.28
	0.49	5	0.46	0.44	0.45	0.37	0.36	0.36	0.33	0.29	0.27
	0.64	5	0.40	0.41	0.42	0.40	0.38	0.39	0.31	0.31	0.28
	0.81	5	0.41	0.40	0.42	0.40	0.41	0.38	0.29	0.32	0.27
	0.98	5	0.33	0.32	0.35	0.33	0.31	0.34	0.28	0.29	0.22
	1a	5	0.31	0.27	0.31	0.24	0.22	0.27	0.31	0.33	0.28
	1b	2.5	0.31	0.28	0.32	0.23	0.22	0.28	0.36	0.37	0.34
S3	0.25	5	0.29	0.32	0.31	0.27	0.28	0.28	0.45	0.44	0.44
	0.36	5	0.33	0.32	0.29	0.34	0.36	0.36	0.46	0.43	0.43
	0.49	5	0.40	0.39	0.37	0.39	0.36	0.39	0.48	0.46	0.44
	0.64	5	0.38	0.35	0.37	0.42	0.38	0.42	0.45	0.45	0.44
	0.81	5	0.39	0.36	0.39	0.42	0.40	0.41	0.42	0.44	0.44
	0.98	5	0.35	0.32	0.34	0.38	0.40	0.38	0.38	0.41	0.40
	1a	5	0.31	0.29	0.33	0.34	0.37	0.34	0.41	0.43	0.41
	1b	2.5	0.31	0.28	0.32	0.33	0.35	0.34	0.42	0.45	0.44

			FL			NI			PW		
Scenario	r^2	MAF (%)	BayesB	GBLUP	RKHS	BayesB	GBLUP	RKHS	BayesB	GBLUP	RKHS
S4	0.25	5	0.31	0.31	0.30	0.30	0.31	0.31	0.35	0.34	0.31
	0.36	5	0.33	0.32	0.32	0.44	0.42	0.44	0.36	0.35	0.44
	0.49	5	0.42	0.34	0.40	0.52	0.49	0.50	0.39	0.39	0.50
	0.64	5	0.45	0.38	0.43	0.48	0.47	0.48	0.39	0.39	0.48
	0.81	5	0.41	0.35	0.40	0.49	0.48	0.49	0.40	0.39	0.49
	0.98	5	0.36	0.37	0.36	0.46	0.46	0.46	0.38	0.38	0.46
	1a	5	0.31	0.35	0.30	0.45	0.45	0.45	0.37	0.38	0.45
	1b	2.5	0.27	0.31	0.28	0.42	0.42	0.43	0.38	0.39	0.43
S5	0.25	5	0.39	0.39	0.38	0.18	0.20	0.20	0.18	0.18	0.17
	0.36	5	0.40	0.39	0.38	0.34	0.32	0.34	0.21	0.19	0.19
	0.49	5	0.48	0.43	0.47	0.43	0.37	0.42	0.27	0.26	0.26
	0.64	5	0.51	0.49	0.51	0.43	0.41	0.43	0.28	0.28	0.28
	0.81	5	0.46	0.44	0.46	0.44	0.42	0.45	0.30	0.30	0.30
	0.98	5	0.39	0.43	0.40	0.41	0.40	0.41	0.29	0.29	0.29
	1a	5	0.30	0.40	0.32	0.38	0.38	0.39	0.31	0.30	0.30
	1b	2.5	0.28	0.34	0.29	0.38	0.36	0.38	0.31	0.31	0.31
S6	0.25	5	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
	0.36	5	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.27 ± 0.01
	0.49	5	0.21 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.28 ± 0.01	0.33 ± 0.01	0.31 ± 0.01
	0.64	5	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.30 ± 0.01	0.35 ± 0.01	0.33 ± 0.01
	0.81	5	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.26 ± 0.01	0.32 ± 0.01	0.36 ± 0.01	0.35 ± 0.01
	0.98	5	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.21 ± 0.02	0.21 ± 0.02	0.24 ± 0.01	0.30 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
	1a	5	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.20 ± 0.02	0.18 ± 0.02	0.23 ± 0.02	0.30 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
	1b	2.5	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	0.23 ± 0.02	0.29 ± 0.01	0.33 ± 0.01	0.32 ± 0.01



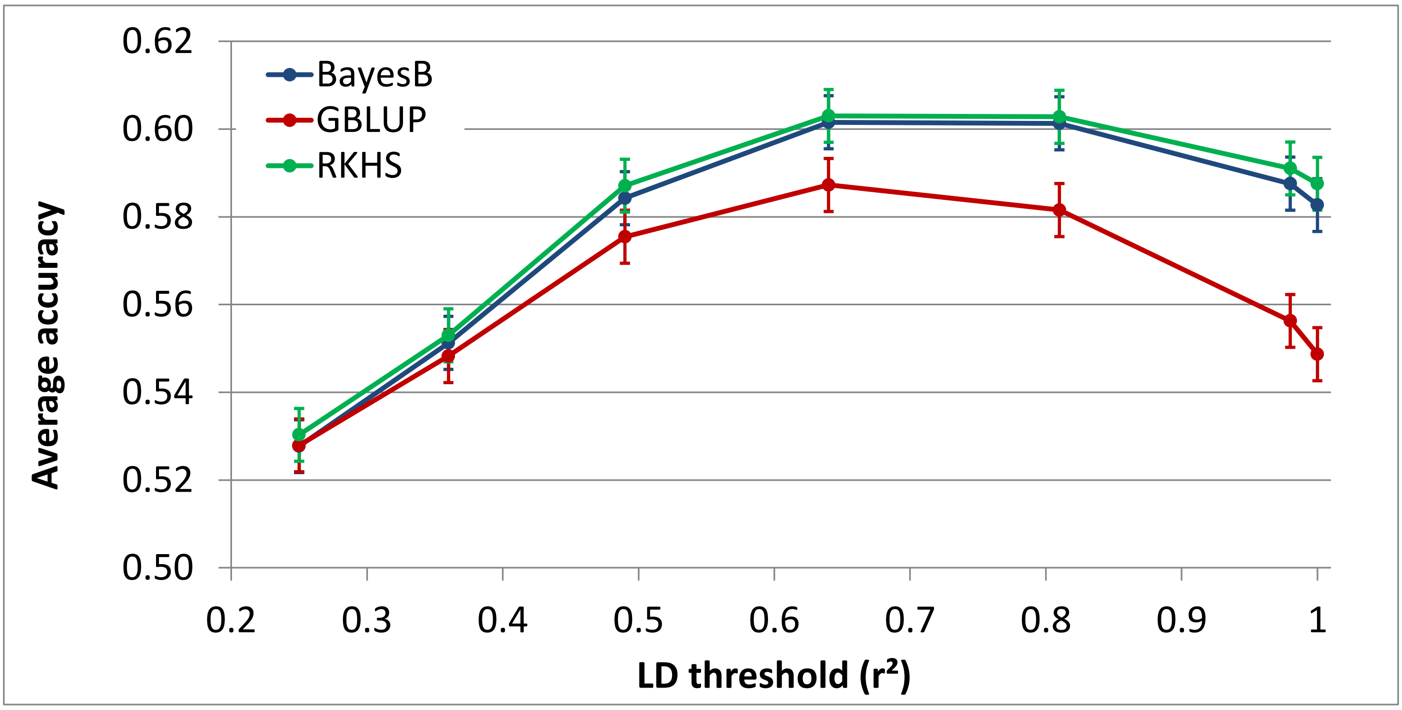
♀ ♂	Aiace	Apollo	Apollo	Carmen	Carnaroli	Centauro	Creso	Delfino	Dimitra	Eurosis /Gladio	Giano	Gigante VC	Gladio	Handao 11	Handao 297	Karnak	Koral	Loto	Luxor	Opale	Perla	Selenio	Vialone Nano	Volano	Total
Aiace																					1				1
Apollo																								1	1
Apollo / Selenio		1																				1			2
Asia						2																			2
Asia x Selenio																						3			3
Augusto												1			2										3
Baldo															4					6					10
Carmen							3											2							5
Centauro									1								4								5
Creso			1																						1
Delfino						1																1			2
Delfino / Centauro						2																			2
Eurosis													1	20											21
Fragrance																1									1
Giano																		1					9		10
Gladio										1										6					7
Handao 297																			2						2
Karnak								3			2														5
Karnak / Giano											2														2
Loto																1									1
Maratelli				2																					2
Pecos								1					2												3
SIS R215					2													2							4
Tejo	1					1																			2
Total	1	1	1	2	2	6	3	4	1	1	4	1	3	20	6	2	4	5	2	12	1	5	9	1	97

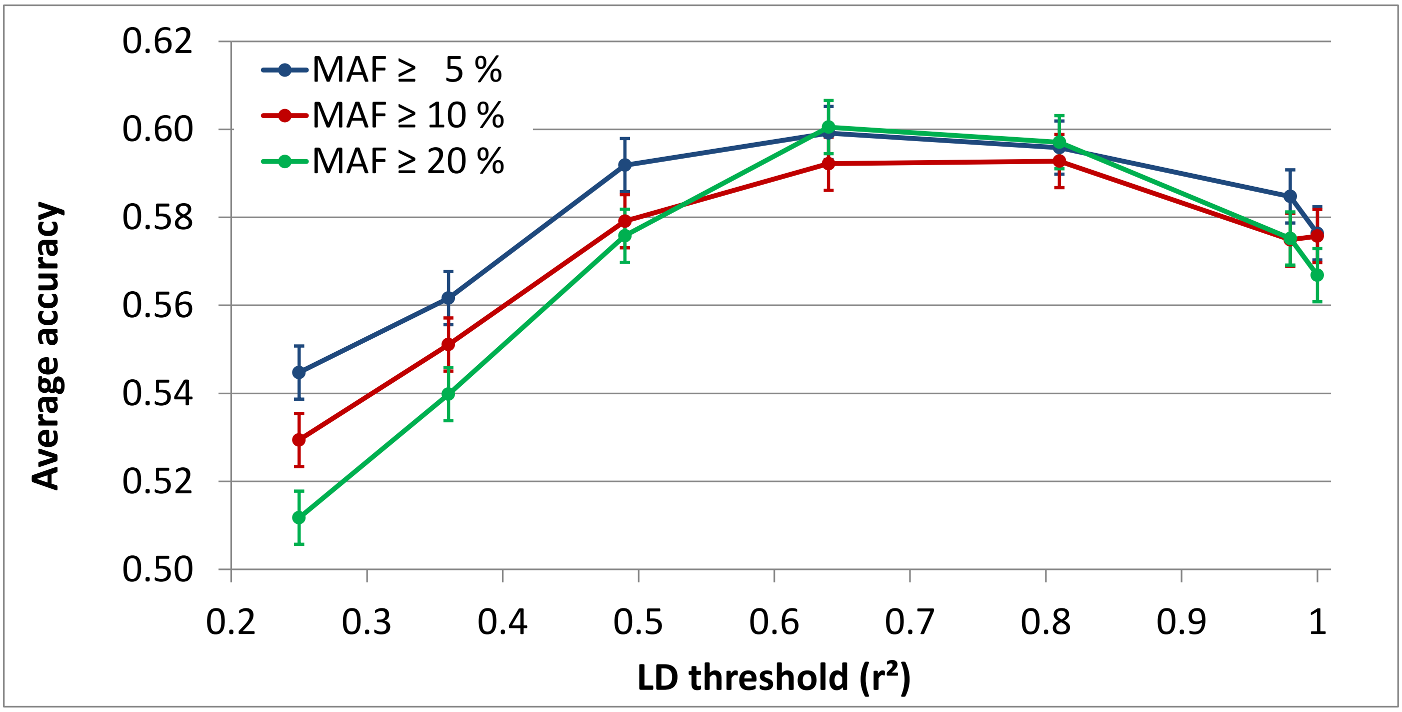
Supplementary Figure 1: Biparental crosses that gave birth to the progeny population. Parental lines belonging to tropical *japonica* group are in green.



Supplementary Figure 2: Distribution of the 43,686 working set SNP markers along the 12 chromosomes.

(A)





Supplementary Figure 3: Differences in average prediction accuracy for (A) the three methods (BayesB, GBLUP and RKHS) and for (B) the three levels of MAF (≥ 5%, ≥ 10%, ≥ 20%) according to the different levels of LD in the reference population (RP)

Chapter 3: Accuracy of genomic prediction accounting for genotype by environment interaction within a rice pedigree-breeding scheme

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In preparation

3.1 Abstract

By 2025, 15–20 million ha of rice lands will suffer some degree of water scarcity due to competition between urban, industrial and agricultural needs for water and due to climate change. It is thus crucial to develop more water use efficient agronomic practices and new rice varieties. Genomic selection has emerged as a promising method to accelerate genetic gain in breeding for complex traits such as water use efficiency. This is the first empirical study evaluating the accuracy of genomic prediction accounting for genotype by environment (GxE) interactions in rice. Using phenotypic data obtained under two contrasted irrigation system, (I) and Aerobic system(A) in a reference population (RP) of 283 inbred *japonica* accessions and in a progeny population (PP) of 97 F5-F7 lines, derived from 31 biparental crosses between members of RP, we tested two set of approaches for predicting response to aerobic system. The first approaches were based on response index and regression analysis, the seconds on explicit modelling of marker by environment interaction. Heritability was high for all target traits under the two environments. Rank-correlation between the performances of the individual entries of the two population was high for flowering time and panicle weight traits, indicating rather limited level of GxE interactions. Accuracy of genomic predictions were much higher when GxE interactions were modelled explicitly. Further progresses in the varietal improvement of adaptation to aerobic system, requires more diverse germplasm and/or more sever drying phase in the aerobic systems.

Keywords: Rice, genomic selection, progeny prediction, GxE interaction, water use efficiency.

3.2 Introduction

Rice is the world's most important staple food and will continue to be so in the coming decades. In the future, the necessary increases in rice production to meet demand, have to come mainly from increases in yield per unit of land, water and other resources. Increasing water scarcity, threatens the sustainability of rice production (Rijsberman, 2006). By 2025, 15–20 million ha of rice lands will suffer some degree of water scarcity (Tuong and Bouman, 2003; Mekonnen and Hoekstra, 2016). It is thus crucial to develop agronomic practices that reduces water use while maintaining or increasing yields. It is also crucial to adapt rice varieties to these new agronomic practices by improving water use efficiency (WUE) at the rice plant level.

One practice that has been shown to reduce water use in rice systems is an irrigation management practice referred to as "Alternate Wetting and Drying" (AWD) where paddy fields are subjected to intermittent flooding (Linquist et al., 2015; Lampayan et al., 2015). Meta-analysis of 56 studies comparing AWD with continuous flooding (CF) reported an overall, yields decrease of about 5% (Carrijo et al., 2017). However, large variations were observed depending, mainly, on the severity of the drying phase (i.e. soil water potential threshold that activate a new phase of re-watering) and on soil physiochemical characteristics (Lampayan et al., 2015; Carrijo et al., 2017). Significant differences of genotypic responses to AWD, measured by change in grain yield, were also reported and attributed to modified biomass partitioning (Bueno et al., 2010). Adapted genotypes had larger sink size at flowering, weaker stems and less unfilled grain number at maturity, suggesting an increase in the sink strength of the filling spikelets (Bueno et al., 2010). Significant genotypic variations for rice leaves' water use efficiency (WUE), based on carbon isotope discrimination ($\Delta^{13}C$) index, was also reported. Quantitative trait loci (QTL) associated with $\Delta^{13}C$, colocalised with photosynthesis parameters, leaf ABA concentration and stomatal conductance (This et al., 2010). Genes networked involved in rice WUE were also involved in regulation of photosynthetic and morpho-physiological responses to environmental stresses such as drought and high-temperature (Ambavaram et al., 2014). Thus, rice WUE and adaptation to alternate irrigation seems to be a typical complex traits, whose improvement require genome-wide breeding approaches that account for genotype by environment (G×E) interaction, i.e. the amplitude of the response of the genotypes to shift from irrigated management system to aerobic system.

Genotypic responses to environment changes translate into change in relative performances of the genotypes according to environment, with or without change in the ranking of the genotypes. The first methods for analyzing G×E, based on analysis of variance was proposed by Fisher and Mackenzie (1923). Later on, Yates and Cochran, (1938), proposed the joint regression approach which consists in a linear regression of the individual genotype performances on the environmental mean or average performance of all evaluated genotypes in the given environment. The joint regression approach was further developed by Finlay and Wilkinson (1963), and Eberhart and Russel (1966). They suggested that the analysis of the interaction should be computed for each variety as a linear regression slope, using a model in which the data of each genotype is regressed on an environmental index estimated as the mean of the environment. Later, Piepho (1997), Smith et al. (2001), Smith et al. (2005) employed the single value decomposition operator for modeling G×E in a context of multivariate linear mixed-effect models. Crossa et al. (2004), Crossa et al. (2006) and Burgueño et al. (2008) considered the framework of mixed model modelling covariance matrices based on pedigree linear mixed models for BLUP estimation to estimate the genotype by environment interactions.

However, those models describe the genotype by environment effect without an explicit modeling of the G×E interaction (Lopez-Cruz et al., 2015). They account mainly for the average interaction effect across the genome. Today, with the availability of genomic and environmental covariate, it is possible to analyze G×E explicitly. Such approach was first used to detect QTL by environment interactions (Moreau et al., 2004; Boer et al., 2007; Malosetti et al., 2004 ; Malosetti et al., 2008) and was then extended to genomic selection (GS) analysis (Meuwissen et al., 2001). Burgueño et al. (2011) were the first to use multi-environment GS. They used the genomic best linear unbiased prediction (GBLUP) to show that including G×E effects increased the accuracy of genomic prediction up to 6% compared to model without G×E effects. Burgueño et al. (2012) showed that multi-environment GBLUP can give higher prediction accuracy than a single environment analysis. Jarquín et al. (2014) implemented a model which consisted of a reaction norm where the genetic and environmental gradients were described as linear functions of markers and of environmental covariates respectively. Results showed that prediction accuracy for grain yield in wheat was higher (17-34%) when including the G×E interaction than prediction accuracy without interaction. This reaction norm model has been widely used in crops data (Pérez and de los Campos, 2014; Crossa et al., 2016). Heslot et al. (2014) compared an extension of the factorial regression model in the framework of GBLUP. This model was further extended to the marker level enabling the modeling of QTL×E on a genome wide scale. When considering the interaction QTL×E, an increased accuracy of 11,1% on average was obtained compared to the results reported by Burgueño et al. (2011). Following the idea of the evaluation of QTL×E, GS models that accommodate the interaction of markers with all the environments were developed Lopez-Cruz et al., (2015), Cuevas et al., (2016). They decomposed the marker effects into components that are common across environments and environment-specific. Lopez-Cruz et al. (2015) included marker environment effect in GBLUP to evaluate the interaction in three wheat populations genotyped and evaluated under simulated environmental conditions. Cuevas et al. (2016) included the M×E effect in the RKHS methodology with two kernel matrices that differed on the way the bandwidth was estimated. Lopez-Cruz et al. (2015) and Cuevas et al. (2016) found higher prediction accuracies when using multi-environment trial than the ones in single environments.

The objectives of this study are to (1) access several expressions of the response to an aerobic system, corresponding to a type of AWD, in two populations of *japonica* rice; (2) evaluate the prediction accuracy of these responses compared with the prediction accuracy in each condition separately (irrigated and aerobic); (3) to evaluate the prediction accuracy when modeling explicitly SNPxE interactions. The two populations used to fulfill the objectives were a diversity panel and a population of F5-F7 progenies derived from bi-parental crosses between several accessions of the diversity panel.

3.3 Material and method

3.3.1 Field trial and phenotyping

The plant material was composed of a reference population (RP) of 283 accessions belonging to the rice *japonica* subspecies, and a progeny population (PP) of 97 advanced (F_5-F_7) inbreed lines. The RP represents the working collection of the Research Center for Cereal and Industrial Crops (CREA), Vercelli, Italy. The PP was derived from 36 biparental crosses involving 31 accessions of RP, the diversity panel, using pedigree breeding scheme (Ben Hassen et al. Submitted).

The two populations were phenotyped separately during two consecutive rice cropping seasons at the experimental station of the CREA experimental station (45°19’24.00”N; 8°22’26.28”E; 134m). RP, during the 2012 and 2013 rice cropping seasons, PP during the 2014 and 2015 rice cropping seasons. Each cropping season, the phenotyping experiment included two independent trials corresponding to the two water management systems tested. The conventional water management or irrigated system (I), where rice is sown in a non-flooded soil and then the field is maintained flooded (10-15 cm of water) from the 3-4 leafs stage (typically 30 day after sowing) until mid-maturity. The aerobic water management system (A), where the field is flooded only when the soil hydric potential reached -30 kPa. The soil hydric potential was monitored with a set of six tensiometers evenly distributed in the field and inserted at 20 cm depth. The two trials were conducted in two separate field distant enough (100 m) to avoid interferences regarding the water regime while other soil characteristics remain identical (sand 47,8%, loam 42,8%, clay 9,4%; pH-H2O 6.4). The experimental design, identical for the two treatments, was a complete randomized design with three replicates for RP and a complete randomized blocks experimental design with three replicates for the PP.

The target traits for both RP and PP were days to flowering (FL), the panicle weight (PW), and the nitrogen balance index (NI) as described in Ben Hassen et al. (submitted).

3.3.2 Analysis of the phenotypic data

The mixed models used to analyze phenotypic plot data for each condition within the RP and the PP were as follows:

$$Y_{ijk}^{c} = \mu^{c} + g_{i}^{c} + y_{j}^{c} + gy_{ij}^{c} + e_{ijk}^{c} \qquad \text{(RP model)}$$

$$Y_{ijk}^{c} = \mu^{c} + g_{i}^{c} + y_{j}^{c} + b(y)_{jk}^{c} + gy_{ij}^{c} + e_{ijk}^{c} \qquad \text{(PP model)}$$

where Y_{ijk}^{c} is the observed phenotype in plot k of year j for accession i for condition c; μ^{c} the mean for condition c; g_{i}^{c}, the accession effect; y_{j}^{c} the year effect; $b(y)_{jk}^{c}$ the replication within year effect; gy_{ij}^{c} the interaction between accession and year; and e_{ijk}^{c} the residual.

In the analysis of variance components, all effects except the mean were considered random in both populations and conditions. The analyzed was performed with the proc mixed procedure of SAS 9.2 (SAS Institute, Cary NC, USA); the estimation method for the variance components was the restricted maximum likelihood (REML).

A model-based diagnostic analysis was run to detect potential outliers among the individual data points (plot level). This procedure led to the elimination of one entire accession in the RP for the aerobic condition, one data point for days to flowering in the aerobic condition of year 2012 and one data point for panicle weight in the aerobic condition of year 2013. The eliminated data were considered as missing data in the next steps of data analysis.

Broad sense heritability H² was calculated for each trait within each population and each condition using the formula of Holland et al. (2003) as follows:

$$H^{2} = \frac{\sigma_{g}^{2}}{\sigma_{g}^{2}+\frac{\sigma_{gy}^{2}}{ny}+\frac{\sigma_{e}^{2}}{nr}},$$

with *ny* being the harmonic mean number of years and *nr*, the harmonic mean number of plots across years per accession.

The adjusted means were extracted by another run of proc-mixed with accession declared as fixed effect and the lsmeans statement specified. The adjusted means, $\hat{Y}_{i}^{c}$, were used to model GxE.

3.3.4 Genotypic data and genotypic characteristics of RP and PP

The genotyping procedures, based on genotyping by sequencing technology are detailed in (Biscarini et al., 2016). After imputation, 246,554 SNPs with a call rate greater than 80% were available. Filtering of this matrix for the rate of heterozygoty (threshold of 5%) and for a minor allele frequency (MAF, threshold of 2.5%) among the RP accessions and PP lines, considered together, led to a final working set of 43,686 SNP loci. The genotypic data are available at http://tropgenedb.cirad.fr/tropgene/JSP/interface.jsp?module=RICE, (Choose Tab Studies) as GS-Ruse_CREA_GBSgenotype_RP&PP.

The genetic structuring of the two populations was analyzed jointly using a distance based method. First, a matrix of 4,824 SNPs was extracted from the working genotypic dataset of 43,686 SNPs, by discarding loci that had imputed data and by imposing a minimum distance of 10 kb between two adjacent loci. Then an unweighted neighbor-joining tree based on a simple matching matrix was constructed using DarWin v6 (Perrier and Jacquemoud-Collet, 2006).

Pairwise LD between SNP loci was calculated separately in RP and PP at the level of the individual chromosome, using the working genotypic dataset of 43,686 SNPs and the r^2 estimator proposed by (Rogers and Huff, 2009) for non-phased genotypic data.

3.3.5 Statistical methods for genomic prediction

a) Methods for single environment and response to aerobic genomic prediction

Prediction accuracies for the three traits and their related response to water treatment (index and slope of the joint regression) were assess with two different validation schemes. The first scheme used only the reference population with random partitions and was referred as cross-validation. The second validation scheme used the information of the reference population to predict the performance of the progeny population and was referred as progeny validation.

Two of the statistical methods used were genomic best linear unbiased prediction (GBLUP) and reproducing kernel Hilbert spaces regressions (RKHS), both implemented in KRMM package (https://sourceforge.net/projects/package-krmm/) and described by Jacquin et al., (2016). The third method used was BayesB implemented in BGLR package (Perez and de Los Campos, 2014).

b) Methods for multi environment genomic prediction

Four different kernel regression methods were used to predict the genomic estimated breeding values. The first method that relies on a linear kernel was the genomic best linear unbiased predictor (GBLUP, (Van Raden 2008)) as it is one of the most popular method for genomic prediction. The three others methods used a nonlinear Gaussian kernel estimated with two different approaches: i) an empirical Bayesian estimation of the posterior mode of the bandwith (RKHS-EB, GK, (De Los Campos et al., 2010, Cuevas et al., 2016) (Cuevas et al., 2017)), ii) an average of three different kernels (RKHS-KA, (De Los Campos et al., 2010, Pérez-Elizalde et al., 2015). These four methods included multi-environment component: For GBLUP, RKHS-EB and RKHS-KA, These model integrate the effects of m environments, and the effects of the markers are separated into two components: the main effect of the markers for all the environments and the effect of the markers for each environment (López-Cruz et al., 2015). For

GK method, multi-environments with random effects considered genetic correlations between environments with two component *u* and *f* in the following random linear model:

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

where μ is the vector with the intercept of each environment, u the random vector the genetic values, f the genetic variability among individuals that was not accounted for as a function of the markers in component u, and ε the radom vector of the error. u, f and ε are independent and normally distributed (Burgueño et al. 2012 Cuevas et al., 2017).

Analysis were performed in the R 3.3.3 environment (R Core Team 2016) with the R packages *BGLR* (Pérez and de los Campos 2014) and *MTM* (de los Campos and Grüneberg 2016). For both packages, a total of 12,000 iterations for the Gibbs sampler were used. For the inference, 1,000 samples were used after removing the first 2,000 samples (burn-in) and keeping one in ten samples to avoid auto-correlation (thinning). Convergence of Markov Chain Monte Carlo algorithm was assess for all parameters of the models with Gelman-Rubin tests (Gelman and Rubin 1992) using the R-package *coda* (Plummer et al., 2006).

3.3.6 Assessing prediction accuracy

Different types of random partitions were performed depending on the phenotypic information used in the statistical mode. For trait in a single environment and for response variables 80% of the 283 accessions of the RP (i.e. 228 accessions) was used as training set and the remaining 20% (57 accessions) was used as the validation set. For multi-environment models, two different method were used. The first method (CV1) used 80% of the observations as a training set and the remaining 20% as the validation set and assumed that individuals composing the training set have observations for all environment. This corresponds to the situation when unobserved phenotypes of untested lines (newly generated lines) have to be predicted based only on their genotypes (Burgueño et al., 2012). The second method (CV2), used also 80% of the observations as a training set and the remaining 20% as the validation set but assumed that individuals in the training set have at least an observation in one environment. This corresponds to the situation when missing phenotypes of lines in an environment were to be predicted with genotypes and phenotypes from other environments (Burgueño et al., 2012)

3.4 Results

3.4.1 Analysis of the phenotypic variation

The analysis was conducted separately within populations and water management treatments. The estimates of all sources of variation are shown (Table 1). However, the Wald test is given for those components that have high number of levels (*i.e.* accession or progeny, accession x year or progeny x year and residual). At most, two and six levels were available for year and replication within year effects respectively, the reason why the Wald test was not performed as recommended by West et al. (2014). The variance for accessions or progenies contributed very highly significantly to the phenotypic variation. The accession x condition or progeny x condition interaction also contributed very highly significantly except for NI trait and aerobic condition in both populations, where the significance level is lower (* and NS respectively).

The heritability based on accession or progeny means was high for FL ($H^2 = 0.84$ to 0.94) and PW ($H^2 = 0.76$ to 0.90), and moderate to high for NI ($H^2 = 0.55$ to 0.80). But there was not a clear trend in heritability between populations or water management conditions. The prediction accuracies are expected to be rather good.

Table 1: Variance components of days to flowering (FL) , nitrogen balance index (NI), and 100 panicles weight (PW) in the reference (RP) and progeny (PP) populations for Aerobic and irrigated conditions

Pop	**Water management condition**	**Variance components**	**Estimate**	**Zvalue**	**ProbZ**		**H^2**	**SE**	**Estimate**	**Zvalue**	**ProbZ**		**H^2**	**SE**	**Estimate**	**Zvalue**	**ProbZ**		**H^2**	**SE**
RP	**Aerobic**		88,96				0,94	0,01	1,80				0,55	0,05	1447				0,85	0,02
		Accession	**54,52**	10,42	<0.0001	***			**5,01**	6,8	<0.0001	***			**3368**	8,8	<0.0001	***		
		Year*Accession	**10,71**	8,77	<0.0001	***			**1,22**	2,14	0,0161	*			**940**	4,99	<0.0001	***		
		Residual	**10,75**	23,69	<0.0001	***			**14,72**	23,45	<0.0001	***			**3145**	22,29	<0.0001	***		
	Irrigated	Year	16,84				0,88	0,01	2,97				0,61	0,05	225				0,76	0,03
		Accession	**46,26**	11,09	<0.0001	***			**6,07**	6,01	<0.0001	***			**4999**	10	<0.0001	***		
		Year*Accession	**4,38**	8,06	<0.0001	***			**4,12**	4,82	<0.0001	***			**905**	5,94	<0.0001	***		
		Residual	**5,97**	23,77	<0.0001	***			**16,73**	23,59	<0.0001	***			**2345**	23	<0.0001	***		
PP	**Aerobic**	Year	81,87				0,85	0,03	1,50				0,80	0,04	1429				0,90	0,02
		Rep(Year)	0,42						0,88						194					
		Progeny	**34,81**	5,78	<0.0001	***			**3,03**	5,25	<0.0001	***			**2488**	6,1	<0.0001	***		
		Year*Progeny	**9,25**	4,91	<0.0001	***			**0,00**	0,00					**466**	4,97	<0.0001	***		
		Residual	**10,52**	13,71	<0.0001	***			**5,32**	15,02	<0.0001	***			**522**	13,77	<0.0001	***		
	Irrigated	Year	55,47				0,84	0,03	4,99				0,76	0,04	16				0,88	0,02
		Rep(Year)	0,16						0,12						296					
		Progeny	**23,20**	5,83	<0.0001	***			**4,12**	5,4	<0.0001	***			**2699**	6,2	<0.0001	***		
		Year*Progeny	**7,38**	6,25	<0.0001	***			**0,70**	2,28	<0.0001	***			**415**	4,68	<0.0001	***		
		Residual	**2,27**	13,78	<0.0001	***			**3,72**	13,26	<0.0001	***			**554**	13,71	<0.0001	***		

Significance with four levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001).

3.4.2 Distribution of adjusted means.

The three traits investigated in both water management conditions and both populations populations exhibited a Gaussian distribution (Figure 1). We noticed a shift of the distribution between the two conditions with later flowering in the aerobic condition (mean value of 100.34 days for A and 93.35 day for I). We had lower NI value in the aerobic condition compared to the irrigated condition (20.04 for A and 23.71 for I) as well as for PW (251.27g for A and 341.84g for I).

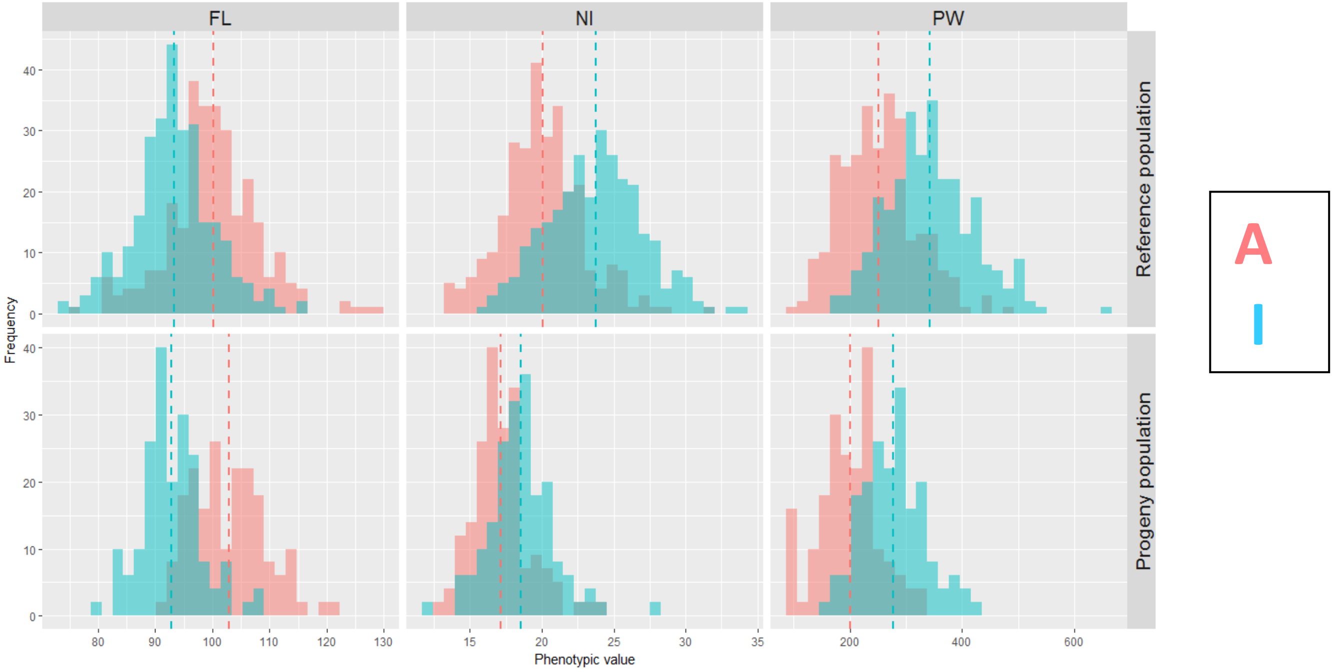
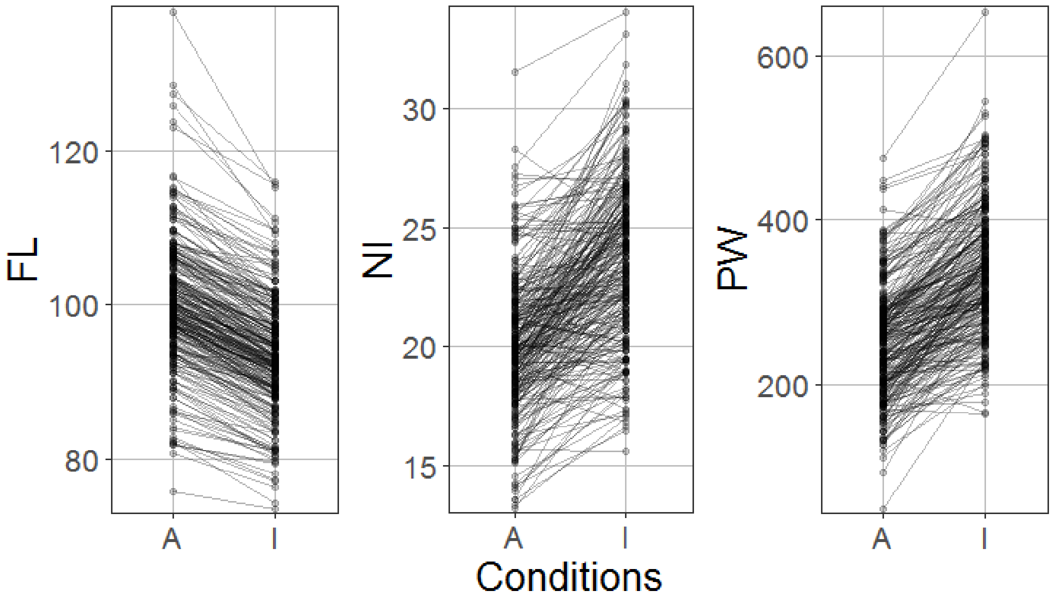


Figure 1: Distribution of adjusted means of days to flowering (FL), Nitrogen balance index (NI) and 100 panicles weight (PW) within the reference and progeny populations in irrigated (I, blue) and aerobic (A, pink) conditions.

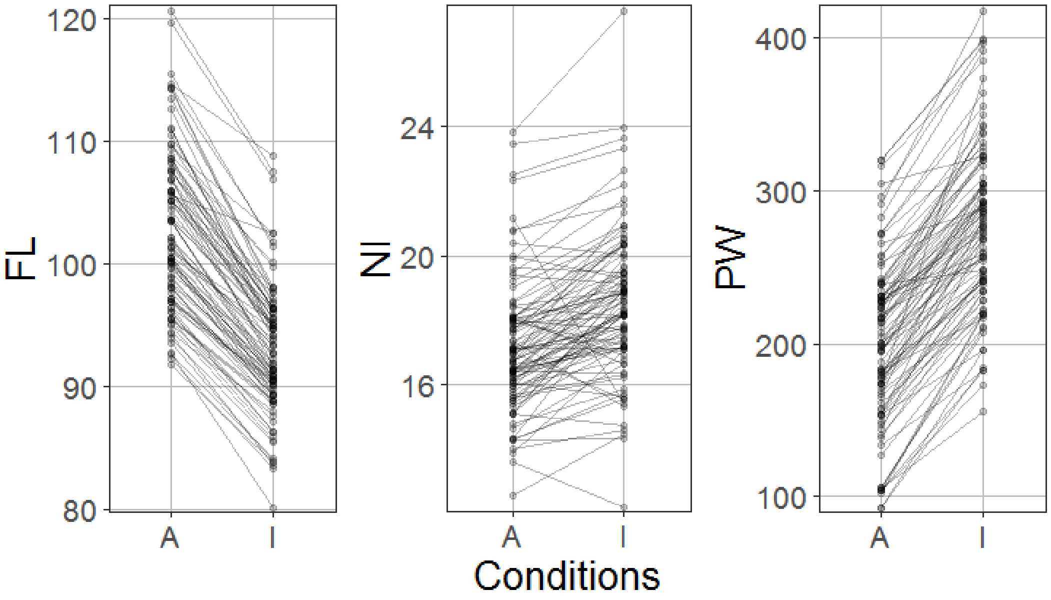
The two conditions are rather highly correlated (Figures 2 and 3). The highest correlations were observed on days to flowering (0.95 for RP and 0.92 for PP) and the lowest on nitrogen balance index (0.54 and 0.64 respectively).



Spearman correlations :

$\rho_{FL}(A,I) = 0.95$ $\rho_{NI}(A,I) = 0.54$ $\rho_{PW}(A,I) = 0.74$

Figure 2: Reaction norm of 283 accessions from the Reference Population to the water management conditions (A: aerobic and I: irrigated) and Spearman correlations between A and I for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW)



Spearman correlations :

$\rho_{FL}(A,I) = 0.92$ $\rho_{NI}(A,I) = 0.64$ $\rho_{PW}(A,I) = 0.84$

Figure 3 : Reaction norm of the 97 lines of the Progeny Population to the water management conditions (A: aerobic and I: irrigated) and Spearman correlations between A and I for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW)

3.4.3 Accuracy of genomic prediction within the diversity panel (RP)

a) Prediction accuracy of the response variables.

The focus here is to compare two ways to model the response to drought (drought tolerance index and joint regression slope). The average prediction accuracy (APA) was analyzed and compared with the ones based on adjusted means within each condition considered as baselines (Table 2) The overall mean of accuracies was 0.51 but the range of values extended from 0.07 up to 0.71, depending on the type of trait, response and prediction method (Table S1a, Figure 4). The most influential factor was the type of response (Table 2a). The drought tolerance index is much less accurate than joint regression slope (0.24 and 0.62 respectively). The prediction using joint regression slope was at least as accurate as the prediction within conditions using the respective adjusted means (0.58-0.60). The prediction accuracy depended secondly on trait architecture, with the most accurate for PW (0.57) and least accurate for NI (0.43). The prediction methods were not significantly different from each other (0.50-0.52). The interactions were not important, except for the one between response variable and trait architecture (Table 2b).

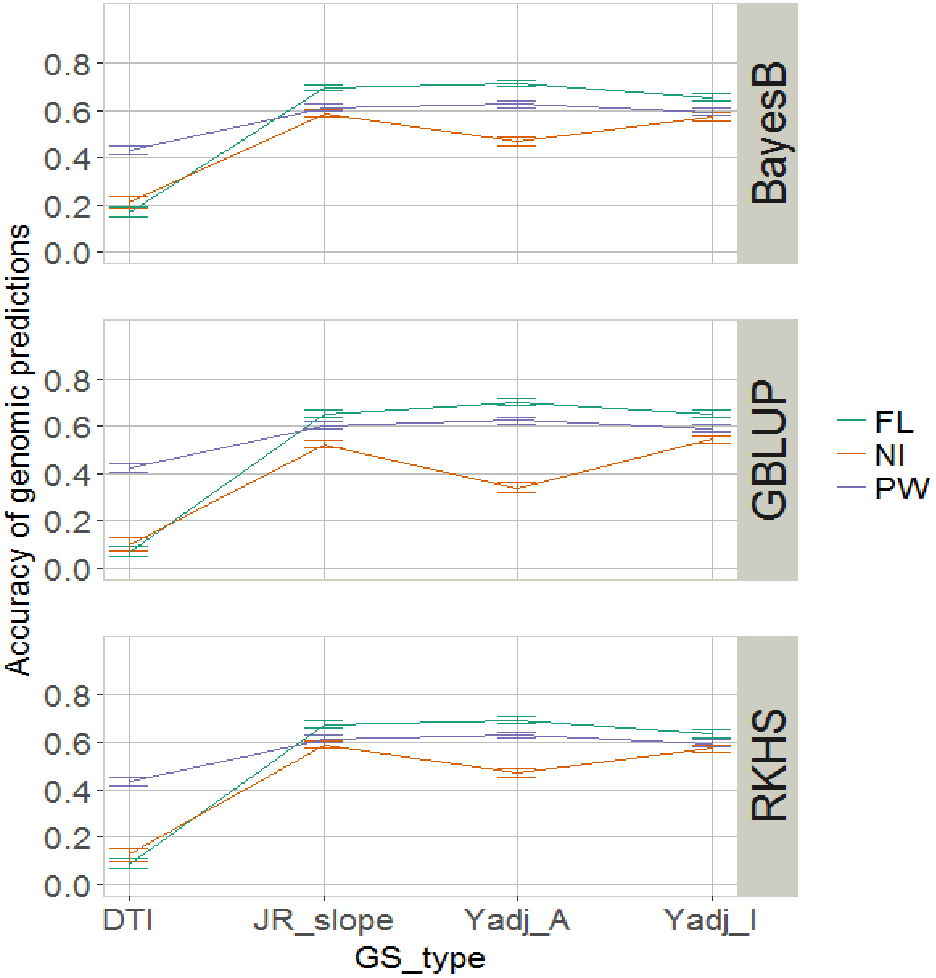


Figure 4: Average prediction accuracy in cross validation experiments within the reference population for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW), using the drought tolerance index (DTI), the regression coefficient of the joint regression (JR_slope) and the adjusted means of the phenotypic traits per conditions (Yadj_A and Yadj_I) Accuracies were obtained with three statistical methods (BayesB, GBLUP, RKHS)

Table 2: Analysis of factors influencing the variation of average prediction accuracy of response variables

a.ANOVA including principal effects and respective adjusted means

Source	DF	SS	MS	F Value	Pr > F
Model	7	1,073	0,153	18,8	<.0001***
Error	28	0,228	0,008		
Corrected Total	35	1,301			

R²	CV	Root MSE	Mean
0,82	17,80	0,090	0,51

Source	DF	Type III SS	MS	F Value	Pr > F	
Response	3	0,778	0,259	31,8	<.0001	***
Trait	2	0,127	0,064	7,8	0,002	**
Method	2	0,004	0,002	0,24	0,7908	NS

b.ANOVA including principal effects and first order interaction

Source	DF	SS	MS	F Value	Pr > F
Model	22	1,285	0,058	47,23	<.0001***
Error	13	0,016	0,001		
Corrected Total	35	1,301			

R²	CV	Root MSE	Mean
0,99	6,93	0,035	0,51

Source	DF	Type III SS	MS	F Value	Pr > F	
Response	3	0,758	0,253	204,42	<.0001	***
Trait	2	0,113	0,056	45,63	<.0001	***
Method	2	0,004	0,002	1,79	0,2062	NS
Response*Trait	6	0,179	0,030	24,18	<.0001	***
Response*Method	5	0,005	0,001	0,74	0,6059	NS
Trait*Method	4	0,004	0,001	0,91	0,489	NS

Source: Trait with three levels (FL: Days to flowering; NI: Nitrogen balance index; PW; Response with two levels, (Drought tolerance index DTI; Regression coefficient of the joint regression JR_slope. regression and the adjusted means of the phenotypic traits per conditions,Yadj_A and Yadj_I; Prediction method (Method) with three levels (Bayes B, GBLUP, RKHS) Significance with four levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001)

b) Prediction accuracy when including explicit multi-environment effects

In this study, the focus is on (1) the sampling strategy (across-environment, CV1 and CV2) using single environment as the baseline, and (2) the target condition (aerobic or irrigated) using single or multiple environments to calibrate the model. The overall mean accuracy was 0.62 and ranged from 0.44 to 0.91 (Table S1b), depending on the sampling strategy, target condition, trait architecture, and prediction method. Indeed, only sampling strategy and trait architecture were significant (Table 3a). The accuracy of the baseline strategy (single) was 0.62. The across sampling was the least accurate (0.56). The CV1 sampling is comparable to the baseline (0.62) while the CV2 was the most accurate (0.69). The range was even larger for traits: only 0.51 for NI, 0.62 for PW and up to 0.73 for FL. Neither target condition, nor prediction method were significant. The ANOVA table shows that three interactions out of six were significant: trait*sampling, trait*target, and sampling*method. The target condition and the prediction method were influential only in interactive mode.

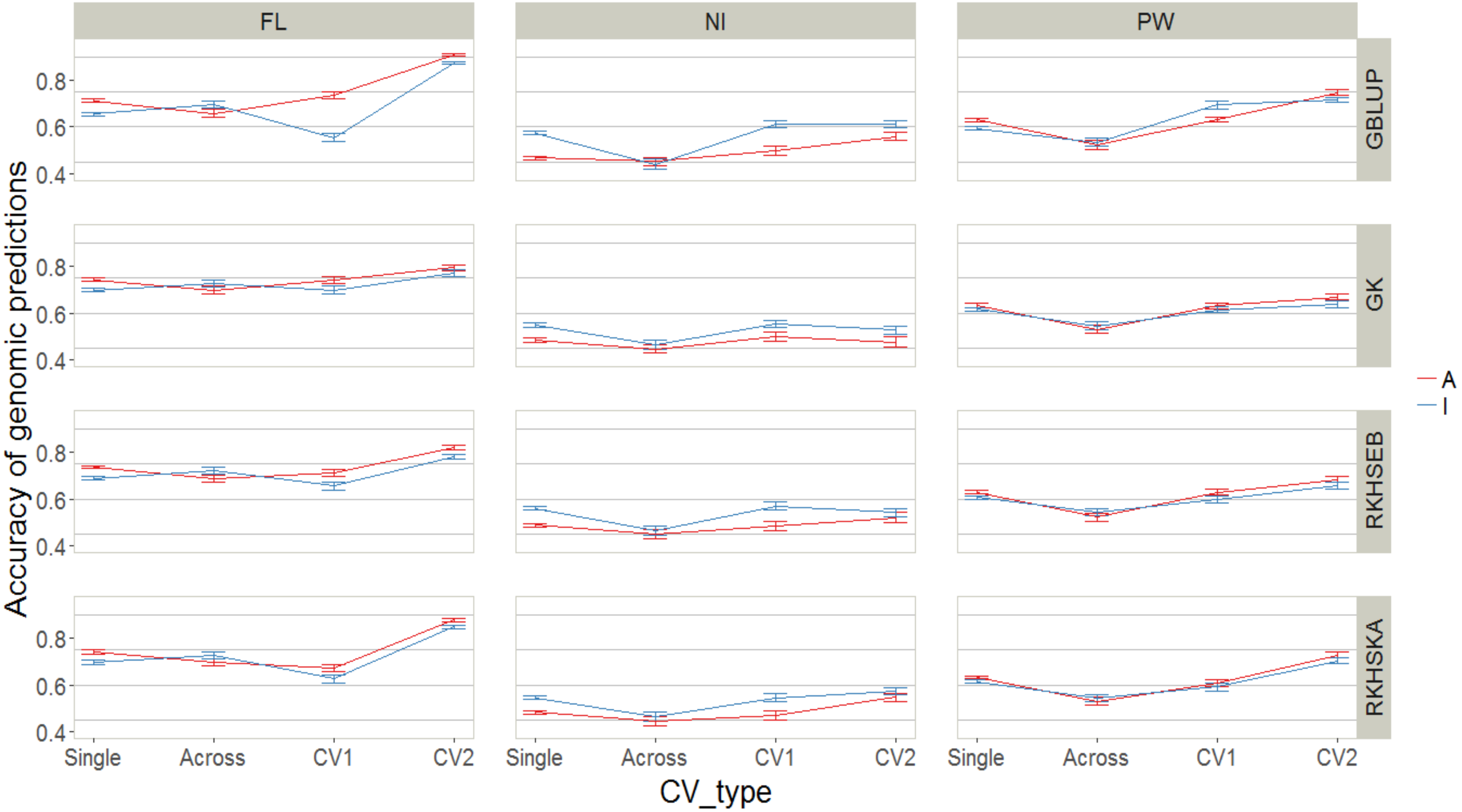


Figure 5 : Average prediction accuracy in cross validation experiments within the reference population for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW), obtained with four statistical methods (GBLUP, GK, RKHS-EB, RKHS-KA) with a single environment, across environment and with multi environment (CV1 and CV2)

Table 3: Analysis of factors influencing the variation of average prediction accuracy within the reference population using multi-environment models

a. ANOVA including principal effects and respective adjusted means

Source	DF	SS	MS	F Value	Pr > F
Model	9	0,970	0,108	53,06	<.0001***
Error	86	0,175	0,002		
Corrected Total	95	1,144			

R^2	CV	Root MSE	Mean
0,85	7,27	0,045	0,62

Source	DF	Type III SS	MS	F Value	Pr > F	
Trait	2	0,768	0,384	189,10	<.0001	***
Sampling	3	0,198	0,066	32,53	<.0001	***
Method	3	0,003	0,001	0,52	0,669	NS
Target	1	0,000	0,000	0,21	0,652	NS

b. ANOVA including principal effects and first order interactions

Source	DF	SS	MS	F Value	Pr > F
Model	38	1,102	0,029	39,39	<.0001
Error	57	0,042	0,001		
Corrected Total	95	1,144			

R^2	CV	Root MSE	Mean
0,96	4,38	0,027	0,62

Source	DF	Type III SS	MS	F Value	Pr > F	
Trait	2	0,768	0,384	521,35	<.0001	
Sampling	3	0,198	0,066	89,68	<.0001	
Method	3	0,003	0,001	1,43	0,242	
Target	1	0,000	0,000	0,57	0,455	
Trait*Sampling	6	0,062	0,010	14,04	<.0001	
Trait*Method	6	0,004	0,001	0,85	0,534	***
Trait*Target	2	0,029	0,014	19,43	<.0001	NS
Sampling*Method	9	0,036	0,004	5,4	<.0001	***
Sampling*Target	3	0,002	0,001	1,03	0,386	***
Method*Target	3	0,000	0,000	0,05	0,986	NS

Source: Trait with three levels (FL: Days to flowering; NI: Nitrogen balance index; PW°; Target , the target condition predicted (Aerobic or Irrigated); Sampling, single across or multi environment with CV1 or CV2; Prediction method (Method) with three levels (GBLUP. GK. RKHS-KA; RKHS-EB); Significance with four levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001)

3.4.3 Accuracy of genomic prediction across populations

a) Prediction accuracy of the response variables

In average, across generations prediction was less accurate (0.27) than prediction within the reference panel (0.51) (Table S2a). Three out of four factors were significant (Table 4a). The most influential was the response (with average of 0.16 for DTI and 0.37 for joint regression slope). The trait factor came in second (with mean accuracy of 0.22 for NI, 0.23 for FL and 0.36 for PW). The scenario was less significant, with levels ranging from 0.24-0.25 (for S1, S2 and S5) to 0.30 (for S3 and S4). Only one interaction was found significant (trait*response).

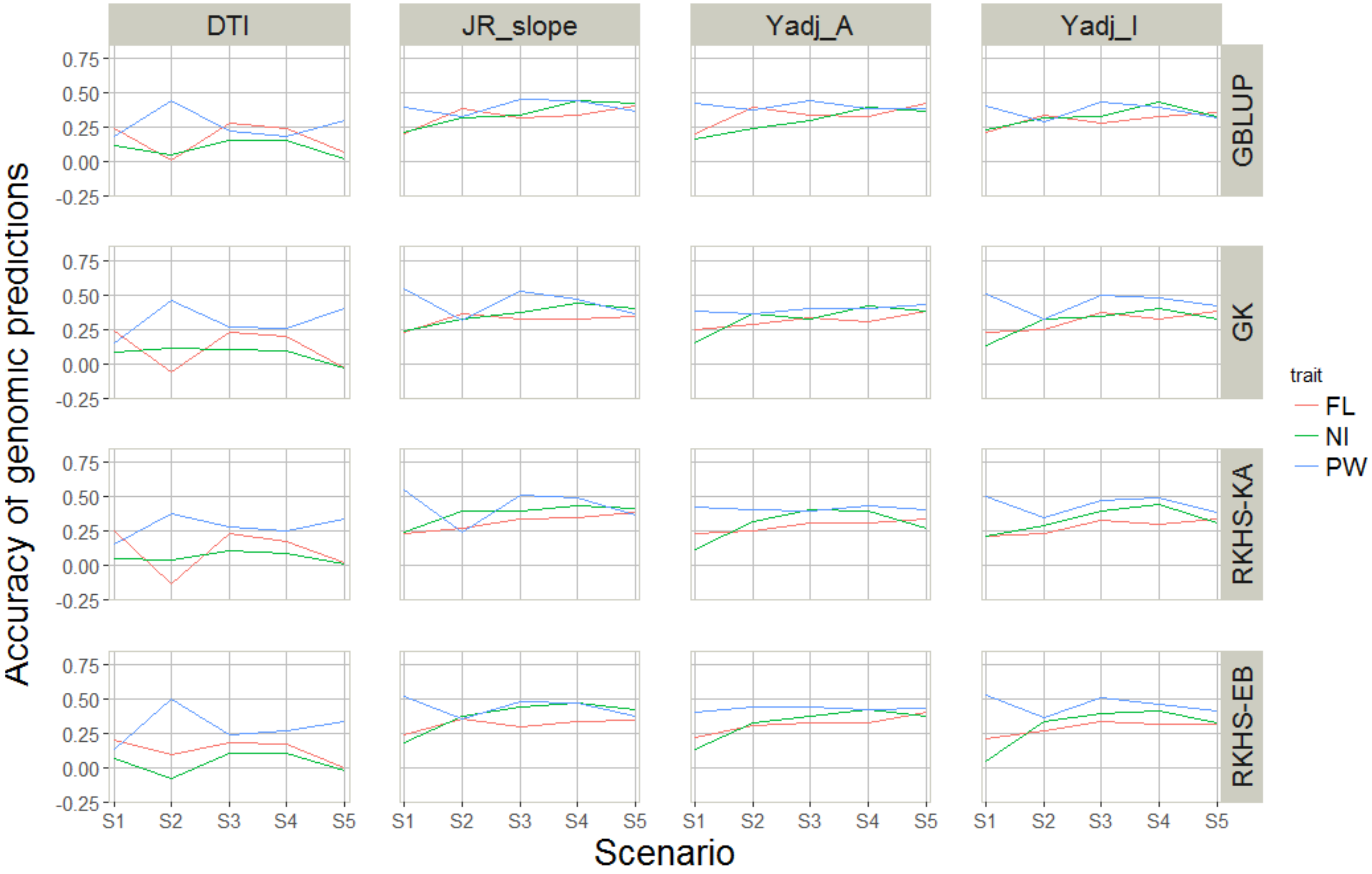


Figure 6 : Average prediction accuracy in across population validation experiments for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW), using the drought tolerance index (DTI), the regression coefficient of the joint regression (JR_slope) and the adjusted means of the phenotypic traits per conditions (Yadj_A and Yadj_I) obtained with four statistical methods (GBLUP, RKHS-KA, RKHS-EB, GK) for scenario S1-S5

Table 4: Analysis of factors influencing the variation of average prediction accuracy of two response variables progenies

a.ANOVA including principal effects and respective adjusted means

Source	DF	SS	MS	F Value	Pr > F
Model	10	1,911	0,191	21,94	<.0001
Error	109	0,949	0,009		
Corrected Total	119	2,860			

R²	CV	Root MSE	Mean
0,67	34,95	0,093	0,27

Source	DF	Type III SS	MS	F Value	Pr > F	
Response	1	1,310	1,310	150,43	<.0001	***
Trait	2	0,504	0,252	28,95	<.0001	***
Scenario	4	0,096	0,024	2,76	0,0311	*
Method	3	0,000	0,000	0,01	0,9979	NS

b.ANOVA including principal effects and first order interactions

Source	DF	SS	MS	F Value	Pr > F
Model	37	2,189	0,059	7,23	<.0001
Error	82	0,671	0,008		
Corrected Total	119	2,860			

R²	CV	Root MSE	Mean
0,77	33,88	0,090	0,27

Source	DF	Type III SS	MS	F Value	Pr > F	
Response	1	1,124	1,124	137,32	<.0001	
Trait	2	0,431	0,216	26,34	<.0001	
Scenario	4	0,082	0,020	2,5	0,0491	
Method	2	0,000	0,000	0,02	0,9817	
Trait*Response	2	0,127	0,063	7,74	0,0008	***
Trait*Scenario	8	0,075	0,009	1,15	0,3400	NS
Trait*Method	4	0,018	0,005	0,55	0,6965	NS
Response*Scenario	4	0,043	0,011	1,3	0,2758	NS
Method*Response	2	0,009	0,004	0,54	0,5852	NS
Method*Scenario	8	0,006	0,001	0,09	0,9993	NS

Source: Trait with three levels (FL: Days to flowering; NI: Nitrogen balance index; PW; Response with two levels, (Drought tolerance index DTI; Regression coefficient of the joint regression JR_slope. regression and the adjusted means of the phenotypic traits per conditions,Yadj_A and Yadj_I; Prediction method (Method) with three levels (GBLUP,GK, RKHS-KA, RKHS-EB) Significance with four levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001)

b) Prediction accuracy when including explicit multi-environment effects

The overall mean accuracy was 0.34, with values ranging from 0.05 up to 0.53 (Table S2b), depending on traits, scenarios, sampling modalities, prediction methods and target conditions. However, only two factors were significant. The first was trait, with average acuuracy of 0.30, 0.31 and 0.40 for FL, NI and PW respectively. The values for scenarios were 0.27, 0.32, 0.36, 0.38 and 0.37 for S1 to S5 respectively. The remaining factors (sampling strategy, target condition and prediction method) influence the accuracy only in interactive mode (table5b).

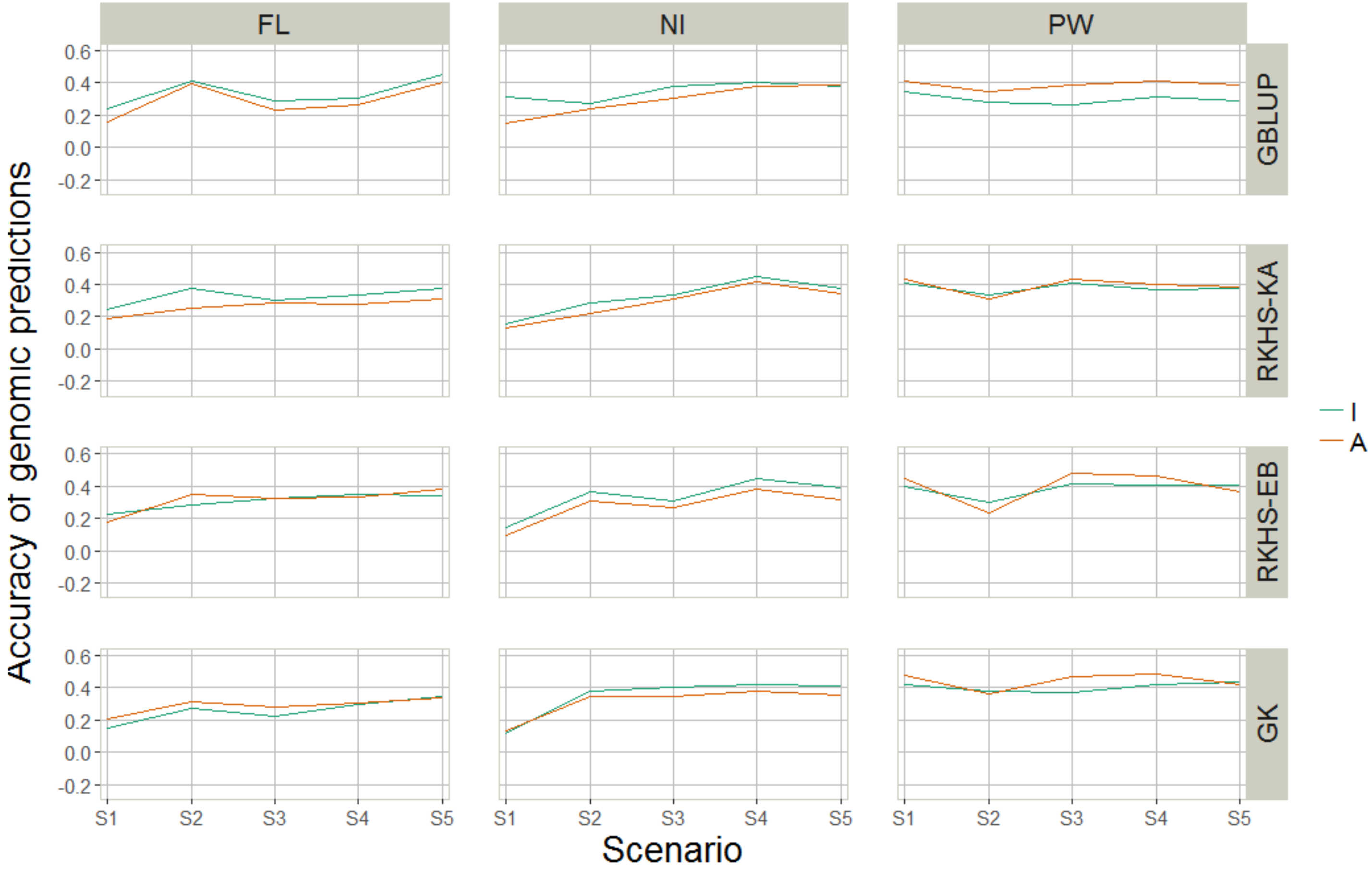


Figure 7: Average prediction accuracy in across population validation experiments with for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW), with CV1, Accuracies were obtained with four statistical methods (GBLUP, RKHS-EB, RKHS-KA, GK)

Table 5: Analysis of factors influencing the variation of average prediction accuray of progenies using multi-environment models

a. ANOVA including principal effects and respective adjusted means

Source	DF	SS	MS	F Value	Pr > F
Model	11	0,941	0,086	21,41	<.0001
Error	228	0,911	0,004		
Corrected Total	239	1,852			

R²	CV	Root MSE	Mean
0,51	18,61	0,063	0,34

Source	DF	Type III SS	MS	F Value	Pr > F	
Trait	2	0,484	0,242	60,62	<.0001	***
Scenario	4	0,436	0,109	27,26	<.0001	***
Sampling	1	0,013	0,013	3,35	0,0686	NS
Method	3	0,007	0,002	0,58	0,6267	NS
Target	1	0,000	0,000	0,11	0,7380	NS

b. ANOVA including principal effects and first order interactions

Source	DF	SS	MS	F Value	Pr > F
Model	56	1,581	0,028	19,04	<.0001
Error	183	0,271	0,001		
Corrected Total	239	1,852			

R²	CV	Root MSE	Mean
0,85	11,34	0,038	0,34

Source	DF	Type III SS	MS	F Value	Pr > F	
Trait	2	0,484	0,242	163,42	<.0001	
Scenario	4	0,436	0,109	73,49	<.0001	
Sampling	1	0,013	0,013	9,03	0,003	
Method	3	0,007	0,002	1,57	0,1978	
Target	1	0,000	0,000	0,3	0,583	
Trait*Scenario	8	0,478	0,060	40,34	<.0001	***
Trait*Sampling	2	0,011	0,006	3,76	0,025	*
Trait*Method	6	0,051	0,009	5,78	<.0001	***
Trait*Target	2	0,029	0,015	9,93	<.0001	***
Scenario*Sampling	4	0,014	0,003	2,29	0,0614	NS
Scenario*Method	12	0,031	0,003	1,72	0,0654	NS
Scenario*Target	4	0,021	0,005	3,52	0,0085	**
Sampling*Method	3	0,001	0,000	0,27	0,8485	NS
Sampling*Target	1	0,001	0,001	1,01	0,3174	NS
Method*Target	3	0,002	0,001	0,36	0,7815	NS

Source: Trait with three levels (FL: Days to flowering; NI: Nitrogen balance index; PW°; Target , the target condition predicted (Aerobic or Irrigated); Sampling, single across or multi environment with CV1 or CV2; Prediction method (Method) with three levels (GBLUP. GK. RKHS-KA; RKHS-EB); Significance with four levels (NS: not significant, *: significant at p= 0.05, **: significant at p= 0.001, ***: significant at p=0.0001)

3.5 Discussion

The objective of the present research was to evaluate the potential of genomic approaches in prediction genotype by environment interactions under two water management systems namely, irrigated (I) and aerobic (A).

The experimental configuration were favorable for the implementation of multi-environment genomic prediction but was less adapted to the objective of selection of ideotype specific to the (A) system. Indeed, heritability H^2 was high for the three traits considered, in both (I) and (A) environments; and rank correlation was very high between the performances under the two environment, spicily for FL and PW traits that exhibited low GxE interaction.

The two modeling approach of response to aerobic environment intend to consider two aspect of this response. The response index provides an estimate of the relative response of the individual accessions but this response dose note integrate the performances of other genotypes under the two environments. Genotypes less susceptible to the effects of shifting from FC environment to aerobic environment are the one with response index close to zero. However, the index does not provide information on the performances of the genotype under each environment. The slope of the joint regression is a response that take into account the performances of the genotypes involved in the experiments, under the two environments. Genotypes less susceptible to the effect of A have a slope close to one. The most stable genotype are the one with response equal to the average response. It would have been interesting to compute the heritability of these response traits. Unfortunately, this was not possible as the I and A experiment were conducted separately.

The response index based genomic prediction resulted in lowest accuracies for all the traits. The joint regression base genomic prediction led to high prediction accuracy, like those obtained with the separated conditions. Similar results were shown by Huang et al. (2016) when studying several environments of wheat trials. They found that prediction accuracy was higher for the response to environment estimated with slop of the joint regression than the accuracy of prediction for performance under individual environment.

The alternative model that account explicitly for GXE interaction rely on the fact that response to different environments are more or less correlated positively and can be utilized to capture a larger proportion of phenotypic variations, thus reducing experimental noise. We used this approach modelling under scenarios. Scenario one (CV1) intended to predict phenotypic performances, in each environment (I and for A), of individuals for whom only genotypic data were available (i.e. individual that were evaluated neither under I environment nor under A environment). In this scenario, the benefit of accounting explicitly for GxE interaction was not very high, as the GxE interaction was rather low and the multi-environment information produced redundancy. Scenario two (CV2) intended to predict the phenotype in one of the environments (I or A) using both the genotypic data and the performance in the second environment. In this scenario, model accounting explicitly for GxE interactions improved significantly the accuracy of genomic prediction, for the two traits (FL and WP) with the highest rank correlations. The benefit much less important for NI trait that exhibited large GxE and low rank-correlation between the two environments.

These results are in accordance with the finding of Lopez-Cruz et al. (2015) who showed that the higher accuracy obtained with CV2 are related to the correlation between environments, therefore a lack of genetic of phenotypic information of one environment can be covered by the other environment. The borrowing of information within lines across environments is permitted by an explicit modeling of marker main and marker specific effects of each environment (Lopez-Cruz et al., 2015; Cuevas et al., 2016). As this approach is not possible with CV1, the model doesn’t perform better than in single environment.

Considering that our rice breeding program is in a transition situation moving progressively from breeding for I system toward breeding for the A system, we can consider that we are in a favorable configuration. Indeed, (i) the best performing germplasm under I, also perform well under A, (ii) rather high genomic prediction can be expected from combination of data for the two irrigation system, and (iii) occasionally genotype with high level of GxE can also be identified. However, more contrasted environments (lower soil water potential during the drying phase of the A system) and more diverse germplasm, exhibiting larger GxE interactions would be need to improve rice stressful A irrigation system.

3.6 Acknowledgments

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3.7 References

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3.8 Supplemental material

Supplemental table 1: Cross-validation experiment within the reference population for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW)

a. Prediction accuracy of ajusted means in aerobic and irrigated conditions (Yajd_A, Yadj_I), joint regression slope (JR_slope) and drought tolerance index (DTI) with methods BayesB, GBLUP and RKHS

	BayesB									GBLUP									RKHS								
	FL			NI			PW			FL			NI			PW			FL			NI			PW		
Predicted phenotypes	APA		CL(1)	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL
Yadj_A	0.71	±	0.01	0.47	±	0.02	0.63	±	0.01	0.70	±	0.02	0.34	±	0.02	0.62	±	0.01	0.69	±	0.02	0.47	±	0.02	0.63	±	0.01
Yadj_I	0.65	±	0.02	0.57	±	0.02	0.59	±	0.02	0.65	±	0.02	0.54	±	0.02	0.59	±	0.01	0.64	±	0.02	0.57	±	0.02	0.60	±	0.01
JR_slope	0.70	±	0.01	0.59	±	0.02	0.61	±	0.01	0.65	±	0.02	0.52	±	0.02	0.60	±	0.02	0.68	±	0.02	0.59	±	0.02	0.61	±	0.01
DTI	0.17	±	0.02	0.21	±	0.02	0.43	±	0.02	0.07	±	0.02	0.10	±	0.03	0.42	±	0.02	0.09	±	0.02	0.13	±	0.03	0.43	±	0.02

b. Prediction accuracy of adjusted means in single or across environment and in multi-environment using two sampling procedures (CV1, CV2), using four methods (GBLUP, GK, RKHS-EB, RKHS-KA)

		GBLUP									GK									RKHSEB									RKHSKA								
		FL			NI			PW			FL			NI			PW			FL			NI			PW			FL			NI			PW		
Target condition	Sampling procedure	APA		CL(1)	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL	APA		CL
Aerobic (A)	Single	0.71	±	0.01	0.47	±	0.01	0.63	±	0.01	0.74	±	0.01	0.48	±	0.01	0.63	±	0.01	0.73	±	0.01	0.49	±	0.01	0.63	±	0.01	0.74	±	0.01	0.48	±	0.01	0.63	±	0.01
	Across	0.66	±	0.02	0.45	±	0.02	0.52	±	0.02	0.70	±	0.02	0.45	±	0.02	0.53	±	0.02	0.69	±	0.02	0.45	±	0.02	0.52	±	0.02	0.70	±	0.02	0.45	±	0.02	0.53	±	0.02
	CV1	0.74	±	0.02	0.50	±	0.02	0.63	±	0.01	0.74	±	0.01	0.50	±	0.02	0.63	±	0.01	0.71	±	0.02	0.48	±	0.02	0.63	±	0.01	0.67	±	0.02	0.47	±	0.02	0.61	±	0.01
	CV2	0.91	±	0.01	0.56	±	0.02	0.75	±	0.01	0.79	±	0.01	0.47	±	0.02	0.67	±	0.01	0.82	±	0.01	0.52	±	0.02	0.68	±	0.01	0.88	±	0.01	0.55	±	0.02	0.73	±	0.01
Irrigated (I)	Single	0.66	±	0.01	0.57	±	0.01	0.59	±	0.01	0.70	±	0.01	0.55	±	0.01	0.62	±	0.01	0.69	±	0.01	0.56	±	0.01	0.61	±	0.01	0.70	±	0.01	0.55	±	0.01	0.62	±	0.01
	Across	0.70	±	0.02	0.44	±	0.02	0.53	±	0.02	0.73	±	0.01	0.46	±	0.02	0.55	±	0.02	0.72	±	0.01	0.47	±	0.02	0.54	±	0.02	0.73	±	0.01	0.46	±	0.02	0.55	±	0.02
	CV1	0.55	±	0.02	0.61	±	0.01	0.69	±	0.02	0.70	±	0.02	0.55	±	0.02	0.61	±	0.01	0.66	±	0.02	0.57	±	0.02	0.60	±	0.02	0.63	±	0.02	0.55	±	0.02	0.59	±	0.02
	CV2	0.87	±	0.01	0.61	±	0.02	0.72	±	0.01	0.77	±	0.01	0.53	±	0.02	0.64	±	0.01	0.78	±	0.01	0.54	±	0.02	0.66	±	0.01	0.85	±	0.01	0.57	±	0.02	0.70	±	0.01

(1)The confidence limits estimated from 100 samples of the 5-fold cross-validation

Supplemental Table 2: Accuracy of genomic prediction across populations for days to flowering (FL), nitrogen balance index (NI) and 100 panicles weight (PW), using four statistical methods (GBLUPGE, RKHSKAGE, RKHSEBGE, GKGE) and under 5 scenarios of relatedness between the training and the progeny set

a. Drought tolerance index and joint regression slope used as phenotypes

		GBLUP			GK			RKHS-EB			RKHS-KA		
Response variable	Scenario	FL	NI	PW	FL	NI	PW	FL	NI	PW	FL	NI	PW
Drought tolerance index (DTI)	S1	0.24	0.12	0.18	0.24	0.09	0.15	0.25	0.05	0.16	0.21	0.07	0.14
	S2	0.02	0.05	0.44	-0.06	0.12	0.46	-0.14	0.04	0.38	0.10	-0.08	0.50
	S3	0.28	0.15	0.23	0.23	0.11	0.26	0.24	0.11	0.28	0.19	0.11	0.24
	S4	0.24	0.15	0.19	0.20	0.10	0.26	0.18	0.09	0.25	0.17	0.10	0.27
	S5	0.07	0.02	0.30	-0.04	-0.03	0.40	0.02	0.01	0.34	0.00	-0.02	0.34
Joint regression slope (JR_slope)	S1	0.20	0.22	0.40	0.23	0.23	0.55	0.23	0.25	0.55	0.24	0.18	0.52
	S2	0.39	0.32	0.33	0.37	0.33	0.32	0.27	0.40	0.25	0.36	0.37	0.36
	S3	0.32	0.34	0.45	0.33	0.37	0.52	0.34	0.40	0.52	0.30	0.44	0.48
	S4	0.34	0.45	0.45	0.32	0.44	0.47	0.35	0.44	0.49	0.33	0.47	0.48
	S5	0.41	0.43	0.37	0.34	0.40	0.36	0.39	0.42	0.37	0.34	0.42	0.37

b. Calibrating with single or multi-environment (CV1) data and predicting of aerobic or irrigated condition

			GBLUP			GK			RKHS-EB			RKHS-KA		
Target condition	Sampling method	Scenario	FL	NI	PW	FL	NI	PW	FL	NI	PW	FL	NI	PW
Aerobic	CV1	S1	0.24	0.31	0.35	0.24	0.15	0.41	0.23	0.15	0.40	0.15	0.12	0.42
		S2	0.41	0.27	0.28	0.37	0.28	0.34	0.29	0.36	0.30	0.28	0.38	0.38
		S3	0.29	0.38	0.26	0.30	0.33	0.41	0.33	0.31	0.42	0.22	0.40	0.37
		S4	0.31	0.40	0.31	0.34	0.45	0.37	0.35	0.45	0.41	0.30	0.42	0.42
		S5	0.45	0.38	0.29	0.38	0.37	0.37	0.34	0.39	0.41	0.35	0.41	0.44
	single	S1	0.20	0.16	0.43	0.25	0.15	0.38	0.23	0.12	0.43	0.22	0.13	0.41
		S2	0.40	0.24	0.38	0.28	0.36	0.36	0.25	0.33	0.41	0.31	0.33	0.45
		S3	0.34	0.30	0.44	0.33	0.32	0.40	0.31	0.41	0.40	0.33	0.38	0.44
		S4	0.33	0.39	0.38	0.30	0.42	0.40	0.31	0.40	0.43	0.32	0.42	0.43
		S5	0.43	0.37	0.39	0.38	0.38	0.43	0.34	0.28	0.41	0.41	0.38	0.43
Irrigated	CV1	S1	0.16	0.15	0.42	0.19	0.13	0.43	0.18	0.10	0.45	0.21	0.13	0.48
		S2	0.40	0.24	0.35	0.26	0.22	0.31	0.35	0.31	0.24	0.31	0.34	0.37
		S3	0.23	0.31	0.39	0.28	0.31	0.43	0.32	0.26	0.48	0.28	0.35	0.47
		S4	0.27	0.38	0.41	0.28	0.42	0.40	0.33	0.38	0.46	0.31	0.38	0.48
		S5	0.40	0.39	0.39	0.31	0.34	0.38	0.38	0.31	0.37	0.34	0.35	0.42
	single	S1	0.21	0.24	0.41	0.23	0.13	0.51	0.22	0.22	0.50	0.21	0.05	0.53
		S2	0.33	0.32	0.29	0.25	0.32	0.32	0.24	0.29	0.35	0.27	0.34	0.36
		S3	0.28	0.33	0.43	0.37	0.35	0.50	0.33	0.40	0.48	0.34	0.39	0.51
		S4	0.33	0.43	0.40	0.33	0.41	0.48	0.30	0.44	0.49	0.32	0.42	0.46
		S5	0.36	0.33	0.32	0.38	0.32	0.42	0.34	0.31	0.39	0.32	0.33	0.42

Suplemmental Table 3 Scenario tested for the progeny validation

Scenario	Training set
S1	31 parents
S2	58 related accessions
S3	31 parents + 58 related accessions
S4	31 parents + 252 accessions
S5	252 accessions, excluding the parents

Chapter 4: Economic Performance of Traditional and Modern Rice Varieties under Different Water Management Systems

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4.1. Abstract

Italian rice production is progressively threatened by water scarcity. Some strategies have been developed to reduce water use. Nevertheless, reducing water irrigation amounts may lower paddy rice production. This publication compares the productivity and the economic performances of traditional and modern rice varieties in northern Italy using two different water management systems. The objective of this analysis is to enhance Italian rice cultivation at the economic, environmental and agronomic levels. Some positive variations of water productivity and economic water productivity were observed for the two varieties when using a lower amount of irrigation water. However, actual production costs and most water supply fees are the same for all the irrigation methods. Furthermore, the study of agronomic traits shows that during the recent years, there were no significant differences or increases of yield among varieties. Consequently, to be adopted by farmers, the irrigation costs coupled with improved rice accessions need to be optimized.

Keywords: rice cultivation; Italy; water saving; water productivity; economic water productivity.

4.2 Introduction

Worldwide, rice is one of the most important crops and it represents a staple food for over half of the world's population, with a global production of more than 700 million tons per year (World Wheat,Corn and Rice, FAOSTAT, 2015) and a harvested area reaching 165 million ha. In Europe, where *Japonica* rice is cultivated, Italy is the leading rice producer, with around 227,300 ha of rice-cultivated areas (Maclean *et al.,* 2002). Additionally, a trend of continuous increase of the rice cultivation surface was observed during the last 30 years; also the area per farm has increased, moving from 20.9 ha of rice per farm in 1983 to 53 ha in 2012, with an increase of 3% to 5% per year (Il bilancio dell'azienda risicola, 2013). Besides, rice cultivation is a high-water-consuming crop and irrigated rice is the most spread-out agrosystem. It represents 53% of worldwide rice-cultivated areas (Tuong and Bouman, 2003). A volume of 2.5 to 5.0 m^3 is needed to produce 1 kg of rice, whereas only 0.4–0.7 m^3 of water is needed for 1 kg of sorghum (Bouman *et al.,* 2009). However, a large amount of total water applied at the field-level is lost by evapotranspiration, seepage and percolation (Cabangon *et al.,* 2004).

Moreover, rice cultivation is threatened by climate change which represents the major challenges that irrigated agriculture all over the world will have to face. It is foreseen that by 2025, 15–20 million ha of rice lands will suffer from water scarcity. As summarized by (Olesen et al., 2012) hot-spots of water scarcity in rice-growing areas have been reported, and temperatures higher than the mean trend have been registered in many European countries. In Italy, the flow of the Po River, which provides water to an extensive network of artificial channels used for rice irrigation, decreased by 20%–25% in the last 30 years, passing from historical values of 1800 $m^3 \cdot s^{-1}$ to 1400–1500 $m^3 \cdot s^{-1}$ (Carrera *et al.,* 2013). This trend caused a reduction of water availability during the dry summers of 2003 and 2012. Therefore, the effects of climate change necessitate an optimization of the water use in irrigated rice areas. To address these problems, new rice cultivation practices are being experimented with worldwide. These approaches, called water-saving technologies, can help to reduce the water irrigation amount associated with traditional rice farming, especially owing to the reduction of water losses at the field level (Tabbal et al., 2002, Belder et al., 2004) and optimize the use of available water. For instance, operations connected to land preparation can help in reducing or regulating irrigation water in rice-fields (Tuong et al., 2003). Specifically, field channels help in controlling the water volume flowing in and out of a rice field; a well-leveled field is necessary for good circulation of the water and good crop emergence, while additional shallow soil tillage before land preparation, as well as saturated soil culture, can decrease seepage and percolation flows (Tuong et al., 2000). Therefore, different cultivation methods have been tested to evaluate the effect on rice productivity and on irrigation. The alternate wetting and drying (AWD) method can reduce irrigation by 15%–30% without any impact on yield (Lampayan *et al.,* 2014). This method consists of applying irrigation a few days after the disappearance of water. Hence, the field is alternately flooded and non-flooded. The number of days of non-flooded soil between irrigations can vary from one to more than 10 days, depending on a number of factors such as soil type, weather and crop growth stage. This method requires varieties selected for cultivation in conditions of reduced irrigation. Asian countries developed a panel of accessions adapted to different methods of alternate or reduced irrigation. In aerobic rice cultivation, varieties are grown under dry land conditions like wheat or maize. This method can reduce irrigation by 30% to 50% (Bouman *et al.,* 2005) Other advantages associated with reduced irrigation exist. It is known that under flooding conditions, there is a higher arsenic accumulation in rice grains compared to rice cultivated in conditions of alternate irrigation. This point is particularly important for areas with a Protected Geographical Indication such as the Verona area in Italy, where agricultural management practices are strongly prescribed. Furthermore, flooded rice produces a high level of greenhouses gases and the shift from permanent flooding to alternate irrigation can reduce CH_4 emissions. A single mid-season aeration can reduce the seasonal CH_4 emissions by 40%

However, the introduction of new cultivation methods requires an economic evaluation of production costs and net returns. It is known that Italian farms are affected by the fluctuation of rice prices. They varied from €186 to €489 per ton in the last 10 years, with many fluctuations between 2005 and 2015 (Ente Nazionale risi relazione annuale, 2016). At the same time, production costs follow a continuous increase (Figure 1).

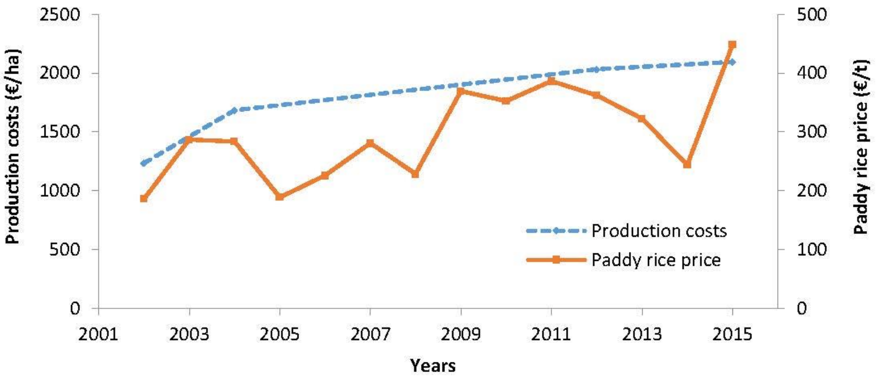


Figure 1. Trend of rice production costs (Il bilancio economico dell'azienda risicola, 2013) and paddy rice prices (Bouman *et al.*, 2005).

To find a more suitable solution for the Italian rice sector, it is necessary to evaluate the productivity and the economic efficiency of these strategies. The effectiveness of a production system can be assessed through the water productivity (WP), which is the ratio of the amount or value of product to the volume or value of water depleted or diverted. The study illustrated in (Cook *et al.*, 2017) compared the WP of flooded, aerobic and AWD conditions and observed an increase in the index when water management alternatives were applied because of a higher reduction of water inputs with respect to the yield reduction. Similarly, the WP of aerobic rice, higher than that of flooded rice (Tabbal *et al.*, 2002, Belder *et al.*, 2004), suggests that this agrosystem can be considered as an adapted solution to water scarcity. However, water productivity does not provide any information about the economic effects of decreased water use. Consequently, it is important to also consider the economic water productivity (EWP) (Pereira *et al.*, 2009, Blanco-Guttiérrez *et al.*, 2013), which defines the production value per unit of water used.

The objective of this study is to explore the effect of different water management methods in paddy rice fields in northern Italy by evaluating their agronomic productivity and economic performances. Field experiments were carried out using traditional and modern varieties under irrigated and alternately irrigated conditions.

4.3. Materials and Methods

4.3.1 Experimental Sites

The field experiments were carried out in two rice research centers in the western Po Valley: The Rice Research Center (RRC) of Ente Nazionale Risi at Castello d'Agogna (Pavia province, Lombardy region) and the Rice Research Unit (RRU) in Vercelli (Piedmont region), which belong to the Council for Agricultural Research and Economics.

4.3.2. Experimental Design and Treatments

The RRC carried out the experiments during four growing seasons (2011 to 2014), using a split-plot design with water management as main plot factor and variety as sub-plot factor (Miniotti *et al.,* 2016). Each water management modality was allocated in two plots, of size 20 m × 80 m each as described below:

1. Standard condition of rice cultivation (referred as standard): broadcasted rice is sown into the water, the field is then continuously flooded;
2. Irrigated condition (irrigated): rice is sown into dry soil, and the field is submerged at the three to four leaf stage;
3. Alternately irrigated condition: rice is sown in rows into dry soil. Irrigation is then applied intermittently, when soil water potential reaches the limit of −30 kPa at 10 cm depth at RRC and −30 kPa at 30 cm depth at RRU.

Four varieties (Baldo, Selenio, Gladio and Loto) were allocated in subplots of size 2.5 m × 10 m within each main plot. In the following, they will be referred as traditional as they were released in Italy in 1977, 1987, 1998 and 1998, respectively.

The RRU carried out four experiments during two growing seasons (2012 and 2013), in two water management modalities (irrigated and alternately irrigated). Each modality was replicated only once per season. Within one season, the two fields were divided in small plots of size 1.33 m² (1.9 m × 0.7 m) to evaluate a diversity panel of 284 varieties released from 1904 to 2012 (90 of which were Italian, including the four traditional varieties grown at RRC). All trials used a completely randomized design with three plots per variety.

4.3.3. Water Balance Monitoring

At the RRC experimental site, elements of water balance were continuously monitored by an integrated multi-sensor system (Chiaradia *et al.*, 2015, Cesari de Maria et al., 2016). The values obtained for the standard, irrigated and alternately irrigated conditions were respectively 2270 mm, 1760 mm, and 680 mm (Miniotti *et al.,* 2016). At the RRU site however, detailed measurements of circulating water volumes were lacking, thus we will use the values measured at the RRC site.

4.3.4. Phenotyping

At RRC, grain yield (tons ha^{-1}) was estimated on the basis of 14% moisture content. It was the only trait used for this site. At RRU, several traits were measured including yield, yield components (panicle number and 50-panicle weight), and other traits (height, earliness) and less correlated traits (grain format).

4.3.5. Water Productivity and Economic Water Productivity

Water productivity is the amount of grain produced for each volume of water used, which can be taken as evapotranspiration, irrigation, or irrigation and rainfall. For the purpose of this study, rainfall and irrigation are considered as the only water volume. Thereby, *WP* is defined as:

$$WP = \frac{Y}{TWU} \qquad (1)$$

WP is expressed in kg m^{-3}, *Y* is the yield (tons ha^{-1}), and *TWU* is the total water used (mm).

A high reduction of available water may affect crop productivity and reduce yield, with important consequences on farmers' incomes (Pereira *et al.*, 2009). Thus it is important to evaluate the economic impact of a reduction of irrigation water relative to the economic water productivity, *EWP* (€ m^{-3}) (Rodrigues *et al.*, 2003) defined as

$$EWP = \frac{HV}{TWU} \qquad (2)$$

where *HV* (€/ha) is the harvest value. A five-year mean (Granaria, 2016) was used to evaluate rice prices in order to reduce the impact of price volatility that characterizes the rice sector (Figure 1).

To go further on the economic analysis, it is possible to evaluate the Economic Water Productivity Ratio (*EWPR*) (Darouich *et al.*, 2012, Pereira *et al.*, 2012) where *IWC* (€) is the irrigation water costs

$$EWPR = \frac{HV}{IWC} \qquad (3)$$

4.4. Results

4.4.1. Agronomic Performances of Traditional and Modern Varieties Using Three Water Management Methods

First, Table 1 shows the results for yield, yield components and water productivity (WP) per site, varietal group and water management condition. In both sites, rice production was significantly reduced when using the alternately irrigated method. Yield differences between modern and traditional varieties were not significant for any condition. However, the groups differed in terms of height and yield components, especially when comparing the two largest groups (63 and 23 varieties). Thus, modern varieties were, on average, smaller and produced more panicles. The amount of water used in the alternately irrigated condition was more than two times lower than the amount used in the irrigated condition. Therefore, a higher productivity was observed in the alternately irrigated condition.

Table 1. Yield components and water productivity (WP) under three water management methods and three groups of Italian varieties in in the western Po Valley (Italy).

Site	Varietal Group	Water Management	Total Height (cm)	Panicles Number/m	50-Panicles Weight (g)	Yield (t ha^{-1})	WP (kg·m^{-3})
RRC	4 Traditional Varieties [1]	Standard	-	-	-	9.7 ± 0.3	0.43
		Irrigated	-	-	-	9.3 ± 0.4	0.53
		Alternately irrigated	-	-	-	7.6 ± 0.4	1.12
RRU	4 Traditional Varieties [1]	Irrigated	81.6 ± 4.6	92.9 ± 11.3	169.9 ± 17.6	11.8 ± 1.3	0.65
		Alternately irrigated	69.5 ± 4.6	87.4 ± 11.5	128.7 ± 17.9	8.0 ± 1.4	1.27
	63 other Traditional Varieties [2]	Irrigated	94.3 ± 1.2	85.3 ± 2.9	183.4 ± 4.5	11.7 ± 0.3	0.63
		Alternately irrigated	84.2 ± 1.2	80.3 ± 2.9	137.8 ± 4.5	8.1 ± 0.3	1.29
	23 Modern Varieties [3]	Irrigated	80.3 ± 1.9	91.2 ± 4.8	160.8 ± 7.4	11.1 ± 0.6	0.66
		Alternately irrigated	70.0 ± 1.9	92.5 ± 4.8	126.7 ± 7.5	8.8 ± 0.6	1.18

[1] Baldo, Gladio, Selenio, Loto; [2] Sixty-three varieties released from 1904 to 1998; [3] Twenty-three varieties released from 1999 to 2012.

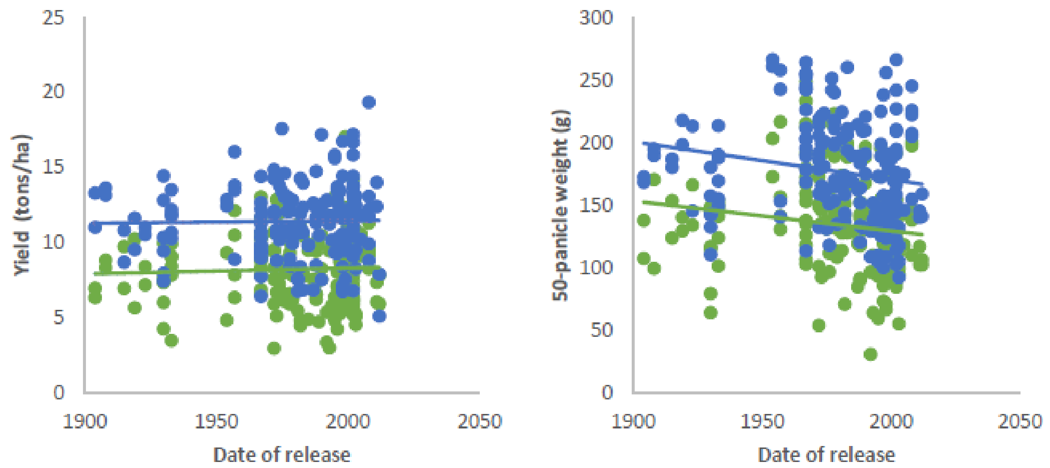
4.4.2. Evolution Trends of Italian Varieties Cultivated in Irrigated and Alternately Irrigated Conditions

Table 2 investigates further the evolution trend of Italian varieties over time. It shows a linear trend for all yield components. The most important information is that the trend seems to not differ between water management methods (slope of the same magnitude). However, the linear trend represented only a fraction of the phenotypic variation among varieties. Phenotypic variation between varieties as a whole was high, including for yield and in both water management methods (Figure 2).

Table 2. Evolution trend of Italian varieties over time evaluated at the Rice Research Unit in Vercelli (Piedmont region, Italy). Mean yield and mean yield components of 90 varieties are regressed on the respective date of release.

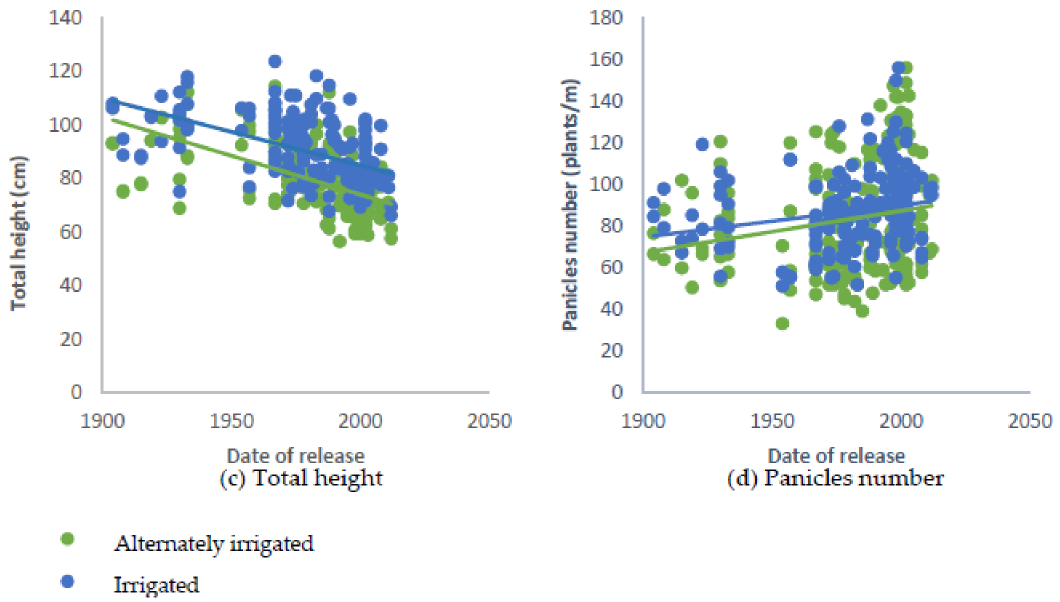
Trait	**Water Management Method**	**Regression Slope [1]**			**Unit**
Total height	Irrigated	−0.274 ***	±	0.030	cm/year
	Alternately irrigated	−0.289 ***	±	0.031	
Panicles number	Irrigated	0.224 **	±	0.066	panicles/year
	Alternately irrigated	0.200 **	±	0.067	
50-panicles weight	Irrigated	−0.383 **	±	0.114	g/year
	Alternately irrigated	−0.239 **	±	0.115	
Yield	Irrigated	0.005 ^{NS}	±	0.007	tons/year
	Alternately irrigated	0.004 ^{NS}	±	0.007	

[1] F test (*: significant at *p* = 0.05, **: significant at *p* = 0.01, ***: significant at *p* = 0.001, NS: not significant) and confidence interval.



(a) Yield

(b) 50 panicles weight



(c) Total height

(d) Panicles number

Figure 2. Distribution of varietal means (90 varieties) along the date of release for irrigated (**blue**) and alternately irrigated (**green**) conditions for (**a**) Yield, (**b**) 50 panicles weight, (**c**) Total height, (**d**) Panicles number.

4.4.3. Sources of Phenotypic Variation among Italian Varieties

The analysis of variance (Table 3) quantifies the amount of variance associated with varieties, water management methods, as well as their interaction. The model was based on varietal means within each trial (season × water management combination) and explained 45% to 95% of the total variation. Tests were constructed using the season effect as the residual. The total height and 50-panicle weight were known with high precision (high R^2 and low CV). Both main factors were very highly significant for all traits, except the number of panicles. Any interaction was detected, meaning that the varieties' ranking did not change from one water management method to another.

Table 3. Sources of phenotypic [1] variation for yield and yield components of 90 Italian varieties evaluated at the Rice Research Unit (Vercelli, Piedmont region, Italy).

	Total Height	**Panicles Number**	**50-Panicles Weight**	**Yield**
R^2	0.95	0.45	0.85	0.70
CV	4.86	28.4	15.4	22.7
Source of variation				
Variety	***	*	***	***
Water management	***	NS	***	***
Variety × water management	NS	NS	NS	NS

[1] Significance level of F test: *: significant at $p = 0.05$, ***: significant at $p = 0.001$, NS: not significant.

4.4.4. Economic Analysis of the Agrosystems

Table 4 shows the economic analysis of gain and production costs for each condition and each type of variety. We noticed that for the alternately irrigated condition, production cost (PC) and irrigation water cost (IWC) were higher for the alternately irrigated condition, due to the additional hours of work and herbicides linked to the alternately irrigated management. The harvest value and net incomes were higher for the irrigated conditions. The EWP was higher for the alternately irrigated condition whereas the EWPR was lower for the irrigated conditions.

Table 4. Economic balances of the varietal groups cultivated in three water management methods.

Site	Varietal Group	Water Management Condition	HV (€/ha)	PC (€/ha)	IWC (€/ha)	NI (€/ha)	EWP (€/m^{-3})	EWP R (-)
RRC	4 traditional varieties [1]	Standard	2 920	2 059	253	861	0.130	11.5
		Irrigated	2 799	2 064	293	735	0.160	9.6
		Alternately irrigated	2 288	2 114	343	174	0.340	6.7
RRU	4 traditional varieties [1]	Irrigated	3 251	2 064	293	1187	0.180	11.1
		Alternately irrigated	2 348	2 114	343	234	0.350	6.8
	63 other traditional varieties [2]	Irrigated	3 335	2 064	293	1271	0.197	11.8
		Alternately irrigated	2 645	2 114	343	531	0.357	7.08
	23 modern varieties [3]	Irrigated	3 483	2 064	293	1419	0.189	11.4
		Alternately irrigated	2 429	2 114	343	315	0.389	7.7

HV: harvested value; PC: production cost which includes IWC; IWC: irrigation water cost; NI: net income. [1] Baldo, Gladio, Selenio, Loto; [2] Sixty-three varieties released from 1904 to 1998; [3] Twenty-three varieties released from 1999 to 2012.

The economic analysis in Table 5 shows that the five most productive varieties in the alternately irrigated condition obtained a higher harvest value than all the varieties cultivated in the irrigated condition. Therefore, NI and EWP were also higher for these five varieties, and the EWPR was nearly the same for both conditions as the higher harvest value compensated the IWC value of the alternately irrigated condition.

Table 5. Economic balances of the 90 Italian varieties in irrigated conditions and the five most productive varieties in the alternately irrigated condition evaluated at the Rice Research Unit (Vercelli, Piedmont region, Italy).

Site	Varietal Group	Water Management Condition	HV (€/ha)	PC (€/ha)	IWC (€/ha)	NI (€/ha)	EWP (€/m^{-3})	EWP R (-)
RRU	90 Italians varieties	Irrigated	3409	2064	293	1345	0.193	11.63
	5 most productive Varieties in Alternately irrigated condition	Alternately irrigated	3980	2114	343	1866	0.600	11.6

4.5 Discussion

This study shows that moving from irrigated to alternately irrigated conditions increases the total production costs. We can also see that the varieties actually cultivated are not adapted to a situation of water scarcity.

First of all, the yield between the modern and traditional varietal groups did not differ significantly but the variation was higher within each group.

It varied only between the water management methods with a higher production for the irrigated conditions. However, a significant reduction of irrigation water was observed for the alternately irrigated condition, inducing higher water productivity. This is in accordance with the data of (Bouman et al., 2001), which reported water savings of 23% under AWD with a yield reduction of only 6%. In another study, (Devkota et al., 2013) showed that AWD induced a reduction of water input of 50%, with a consequent increase in the WP. In many Asian countries, agronomic practices for growing rice provide puddling before sowing with the objective of the disruption of its structure. These operations lead to greater compaction of the soil which results in a reduction of water losses by percolation, and therefore it leads to an increase in the efficiency of irrigation and WP. The situation is different in southern Europe, where puddling is not applied.

Calculation of the EWP shows that the alternately irrigated condition is the economically more efficient method because the water volume is sufficiently low to permit a cost-effective production. The calculation of EWPR shows a higher value for the irrigated conditions, suggesting that the production increase is high enough to cover the IWC. These results agree with the values of NI obtained.

We noticed differences in PC and IWC due to weeding interventions and the number of irrigation cycles associated with each management method. For the standard and irrigated conditions, the differences in field management are very low, as they differ only in the moment of the first field irrigation. Nevertheless, water supply fee set by the Water Use Association (WUA) of the study area depends on the irrigated area and not on the water volume; the water supply costs are thus the same for all methods, despite a large difference in the irrigation water volume used. In Italy, the watering contribution cost is independent from the water amount applied. It should be evaluated considering the size of the areas that have to be irrigated or the volume of water used, as each irrigation method requires a different volume of water, but this contribution depends on the water policies of each country. In the Ebro delta in Spain, the irrigation contribution is dependent on the quantity of water used (Garrido *et al.*, 2009). In this case, the reduction of irrigation water can also reduce the cost of rice production. In the case of northern Italy, the cost of the watering contribution should be adapted to each water management method.

In Italy, some farmers already practice rice cultivation under alternate irrigation, e.g., in Pavia [R] where other high-water-demanding crops are cultivated, such as maize, farmers alternate rice field irrigation. Water scarcity would also impact other sectors. Indeed, a part of the water managed by the Water Use Association (WUA) is used to supply hydroelectric stations and another part is used to produce potable water for the district towns. However, the actual yield level of varieties used in alternate irrigation does not reach the levels obtained in continuous irrigation conditions. To encourage farmers to use alternate irrigation, it is necessary to have adapted varieties, with yields equal to or higher than those of the traditional method. However, the two-season experiment carried out by the Rice Research Unit in Vercelli, based on a large diversity panel including 90 Italian varieties, did not allow us to highlight the specific adaptation to reduced irrigation. Furthermore, little is known about rice cultivation under alternate irrigation in Europe. Even with the increasing problem of climate change, water scarcity is not actually the main research subject and research activities are concentrated on other topics, such as rice diseases, e.g., infections by fungi (Titone *et al.*, 2015), or grain quality (Biselli *et al.*, 2015). In the panel of accessions studied here, the differences between varieties are significant. It was not possible to denote differences for yield when considering the mean production of the two main groups of varieties in each condition. However, some varieties can tolerate a situation of water scarcity. This was confirmed by the economic analysis of the most productive varieties in the alternately irrigated condition. Thus, these varieties can be exploited to produce a reasonable quantity of rice. This positive variability can also be exploited for rice breeding for adaptation to water scarcity.

4.6.Conclusions

The applicability of the different systems depends on many factors such as the availability of water, production costs, IWC and the varieties used. The genetic variability of these varieties has to be studied to breed for other adapted rice varieties that can produce the same quantity or more.

However, other factors may affect the the applicability of those systems. The irrigated system may lead to a competition of water availability with other crops such as maize during the irrigation period in June. Additionally, it would lead to a decrease in the recharge of the phreatic aquifer and therefore to the lowering of groundwater levels. As the availability of water depends on the groundwater depth, a conversion of flooded rice to alternate irrigated rice would result in lowering water savings. On the other hand, flooded rice cultivation can provide important ecosystem services such as the preservation of wetland habitats for a range of aquatic and semi-aquatic wildlife, or of the local traditional landscapes. Consequently, the applicability of these methods at a larger scale depends on the district of rice cultivation, and may be more profitable where rice is the monoculture.

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Abbreviations

The following abbreviations are used in this manuscript:

RRC	Rice research center
RRU	Rice research unit
WP	water productivity, kg·m^{-3}
EWP	economic water productivity, €·m^{-3}
Y	Yield, t.ha^{-1}
TWU	Total water use, mm
CV	Coefficient of variation
HV	Harvest Value, €·t^{-1}
PC	Production cost
IWC	Irrigation water cost, €·ha^{-1}
EWPR	Economic water productivity ratio, dimensionless
WUA	Water use association

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Chapter 5: Water Management Options for Rice Cultivation in a Temperate Area: A Multi-Objective Model to Explore Economic and Water saving Results

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5.1 Abstract:

Due to the changing climatic and environmental conditions, modifications in agricultural and water policies have been made, and irrigated agriculture has to face the challenge of making a rational and optimal use of the water resource effectively available. This urges rice farming, strongly and traditionally linked to water, to change the modalities for the use of the resource. If on one hand water saving techniques should be preferred, a different water management in paddy fields may lead to lower yields and higher production costs, with consequent repercussions on farm incomes. The paper recognizes the disagreement between environmental and economic concerns and aims at contributing to the discussion about how to reconcile them by adopting alternative irrigation strategies. From this perspective, a multi-objective linear optimization model is used to explore the trade-offs between conflicting objectives in a rice-growing area in Northern Italy. The model returns the optimal allocation of land subject to three different irrigation strategies, as those previously performed in experimental fields; in addition, a scenario analysis is run to simulate reduced resource availability. Results demonstrate the key role of prioritizing one objective over the other, while introducing cultivars more suitable for dry cultivation enables enlarging the frontier of optimal solutions.

Keywords: irrigation water; water saving; rice-cultivation; Italy; multi-objective optimization models; linear programming

5.2 Introduction

Rice is the second most important cereal and staple food in the world. Despite the fact that the majority comes from Asian countries, a significant extent of intended agricultural areas can also be found in the Mediterranean basin and in temperate Europe. Italy, with around 227,300 ha of rice land (Ente Nazionale Risi, 2015), is the European leading producer. In the western Po Valley the rice-growing district across Lombardy and Piedmont regions ensures 90% of the national production. Here, rice farming is currently facing manifold constraints (Ferrero, 2007) that limit the possibility in getting adequate productive and economic results.

The main critical issue affecting regional rice-farming concerns water availability. Even in irrigated agriculture, for which water resources have not been traditionally a limiting factor, we must recognize that things are changing. The effects of climate change (Fischler et al., 2007) are in fact strongly affecting the amount of water available for agriculture (Mancosu et al., 2015). Hot spots of water scarcity have been observed in many countries (Bouman et al., 2007) whilst in several European areas a further raise is expected in the next decades (Strosser et al., 2012). According to APAT, the level of the Po River, which water are diverted to irrigate the largest part of Italian rice-fields, has fallen by 20%–25% since the last 30 years, whilst a further lengthening of the dry season and the increase of water stress are expected as well (Coppola et al., 2014). At the same time, a different distribution of available water over spring and summer may not always meet crop water requirements (Bischetti et al., 2014). The competition for the resource amongst different sectors (Elliot et al., 2014) and purposes (Garcia de Jalon et al., 2014) thus calls for integrating water requirements with availability.

On the other hand, the amount of water supplied tends to proportionally affect agricultural yields (Maeda et al., 2011), with consequent repercussions on revenues and economic results of rice-growing farms, especially if small-sized (Blanco-Gutierrez et al., 2013). Added to this, are the production costs of rice in Western Europe, which are generally much higher than both in most Asian countries and in the USA. ; following the marked increase in the price of fossil energy, in recent years they have undergone a further significant augmentation. As a further concern, rice-cultivation, being traditionally and strongly linked to water resource, is profoundly related to water policy. In first instance, water supply cost is a key element affecting total production costs. In general terms, irrigation water tariffs are much lower than those regulatory framework calls for (e.g., the Water Framework Directive); it is then reasonable that in an increase in the cost of water supply shall occur. Thereafter, farmers could be in the condition of managing less water at a higher cost, with possible negative consequences on their own incomes.

In addition, rice farming may be further threatened by different regime of CAP payments following the latest CAP reform (CAP 2014). A higher uncertainty in the amount of financial supports drives rice growers adopting strategies to exploit higher margins and counteract possible diminutions in subsidies.

These changed conditions are pushing the rice sector to seek new cultivation modalities and systems, which make a more rational and sustainable use of the resource. A large variety of options can be undertaken at the farm-level to (i) counteract water scarcity; (ii) adapt to the effective water availability and (iii) get adequate productive and economic returns. From this perspective, the issue of water saving is being paid much attention. In irrigated agriculture the manifold solutions that can be adopted range from a crop mix change, to the cultivation of less water-demanding crops, the reduction of irrigated farmland (Cortignani et al., 2010), the use of additional water sources (Dono et al., 2007, 2010) to mitigate shortages from collective supply, the adoption of different irrigation systems (Rodrigues et al., 2013). In rice farming, the water problem is particularly addressed through either the cultivation of new rice varieties with improved traits, or the adoption of more efficient water management strategies. In the former case, valid options include the amelioration of crops (Clément et al., 2013) with selected varieties more resistant to water stress or more suitable for discontinuous irrigation, the adoption of hybrid rice cultivation (Tesio et al., 2014), the introduction of short-cycle and high yielding cultivars. On the other hand, alternative water management options plays a central role. In European countries, more often, rice-fields are submerged immediately after tillage operations, seeds broadcasted in flooded fields and the crop maintained continuously submerged; this conventional technique makes water requirements of rice far higher than any other cereal (Tuong et al., 2005). Different irrigation systems, e.g., sprinkler (Lopez-Pineiro et al., 2016,), drip (He et al., 2010) or flush irrigation (Cesari de Maria et al., 2016) are opportunities to lower the massive amounts of water associated with traditional rice farming, thanks to higher irrigation efficiency. Water saving cultures for rice also include the possibility to make a different use of water, which contributes in reducing outflows (i.e., leakages and percolation). Such techniques have been spreading worldwide and in temperate rice systems as well. In the Po Valley, both site-specific conditions and water availability levels determine multiple versions of alternative irrigation strategies (Bischetti et al., 2014). In general terms, two are the main typologies that can be distinguished, namely (i) dry seeding and delayed flooding, which implies that rice is planted in dry soil and generally managed as a dry crop until the tillering stage; after then, the ponding water depth is maintained until the final drying, except for drying periods needed to apply fertilizers; and (ii) dry seeding and intermittent irrigation, i.e., aerobic rice (Nie et al., 2012)). It is a particular form of the previous strategy, operated especially in areas close to urban settlements and in presence of water scarcity. Rice is compared to other irrigated cereals, typically maize, with border irrigation interventions practiced every 7–15 days.

If on one hand, such strategies are recognized as water saving techniques, scarce direct indications about their economic implications are provided through out dedicated literature. The adoption of such alternative irrigation options is likely coupled with reduced productive performances (Dunn et al., 2011) and to additional labor costs (Bischetti et al., 2014, Biogesteca, 2014), given the enhanced presence of weeds (De Vries et al., 2014). More research is needed; this can provide sets of data to be used for quantifying the economics of rice production, to finally quantify the trade-offs between economic and environmental concerns.

5.2.1 Addressing Conflicting Objectives in Irrigated Agriculture: A Brief Review

Limited resources are more often to be managed in presence of conflicting objectives. Few are, however, the studies concerning this topic in irrigated agriculture. Despite this, they mostly recognize a disagreement between water saving and economic results, as evident by methodological approaches adopted to rank the most feasible alternatives or select their best allocations.

A first approach refers to multi-criteria analysis. It is particularly useful when participatory processes are carried out, with quantitative and qualitative information, as long as users' preferences are to be taken into account. For each proposed alternative, the method allows the calculation of a global utility value, which represents the integrative score based on the prioritization schemes related to one of the objectives or a balanced situation between them.

The methodology was performed to compare different irrigation systems scenarios for a same crop, namely maize in a Mediterranean area (Rodrigues et al., 2013) and cotton in a semi-arid region (Darouich et al., 2012; 2014), taking into account water saving and economic benefits as the main concerns. In the work of García de Jalon et al. 2014, MCA served for evaluating adaptation measures to water scarcity in an area of southern Spain, where rice farming and protected wetlands are closely linked. In this case, authors input results from MCA to an optimization model, aimed at maximizing global utility and land-use diversification, recognized this latter as a relevant adaptation strategy.

Multi-objective problems are well suited to address trade-offs and synergies between conflicting objectives (Groot et al., 2012). In applying them to irrigated agriculture, the best allocation of water and land resources is the main goal pursued. From this perspective, Latinopoulos et al., 2009 formulated a multi-criteria decision-making model for irrigated agriculture in a rural area of Greece. Based on multi-objective programming, it was aimed at simultaneously optimizing five objectives, related to the socio-economic and environmental spheres. A further optimization was implemented to allocate irrigation water in a Japanese rice paddy area with water saving practices (Maeda et al., 2011). The authors applied it to a hypothetical irrigation network considered representative for the region; objectives functions defined referred to the increase of profit (i.e., maximize total yield and water-saving costs) and water saving (i.e., maximize equity of water allocation and safety of water supply). A very similar model was proposed for a Chinese rice-growing area (Zhang et al., 2007). Still considering production and saving of agricultural water, it was applied to a hypothetical irrigation system to investigate how to allocate irrigation water to paddy fields in the district. Conversely to the other models previously mentioned, it emerged the stochastic nature of the model. Irrigation scenarios were also assumed considering the uncertainty of hydrologic and hyraulic factors.

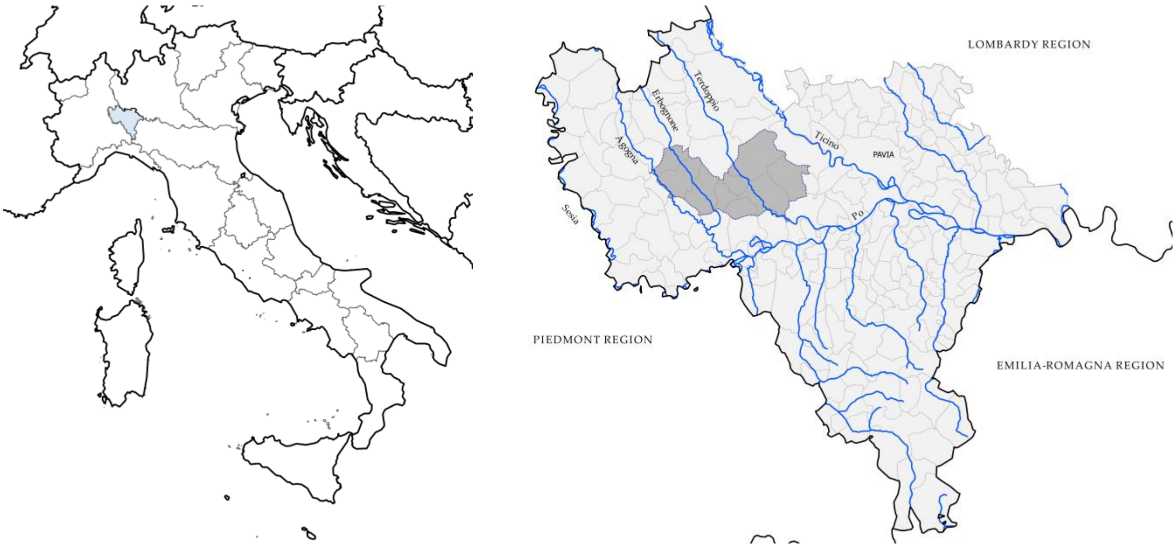
These kinds of investigations into rice farming in temperate areas are instead still underdeveloped. Up to now, based on our knowledge, the only evidence of a multi-objective problem applied to rice is included in Bartolini et al., 2007, who developed a multi-attribute linear programming model to simulate the impacts on Italian irrigated farms of modifications in water and agricultural policy.

To fill this gap, the paper introduces a deterministic multi-objective optimization model, based on real data obtained from field experiments. The model is applied to a homogeneous area in the rice-growing district of Northern Italy, and aims at investigating how competing objectives can be reconciled by managing irrigation water. Economic and environmental returns, i.e., gross margins and water saving, are included in a linear programming problem and simultaneously maximized under different levels of water availability, which in turn simulate water use conflicts and climate change repercussions.

5.3 The Study Area

Programming models for water allocation in agricultural production mostly operate at the regional scale. This allows exploring the optimal solutions from a policy-makers perspective, especially when sufficient homogeneity is observed across the region and it can be considered as a large farm (Graveline, 2016). Each study area should be large enough to contain a significant number of farms, while the focus on an "irrigation unit" would avoid introducing sources of variation related either to agro-climatic or economic conditions (Berbel and Rodrigues-Ocana, 1998).

On these bases, a homogeneous rice-cultivated area has been circumscribed within the Pavia province, Lombardy region (Figure 1a), as the largest part of the rice-cultivated district in Northern Italy.



(**a**) (**b**)

Figure 1. Location of the study area: (**a**) Pavia province, Lombardy region; (**b**) the study area.

With more than 80,000 ha in 2015 (Database, Ente nazionale risi), the province is the first area in Italy for rice production, though other irrigated crops are also present, from maize to permanent grasslands and short rotation forestry (i.e., poplar groves). This combination in the production pattern may determine conflict in the use of water resource (Cesari de Maria, 2016), given the specific water demand during the cropping season and the weather-climate trend; despite this, the largest part of irrigation water is here required by rice, since it is the most spread irrigated crop. In this sense, interventions targeted to this sector would have strongly repercussions on regional agriculture as a whole.

Water administration is assigned to the Water User Association *Associazione Irrigazione Est Sesia*, which annually supplies water to 161,880 ha of farmland in the period April-September. It operates at a smaller scale through its Local Units that manage a collective and consolidated irrigation network system made up of rivers, primary and secondary channels. Once supplied with water, rice-growers may decide to make a different use of it, based on to their actual needs; thus, alternative strategies for water management in paddy fields are already practiced and have been progressively spreading: since the last decade, the conventional cultivation technique, namely water seeding and continuous flooding, has been observing a reduction (−38%) in favor of dry-seeded drill-sown rice (+53%), which reached more than 50,000 ha in 2015 (Database, Ente nazionale risi).

In this wider context, the study area is a portion of territory within the same irrigation unit, still pertaining to the aforementioned WUA. The focus on such a local scale better allows assessing the sustainable use of water, as suggested by Massarutto et al., 2003. The area encompasses nine contiguous municipalities west to Pavia city (Figure 1b), with a total land size of 189 km^2. A strong orientation to agriculture characterizes the area, with the 0.59 hectares of utilizable agricultural area available per capita mainly intended for permanent grassland and arable crops (Figure 2). In this irrigated farming system, a large number of farms with a similar productive specialization are mixed with others not considered in the analysis. In particular, rice cultivation, which involves 270 farms, prevails over other agricultural land-based activities: with an incidence on arable land at municipal-level ranging from 64% to 90%, it counts for total 10,207 ha (75.4% of arable land) [41].

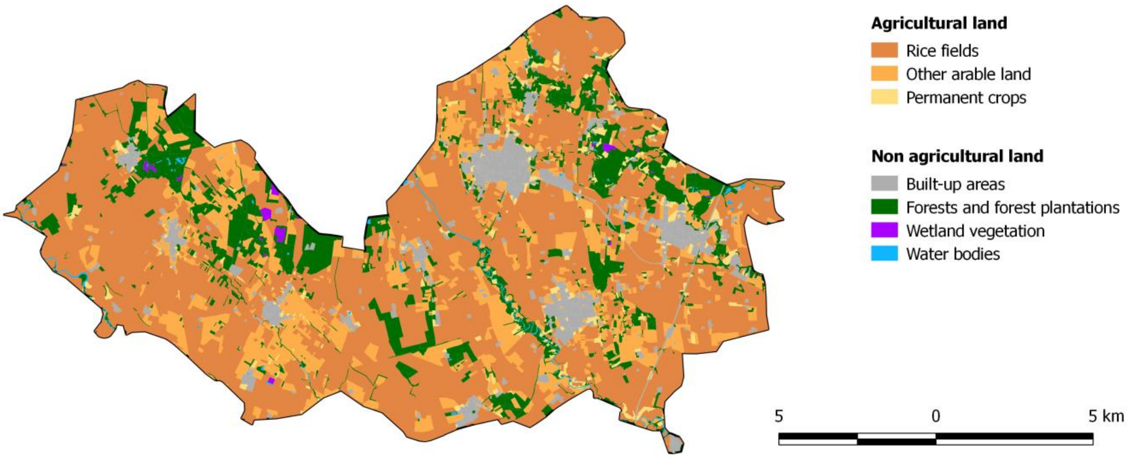


Figure 2. Land use in the selected area (source: own elaboration on DUSAF) .

Rice cultivation is mainly performed under continuous submergence, with a ponded water depth maintained for most of the growing season. A minority of paddy fields are subject to border irrigation, especially whenever water is delivered on rotation of 7 or 15 days (Bischetti et al., 2014, Sali et al., 2014).

5.4 Water Management Options

As pointed out by Graveline et al., 2016, the regional-scale modeling permits the reduction of data needed and ensures the quality of data employed. At the same time, field-level water management strategies play an important role as basic elements for water use efficiency

(Lilienfield et al., 2007). From this perspective, water management options considered in the analysis and their respective irrigation amounts, specifically refer to field experiments performed at the Rice Research Centre of Ente Nazionale Risi at Castello d'Agogna (Pavia province), over the cropping seasons from 2011 to 2014. The experiment site is nearby the study area, and this makes sure that their own results—imputed to the model as described in the following paragraphs—may find practical adoption.

Treatments carried out concerned cultivation practices differing for sowing modality, water management and irrigation method (Bischetti et al., 2014,Giorgi et al., 2008):

- Traditional method (FLD). Rice is sown directly into the water that submerges fields immediately after tillage operations, typically in April. Water is maintained on the field for the whole crop cycle, except for brief periods when treatments with herbicides or fertilizations are operated;
- Semi-traditional cultivation (DFL), which implies the dry seeding of drill-sown rice and the delayed flooding of paddy fields at the 3-leaf stage. The complete submersion of fields is completed approximately in late May-early June, then water management is similar to the previously described condition;
- Dry cultivation, or aerobic rice (IRR). Rice is sown into dry soil before the first irrigation intervention, without any flooding taking place; rather, indeed, the field is irrigated intermittently, typically by border irrigation. The terrain modeling carried out over the decades led to the creation of large and horizontal sections; water management thus likely consists in short submersions, which may last few days, alternate to longer dry periods.

Their respective irrigation amounts, as measured and monitored at field scale (Chiaradia et al., 2015; Miniotti et al., 2016), are shown in Table 1. The irrigation volume applied actually consists in providing more water than the amount needed, which means draining the excess (Cesari de Maria et al., 2016, Watanabe et al., 1992). Such water outflow from the field should not be however considered a loss; rather, water surplus is reused in either downstream or adjacent fields, and thus contributes in their irrigation, either by flooding irrigation or not. This way, water is re-circulated within a closed system, such as that managed at district- or irrigation area-level. In addition, because of this, more reasonable water volumes correspond to the net irrigation amounts, as the difference between irrigation inflows and outflows discharges. In the second instance, the amounts of water saved have been calculated for both the alternative management strategies, namely DFL and IRR, with respect to the conventional flooded condition (FLD).

Table 1. Irrigation and water saving amounts of different water regimes. Both the elements are expressed in mm.

Water Management	Net Irrigation	Water Saved
FLD	2275	–
DFL	1760	5150
IRR	680	15,950

To each irrigation modality, the respective productive results have been associated, by quantifying both total revenues and production costs and combining them into the gross margin. More often, crop yield enters predictive and simulation models by means of a crop-water production function; it thus derives that the effect of water saving (if any) can be explicitly expressed in comparison with farmers' net revenue achieved with that yield. Thus, the revenue of each water management combines the respective obtained yield (metric tons/ha) with the post-harvest farm-gate price (€/t) of paddy rice (Granaria, 1997).

Two different sets of yields for each irrigation strategy have been included in the analysis (Table 2). The former refers to the average yields of a panel of four rice varieties (i.e., *Baldo, Gladio, Loto* and *Selenio* cultivars) representative of the main grain types, measured in the time span 2011–2014 at the aforementioned experiment fields (Darouich et al., 2014, Miniotti et al., 2016). As Borrell et al., 1997 observed in a semi-arid tropical environment, irrigation water strongly influences the yield of rice. Similarly, in the study area, though a temperate rice-system, various irrigation practices allowed obtaining grain yields significantly different, with productions of submerged conditions higher than periodic irrigations (Miniotti et al.,2016). This latter technique is in fact associated with a lower production potential, due to the greater environmental stresses the crop is subject to.

In addition, it is also considered the possibility of introducing for DFL and IRR innovative cultivars more suitable for dry cultivation. In this regard, the respective yield refers to the average yield of the top-5 most productive breeding, selected by CREA amongst 100 experimental varieties specifically intended for semi-aerobic and aerobic conditions: a higher productivity for the DFL condition is evident, whereas grain production in IRR is quite similar to traditional cultivars.

Table 2. Average yield of the three water management options, for traditional and innovative cultivars. Terms expressed in metric tons/ha.

Water Management	Traditional cv.	Innovative cv.
FLD	9.6	–
DFL	9.3	10.8
IRR	7.7	7.8

An array of direct costs related to rice cultivation, as included in Camera di Commercio di Vercelli (Camera di commercio di Vercelli, 2013), has served as the basis to quantify rice production costs under different water management options. Starting from elaboration of data provided, production costs for FLD have been found to be 1998 €/ha; this is consistent with quantifications that demonstrated they vary between 1470–1500 €/ha (Gioia et al., 2016, Regione Lombardia. Programma di sviluppo Rurale 2007-2013) and 2430 €/ha (Castellani et al., 2007). Total production costs have been explicitly derived for the other water management practices as well, resulting in 1975 and 2052 €/ha for DFL and IRR respectively, which mean +1.2% and −2.7% if compared to FLD. Differences amongst treatments are not to be found in the cost of water supply, since the WUA applies a water tariff based on the extent of irrigated farmland and not on the actual volumes delivered to farms. Production costs rather primarily depend on the expenses related to water management. The various options, in fact, provide that a different number of watering operations occur during the cropping season. These interventions are coupled with labor related to the control and the regulation of the operations themselves. Thus, the adoption of DFL enables savings in labor needed to water management; in contrast, the more irrigation interventions, the higher water management costs, as evident especially in the IRR condition. In addition, as the result of water practice and especially in aerobic conditions, different agronomic and weed control operations may be necessary. In this case, they affect total production costs in terms of labor, mechanization, energy (i.e., for consumables) and other technical factors utilized, namely chemical fertilizers and herbicides.

Finally, the gross margin (€/ha), used as a proxy for farmers' income, has been calculated accordingly, as the difference between revenues and production costs.

5.5. The Methodological Approach

5.5.1. Multi-Objective Optimization

When optimal decisions need to be taken in the presence of trade-offs between conflicting objectives, the adoption of a multi-criteria approach is encouraged. From this perspective, the final choice represents a compromise between different objectives (Romero et al., 2003). In the wider context of decision modeling, mathematical programming assumes the role of a privileged instrument for providing general solutions to such complex problems, by formulating a multi-objective design problem.

Different techniques can be used to estimate all the feasible solutions, i.e., the optimal levels of resources allocation, for a certain number of alternative scenarios. From this perspective, linear programming-based models have been widely adopted in managing irrigated agriculture concerns, thanks to their easy formulation and use (Hallaji et al., 1996, Singh et al., 2015). Multi-objective linear programming implies that both the objectives and the constraints they are subject to should be mathematically expressed in linear terms. In particular, each objective function $J_{\mathrm{i}}(x)$ is a function of the decision variable x_{n} to be optimized

$$\text{Max (or Min)} \begin{bmatrix} J_1\,(x) \\ J_2\,(x) \\ \vdots \\ J_{\mathrm{i}}\,(x) \end{bmatrix} = \begin{bmatrix} r_{11} & r_{21} & \cdots & r_{\mathrm{n}1} \\ r_{12} & r_{22} & \cdots & r_{\mathrm{n}2} \\ \vdots & \vdots & \cdots & \vdots \\ r_{1\mathrm{i}} & r_{2\mathrm{i}} & \cdots & r_{\mathrm{ni}} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_{\mathrm{n}} \end{bmatrix} \tag{1}$$

where $J_1(x), \ldots, J_{\mathrm{i}}(x)$ are the objective functions that are simultaneously maximized or minimized, n the number of decision variables and r_{ni} their known coefficients. Decision variables, once adjusted, allow defining different efficient alternative configurations of the system under analysis. They may be subject to the non-negativity condition

$$\begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_{\mathrm{n}} \end{bmatrix} \geq 0 \tag{2}$$

whereas the multi-objective problem (1) is subject to a set of linear constraints

$$\begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1\mathrm{n}} \\ a_{21} & a_{22} & \cdots & a_{2\mathrm{n}} \\ \vdots & \vdots & \vdots & \vdots \\ a_{\mathrm{m}1} & a_{\mathrm{m}2} & \cdots & a_{\mathrm{mn}} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_{\mathrm{n}} \end{bmatrix} \leq \geq \begin{bmatrix} q_1 \\ q_2 \\ \vdots \\ q_{\mathrm{m}} \end{bmatrix} \tag{3}$$

with a_{mn} the known coefficients of decisional variables and q_{m} the upper or lower limits of the constraints.

5.5.2 The Optimization Model

Ensuring the profitability of rice-cultivation and water saving are prime objectives in this study. Based on linear programming, a multi-objective problem has been implemented accordingly. It concerns the optimal allocation of rice land (xrice) amongst different water management options, performed under progressive reductions in the amount of irrigation water (i.e., the *s* scenarios) supplied by the WUA. Irrigation water thus enters the model both directly, through available water amounts, and indirectly, by means of net water volumes distributed through each *m* irrigation practice.

The implemented model was aimed at exploring the trade-offs between economic and environmental objectives in each scenario *s;* in addition, the effects of a possible introduction of cultivars selected for dry-cultivation have been estimated. More in detail, the first objective function maximizes total regional income (€), as related to gross margins

$$J_{1,\mathrm{s}} = \sum_{\mathrm{m}} \mathrm{GM}_{\mathrm{m,s}} \times x\mathrm{rice}_{\mathrm{m,s}} \tag{4}$$

while the second concerns the maximization of irrigation water saved in the area (m^3)

$$J_{2,\mathrm{s}} = \sum_{\mathrm{m}} \mathrm{WS}_{\mathrm{m,s}} \times x\mathrm{rice}_{\mathrm{m,s}} \tag{5}$$

In order to avoid any scale dependency due to different dimensions of the two objective functions, they have been normalized into the [0–1] interval by using the respective maximum achievable values as the normalization constants. Equations (4) and (5) become

$$J^*_{1,\mathrm{s}} = \frac{\sum_{\mathrm{m}} \mathrm{GM}_{\mathrm{m,s}} \times x\mathrm{rice}_{\mathrm{m,s}}}{\max \sum_{\mathrm{m,0}} \mathrm{GM}_{\mathrm{m,0}}} \tag{6}$$

and

$$J^*_{2,\mathrm{s}} = \frac{\sum_{\mathrm{m}} \mathrm{WS}_{\mathrm{m,s}} \times x\mathrm{rice}_{\mathrm{m,s}}}{\max \sum_{\mathrm{m,0}} \mathrm{WS}_{\mathrm{m,0}}} \tag{7}$$

Therefore, the values of the individual objective functions have been assumed to vary within the predefined ranges and express the percentage of achievement of their respective maximum levels.

In order to solve the multi-objective problem, the weighting method has been adopted. It consists in assigning a weight $w_{\mathrm{i,s}}$ to each $J^*_{\mathrm{i,s}}$, as the expression of the relative importance of the various objectives, and can be solved for various sets of weights. The function of the optimization model is then expressed in the form

$$\mathrm{Max} \sum_{\mathrm{i}} w_{\mathrm{i,s}} J^*_{\mathrm{i,s}}(x) = w_{1,\mathrm{s}} \times J^*_{1,\mathrm{s}}(x) + w_{2,\mathrm{s}} \times J^*_{2,\mathrm{s}}(x) \tag{8}$$

The weights $\mathrm{w}_{\mathrm{i,s}}$ relating to each J^*_{i}, are assumed to vary within the [0–1] interval

$$\sum_{\mathrm{i}} w_{\mathrm{i,s}} = 1, \tag{9}$$

with 0 for the more adverse and 1 for the most advantageous result, respectively.

The number of alternative problems the method should solve is equal to k^{i-1}, where k is the number of values given to the weights and i the number of the objective functions included in the model. Different sets of weights have been adopted to assign priorities to economic result, water saving or a balance between them. In particular, all the possible weighting schemes included in the defined range have been initially used for the computations, to reflect the effects of objective prioritization on the final resolution and depict accordingly the trend of the multi-objective function.

The model is subject to the non-negativity condition of decision variable:

$$x\mathrm{rice}_{\mathrm{m,s}} \geq 0, \forall\, m, s \tag{10}$$

and further two different constraints, regarding the availability of land and water resource.

Firstly, the land balance ensures that total rice-growing area may either decrease or increase, up to, in this latter case, cover all the available arable land UAA_arable

$$\sum_{\mathrm{m}} x\mathrm{rice}_{\mathrm{m,s}} \leq \mathrm{UAA\_arable}\,, \forall\, m, s \tag{11}$$

Secondly, the availability of water is run in all the scenarios under the condition that net irrigation volumes NI (m^3/ha) should not exceed the q (m^3/ha) seasonal amount of water supplied by the WUA:

$$\sum_{\mathrm{m}} (x\mathrm{rice}_{\mathrm{m,s}} \times NI_{\mathrm{m}}) \leq q \times \mathrm{UAA\_arable}\,, \forall\, m, s \tag{12}$$

5.6. Results

5.6.1. Optimization of Current Situation

The optimization of the original configuration under the current level of water availability (q = 3.6 L/s·ha^{-1}), reveals how the objective function varies according to the set of weights assigned to the final formulation (Figure 3).

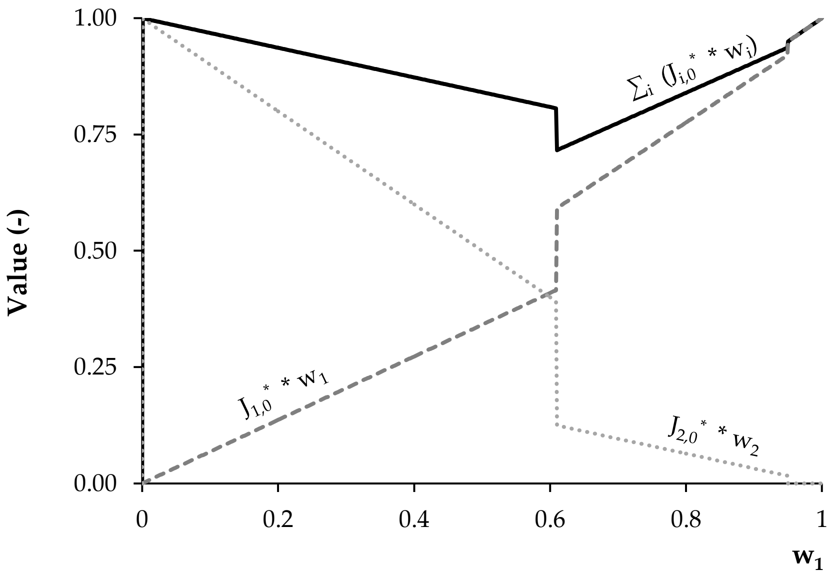


Figure 3. Performance of the two-objectives function and its components in the current situation.

The maximum value of the objective function is observed whenever the optimization is run with w_1, or alternatively w_2, equal to 0, which means reducing it to a one-objective problem. In these cases, the respective components J_i achieve their maximum (minimum) possible value. Given the aforementioned behavior, it derives that with all the other weights sets, both the J_i do not satisfy such a condition; rather, indeed, they show an opposite and complementary trend, which is reversed for w_1 close to 0.60. Before this benchmark, the optimal value of water saving (i.e., J_2) prevails over economic returns; conversely, a higher contribution in the final value of the objective function itself is due to the economic aspect. Over this inflection point the objective function further decreases up to get to its lowest value (0.75) for w_1 = 0.61. A marked change in the performances of individual J_i then occurs, as well as for objective function as a whole. This latter follows an ascending trend and in correspondence of w_1 = 0.95 another inflection point is evident, with the function reaching the upper limit.

This behavior is the basic element that determines the optimal allocation of land amongst different irrigation practices (Figure 4). On one hand, given data input and the constraints imposed to the model, the maximization of water saving (w_1 = 0) would suggest not to practice irrigated agriculture. However, to avoid gross margin not to be generated ($w_1 > 0$), the area intended for rice increases by 33% if compared to the current extent, up to cover all the available arable land.

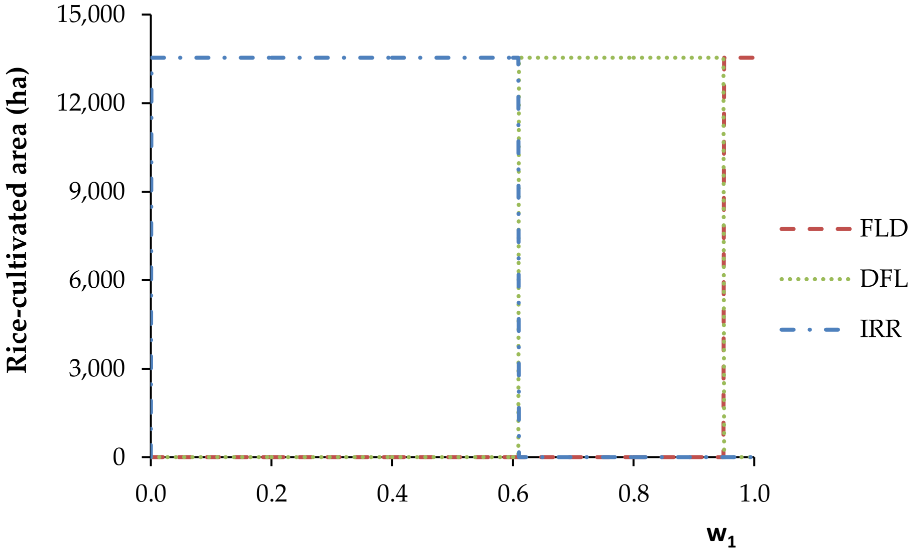


Figure 4. Rice-cultivated area under different irrigation practices.

From this perspective, any coexistence between either different agronomic managements or irrigation practices is favored. These latter are rather sequentially returned starting from the benchmarks previously identified, namely $w_1 \in \{0, 0.61, 0.95, 1\}$, which determine different system configurations.

Dry-seeded rice (DFL and IRR) can benefit from both water saving and economic return, being preferred when w_1 ranges up to 0.95. More in detail, aerobic rice and flush irrigation (IRR) is encouraged for most of the possible weights combinations, with particular regard to prioritization of water saving, but also if it slightly prevails over economic results ($0.5 \leq w_1 \leq 0.6$): this is the strategy a balance between the two conflicting objectives corresponds to.

The prevalent role of maximizing gross margins suggests adopting flooding irrigation, possibly delayed, which ensures highest revenues and lower production costs. In the case of FLD, given that no water is saved and the variation in gross margin over DFL is scarce (+74 €/ha) (Figure 5), it is preferred only if to the economic component is given much more importance (w_1 close to 1).

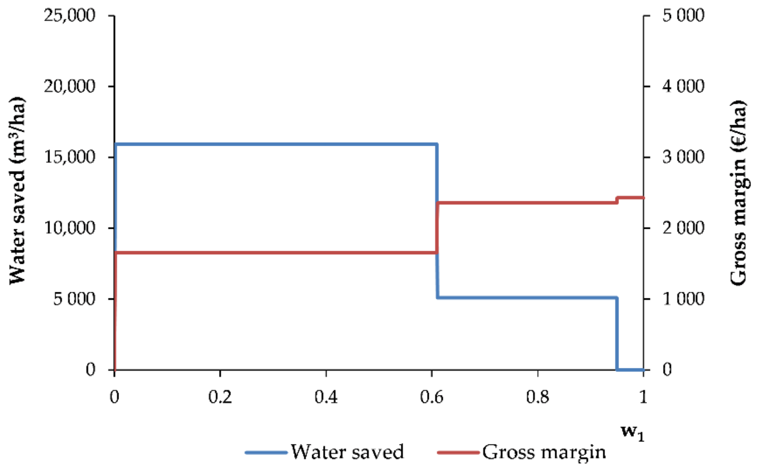


Figure 5. Water saved and gross margin with different sets of weights.

Still ensuring the same amount of water saved, the introduction of more productive cultivars increases the regional gross margin. Such a possibility is however limited to dry and semi-dry cultivations. Introducing the innovative cultivars specifically selected would enhance economic results especially because of a higher yield, rather than different production costs. From this perspective, gross margins undergo an augmentation by 16% in the DFL condition, whilst by only 1.3% in IRR (Figure 6). On the other hand, no additional gross margins can be obtained with the maintenance of the traditional flooded condition. In this case, higher profits should derive from a different exploitation of available resources.

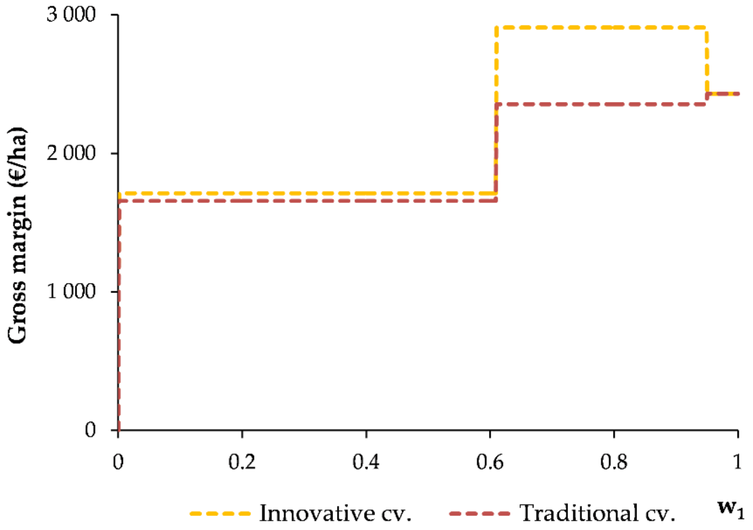


Figure 6. Trend of gross margins following the introduction of innovative cultivars.

To provide indications about the response of the economic performance under a real (if any) water saving, the trade-offs between the two targets have been quantified (Table 3). They express the additional gross margin following a unit variation in the water saved, compared to the FLD condition. This indication once again demonstrates how the economic and the environmental aspects are conflicting one another, given their opposite trends. Both DFL and fIRR are in fact coupled with a loss of 0.01 and 0.05 €/m^3 of water saved, respectively. This denotes productions to be unprofitable at the given prices and irrigation levels, with total losses quantified in 52 € and 798 €. Following the adoption of innovative cultivars, their respective trade-offs shift to positive in the case of DFL (+0.09 €/m^3) and increase to −0.04 €/m^3 for intermittent irrigations.

Table 3. Trade-offs between conflicting objectives per alternative water management and typology of rice variety.

Water Management	Trade-off (Traditional cv.) (€/m^3) (€)	Trade-off (Innovative cv.) (€/m^3) (€)
DFL	−0.01 (−52)	+0.09 (+464)
IRR	−0.05 (−798)	−0.04 (−638)

5.6.2. Scenario Analysis

Solutions from the adoption of four sets of weights are comparatively examined under different levels of water availability, i.e. different scenarios (Table 4). Selected water flows correspond to those needed to sustain from time to time one of the management options (i.e., s_1, s_3 and s_5), and are integrated with other intermediate levels, represented by the second and the fourth scenario.

Table 4. Key parameters of water availability scenarios.

Scenario	q_s (L/s·ha^{-1})	q_s (m^3/ha)
s_1	2.19	22,706
s_2	1.95	20,218
s_3	1.70	17,626
s_4	1.18	12,234
s_5	0.66	6843

As previously demonstrated (see Figure 4), land allocation amongst the possible cultivation strategies remains unchanged within specific ranges of values bounded by precise combinations of weights. On this basis, the analyses introduced in this section focus on economic and environmental results observable at the lower bounds of these ranges; in other words, it means adopting the analytical approach only in correspondence of the different weights sets for which the optimization process determines, from time to time, a different configuration of the system. More in detail, they are identified as the benchmarks described above, obtained for $w_1 = 0.61$, 0.95 and 1 respectively; in addition a balance between the two objective functions ($w_1 = 0.5$) has been taken into account as well, while the condition that maximizes water saving ($w_1 = 0$) is excluded from the analysis, as it returns null areas in all the scenarios, suggesting not to practice irrigated agriculture. Finally, two different initial conditions are optimized. In the former only traditional varieties are included; in the second configuration, the model can choose if adopting them or not, preferring in this latter case, breeding more suitable for dry cultivation. In general terms, as shown in Figure 7, optimization suggests to make use of all the available agricultural area, despite different conditions of both water availability and priority given to the objectives considered. A decreased irrigation amount available to farmers, encourages less water-demanding techniques, with flush irrigation (IRR) particularly favored when priority is given to water saving. This is also the most favored option when environmental and economic concerns are equally accounted for (w_1 up to 0.5). On the other hand, more positive economic results are to be found in presence of submerged rice fields, whenever this practice is effectively sustained by the irrigation water available. Different irrigation volumes supplied may, in fact, enable only one or more resource management strategies. The water flow set in s_1 (2.9 $\text{L/s}\cdot\text{ha}^{-1}$) leads the model to choose optimal land allocation amongst all the management options, given that it corresponds to a volume per hectare (22,706 m^3/ha) higher than each net irrigation amount needed (see Table 1). In this case, water management options are sequentially returned according to the relative importance recognized to each objective function. Conversely, lower water availability (s_2), if mainly aimed at maximizing gross margins, primarily suggests to introduce delayed flooding at the expense of FLD. The area intended for conventional cultivation is further gradually replaced by alternative strategies, the less is the water available. 1.70 $\text{L/s}\cdot\text{ha}^{-1}$ (q_3) permits to adopt DFL and IRR techniques only. In this sense, the former is encouraged when maximization of gross margin is preferred over water saving, the latter in the opposite situation. All these consideration are not entirely valid when optimization concerns a production pattern based on innovative cultivars. In such conditions DFL is the modality prevailing over the other irrigation practice (i.e., IRR), independently from both weights sets and water flow amounts: as demonstrated so far, the same allocation of resources is in fact returned by different scenarios.

Similarly to s_2, the fourth scenario returns the possibility to practice two different water management strategies at once, with half of the area intended for DFL and the rest for IRR, despite different priority given to the objectives, especially when the introduction of ameliorated cultivars is considered. Finally, the last condition simulated (s_5, 0.66 $\text{L/s}\cdot\text{ha}^{-1}$) implies that all the arable land is cultivated with drill-sown rice and its border irrigated, irrespective of both the rice variety and the main objective pursued. Actually, this is the only possible option, being the respective water amount (6843 m^3/ha) able to effectively ensure only such irrigation modality. Therefore, in this case the prioritization of the J_i functions does not affect the optimal solution, as well as the use of resource is not aimed at water saving as such, but rather at making the best use of irrigation water available.

Both irrigation water amounts and gross margins vary accordingly to the optimal allocation of rice-growing area, within a same scenario and across simulated conditions for a same initial configuration (Figure 8).

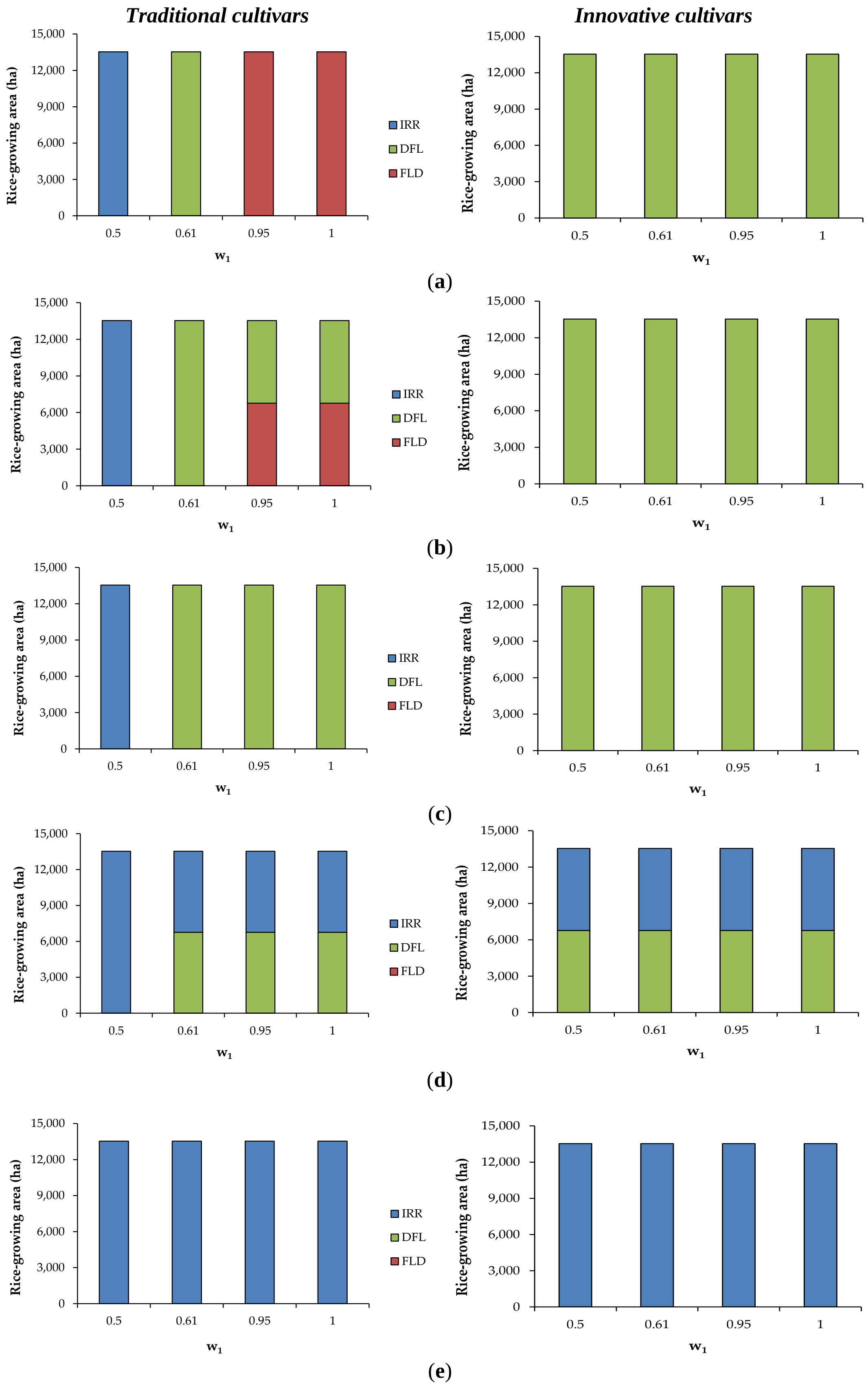
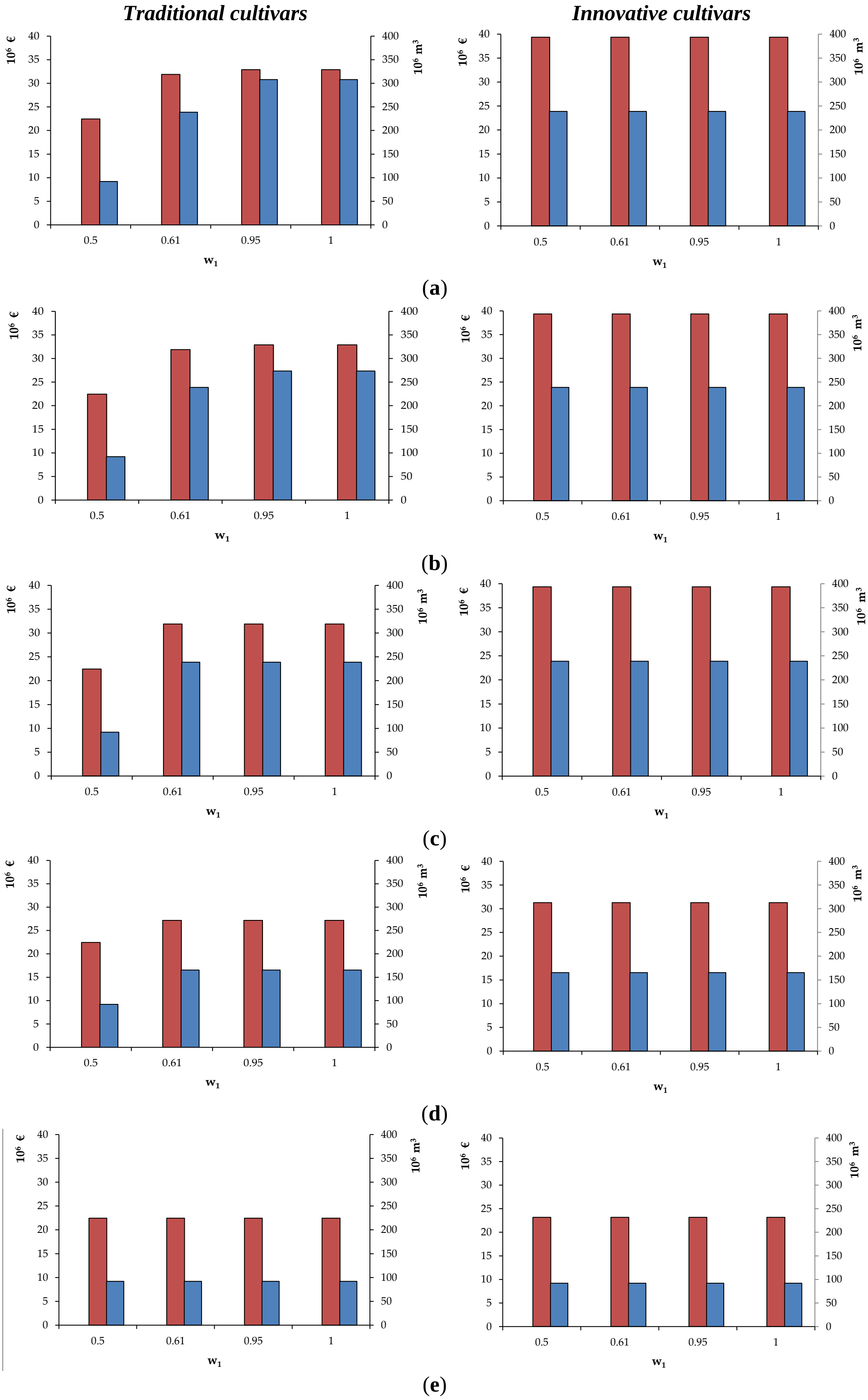


Figure 7. Optimal allocation of rice-growing areas under different water availability levels (i.e., scenarios): (**a**) 2.19 L/s·ha^{-1}; (**b**) 1.95 L/s·ha^{-1}; (**c**) 1.70 L/s·ha^{-1}; (**d**) 1.18 L/s·ha^{-1}; (**e**) 0.66 L/s·ha^{-1}. Each scenario shows results of both prioritization weights

and specific constraints: cultivation of traditional cultivars only or possible introduction of innovative cultivars.



(a)

(b)

(c)

(d)

(e)

Figure 8. Gross margins (Mio. EUR, red series) and irrigation water amounts (Mio. m^3, blue series) in the study area, under different water availability levels: (**a**) 2.19 L/s·ha^{-1}; (**b**) 1.95 L/s·ha^{-1}; (**c**) 1.70 L/s·ha^{-1}; (**d**) 1.18 L/s·ha^{-1}; (**e**) 0.66 L/s·ha^{-1}. Each scenario shows results of both prioritization weights and rice varieties cultivated.

With particular regard to the original configuration of regional agriculture (see Figure 8, left column), they both increase within a same scenario and decrease with the level of water availability, except when water flows ensures only IRR (i.e., s_5) or either the economic and the environmental dimensions remain constant. In addition, the range of gross margins progressively decreases from 10.5 Mio. EUR in s_1 to 4.7 Mio. EUR in s_4, up to be cancelled when water availability represents the strongest constraint for rice-cultivation (Table 5), leading each farm to benefit from economic results that range between 83,103 and 121,874 € (1658–2431 €/ha).

Table 5. Gross margins (Mio. EUR) in the original configurations.

Scenario	Min	Max	Range
s_1	22.4	32.9	–
s_2	22.4	32.9	10.5
s_3	22.4	31.9	9.5
s_4	22.4	27.2	4.7
s_5	22.4	22.4	0

In contrast, when optimization concerns the introduction of more productive varieties, the variation in the total gross margin is noticeable. A diminution occurs with a decreasing water availability, with −21% when passing from s_3 (or, equivalently s_1 or s_2) to s_4, and −26% from s_5 to s_4 (−41% compared to the first three conditions), whilst still remaining the same irrespective of the importance of the set objectives. For irrigation volume disposed, too, the same trend is evident.

5.7. Discussion

In general terms, the use of linear programming is a relatively simply approach to simulate a large variety of modifications in a regional agricultural system. Scenario analysis showed the role of modeling in deepening the possibility to still perform rice-cultivation; this could be strategic in economic terms not only for the whole area, but also for the resilience of specialized farms—which number, at least in Pavia province, has been suffering from a reduction (Database Ente Nazionale Risi)—and the achievement of targets set by water policies.

A varied array of water management options can be adopted in rice-cultivation, to stimulate the rational use of water resources. This is extremely necessary to face water scarcity and represents only a part of the sustainability applied to irrigated agriculture, since it concerns conflicting objectives and strengthens the wicked nature of the sustainability problem.

The innovative irrigation practices included in the analysis are coupled with different gross margins and water saving amounts, considered as simplified indicators for economic and environmental results in the study area. From this perspective, water price has a relevant role and impacts on both profit and water use (Bartolini et al., 2007). In the context analyzed in the paper, water is supplied according to a tariff based on the extent of irrigated farmland. This has currently positive repercussions on the production cost of rice-growing farms, leading gross margins to be affected only marginally by water tariff, benefitting from it regardless the amount of water used. Thus, if farmers were charged with a volumetric fee, the production costs following both traditional and semi-traditional cultivation would be higher, due to increased water supply costs and decreased gross margins. It is also discussed that, despite such an increase, this would lead to a better resource allocation. The role of such a kind of tariff in encouraging water saving is widely recognized and promoted by international regulations, e.g., the Water Framework Directive (2000/60/EC). Future insights on the role of a volumetric water tariff are needed, in order to deepen how it concretely affects the regional economy and to reveal if and under which conditions rice-cultivation is still economically convenient.

Alternative water managements imply different production costs, according to the operative modalities adopted to conduct paddy fields, from fertilizations to weed control treatments. From this perspective, the chance emerges for applied research in selecting varieties that best suit dry conditions and perform higher yields, to counteract possible major production costs. Indeed, the introduction of short-cycle crops and genetically selected varieties, as well as the implementation of efficient methods of weeds control, would make such options more economically convenient for many farms.

Along with economic aspects, it is to be considered that in districts where irrigation has a long tradition, water supply systems are coupled with environmental concerns also related to multiple uses of the resource (Cadario et al., 2006). Rice-cultivation in the study area is the result of a long process of adaptation undertaken by agriculture to site-specific conditions, which has allowed over time to build a consolidated and traditional system made up of the interactions amongst natural resources and human activities. Such a complex stability is continuously managed to create an optimized system that reuses in downstream areas the losses of the upstream ones, thanks to springs, wells and leakages. It therefore derives that alternative allocations of both land and water may lead to significant modifications in the regional irrigated system. They are not entirely the results of farmers' decisions and their change could cause ecological and environmental effects not immediately identifiable. In this sense, the practical feasibility of suggested options should be carefully taken into account when pursuing specific aims: such marked changes could not be actually sustained by the current agricultural practices nor easily accepted by farmers.

Based on model results, some considerations about that in fact arise. When rice cultivation is not advantageous from a water saving point of view, all the arable farmland should be intended for non-irrigated crops or reconverted to permanent land uses (e.g., forest and forest plantations); such a reconversion, as long as the possibility in changing the crop mix, are actually subject to a wider set of other economic, environmental and cultural constraints, which lead to a more complex cultivation pattern variable over time. On the other hand, the expansion of rice-cultivated area is difficult to achieve. The chance to intend all the arable land for rice would moreover have strong economic and managerial repercussions on not specialized farms, which should adapt their cultivation methods in terms of agronomic strategies, use of machinery and technical factors to other or newly-implemented agricultural practices.

Intermittent irrigation is encouraged especially when water saving is preferred over the economic dimension, and whenever water availability becomes the limiting factor for ensuring traditional cultivation. The adoption on large areas of such a modality may also lead to encounter profound modifications in the soil water dynamics and balance. It would enable a significant decrease in irrigation requirements and, at the same time, lead water table to suffer from a reduced recharge, with cascading effects on groundwater resources that are further exacerbated by water scarcity. In a very similar way, an enhanced technical and infrastructural efficiency able to reduce water losses along distribution system, as long as a higher water use efficiency, operate as driving forces to optimize the use of resource under either water scarcity or droughts. Further implications in recharging and supplying water sources in general arise, possibly cancelling, especially at district-level, the potential benefits of water reallocation.

Notwithstanding, performing water saving techniques in rice-cultivation is a strategy that enables increasing water resource availability for augmented irrigated farmland, mitigates the effects of climate change or prevents them thanks to reduced greenhouse gasses emissions (Miniotti et al., 2016, Said-Pullicino et al., 2015). As a further environmental concern, submerged paddy fields and their long-term maintenance have created not only traditional local landscapes, but also agro-environmental habitats with peculiar ecosystem services. Water supply systems are here coupled with environmental aspects, therefore multiple uses of the resource should not be ignored (Garcia de Jalon et al., 2014). Such high value areas are recognized at the community level, as part of the NATURA 2000 network, as well as on the list of both the Special Protected Areas and Site of Community Importance (Habitats Directive, 92/43/EEC); a conflict between irrigation water and water for natural ecosystems thus becomes more evident (Garcia de Jalon et al., 2014). DFL seems to be the best compromise solution to be adopted, since it shows positive performances in terms of water saving (−23% compared to DFL), whilst ensuring adequate gross margins, especially if innovative cultivars are adopted. In this case, in fact, margins obtained with selected breeding are higher than with traditional ones, while maintaining water on the field for most of the growing season allows the permanence of wetlands and humid areas favorable to aquatic biodiversity.

As demonstrated by manifold experiments carried out at Rice Research Centre, just nearby the study area, water management options on temperate rice-fields differently affect the environment as a whole, in terms of water pollution, heavy metals concentration and greenhouse gasses emissions. Especially in dry seeded cropping systems, nitrates represent strong concerns for the quality of surface and ground water (Miniotti et al., 2016), Cadmium concentration in rice grain has turned out to be higher than the standard limit (Cattani et al., 2016). All these elements are to be properly taken into account when analyzing the environmental advantages of different irrigation practices, as well as when environmental cost of water resource needs to be quantified.

5.8. Conclusions

The approach presented serves as a contribution for the discussion about the efficient and sustainable use of water resource in agriculture. Along with innovative water management modalities, the adoption of ameliorated crop varieties was considered in this study as possible solution to address the topic.

In deepening the optimal allocation of resources in irrigated agriculture, few studies takes simultaneously into account both economic and environmental concerns; even less are those focusing on agriculture in temperate areas. The model implemented tries to fill this gap, with particular attention to a sector, i.e., rice-cultivation, which is particularly distinctive of the Po Valley, important for the regional economy and representative of a water-consuming activity that has been suffering from reduced resource availability.

Despite the homogeneous area used as reference, the lack of detailed information about soil characteristics makes the model not properly spatially-dependent. Rather, indeed it is limited to suggest the optimal management of rice-growing area, and indirectly the allocation of irrigation water. Therefore, further research is to be put into the characteristics of the region, as well as into the interaction between agricultural activity and the environment as a whole. The approach adopted required to extremely simplify the representation of the regional system, while all the economic and environmental implications at district level should be accounted for before putting into practice the land use conversion suggested by the optimization model. In addition, it was developed starting from results of field-level experiments and the actual suitability of scaling them up at regional level is a further concern to be properly investigated.

It is then necessary to overcome the purely deterministic nature of the model, which only suggests a set of options not always practically feasible. On the other hand, the need emerges to identify adequate tools for estimating the environmental value of paddy fields, given the manifold interactions between water management and the environment, and their effects.

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Abbreviations

The following abbreviations are used in this manuscript:

APAT	Agenzia per la Protezione dell'Ambiente e per i servizi Tecnici (i.e., Italian Environment Protection Agency)
CAP	Common Agricultural Policy
CREA	Council for Agricultural Research and Economics
MCA	Multi Criteria Analysis
WUA	Water User Association
UAA	Utilisable Agricultural Area
FLD	Water seeded rice and continuous flooding
DFL	Dry-seeded rice and delayed flooding
IRR	Dry-seeded rice and intermittent irrigation
WS	Water Saved
GM	Gross Margin
NI	Net Irrigation

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General Conclusions

General conclusions

The development of rice varieties adapted to water scarcity while taking into consideration economic and environmental factors is possible. In the first publication. We assessed the performance of genomic prediction across rice population and evaluate the effect of different factors (MAF, LD, GS methods, size of the training set) on the prediction accuracy. Prediction accuracies were affected by interaction of those factors in the cross validation experiment, for example the interaction of LD and MAF. Prediction across generation showed the importance of having genetic links between populations. However, when choosing the training set, breeders should test varieties that present an equilibrium between diversity and narrow genetic link between individual of the population. Furthermore, as rice represent a model system and an organism of choice for the study of cereal genome, this approach could be transferable to other crops.

The second publication provided information about how to reach good prediction accuracies when evaluating environment with contrasted irrigation/field management. Different approaches were tested as the evaluation of the genotype response through regression, and by building a multi environment model. Evaluating the genotype response permitted to reach good accuracies. Multi environment prediction were implemented with two cross validation methods, one that considered a situation when breeders want to evaluate untested lines, the second provided a situation when accessions are not present in every environment. This last situation can occur when breeders cannot test all the accessions due to financial issues or when some accessions or trials are lost because of climatic or environmental problems (rain, pests, disease). The first cross validation method gave slightly lower accuracy than when evaluating the separated condition whereas the second cross validation method gave the highest accuracy due to the borrowing of information between environments permitted with this last approach. Considering that our rice breeding program is in a transition situation moving progressively from breeding for irrigation system toward breeding for the aerobic system, we can consider that we are in a favorable configuration. However, urther progresses in the varietal improvement of adaptation to aerobic system, requires more diverse germplasm and/or more sever drying phase in the aerobic systems.

It is known that Italian rice production is threatened by water scarcity, volatile rice prices and a constant increase of production costs that can affect farmers incomes. The third publication explained that alternating field irrigation during the cropping season could be a solution, however it decreases yield. During the last century, with the beginning of green revolution, breeding programs had mainly target the increase of the number of panicles while conserving a constant production, and the use of chemical fertilizer. Rice varieties became shorter with smaller panicles and smaller grain that permitted a resistance to lodging. This approach permitted to get higher rice production in conventional condition of rice cultivation. Production costs increased, with the increase of the use of fertilizer, the mechanization. In the actual situation, reducing irrigation is not affordable for farmers. Therefore, many programs are being implemented to select rice variety tolerant to water scarcity.

The fourth publication presented a multi objective linear optimization is used to explore the trade-off of the conflicting economic, agronomic situation encountered in irrigated rice cultivation. The model evaluated the effect on prioritizing one objective over the other. The simulation model showed that introducing rice accessions adapted to a scarcer irrigation can enable enlarging frontiers of optimal solutions. With accessions adapted to water scarcity adopting alternative strategies of irrigation that may lead to constant or higher yield and lower production costs and lower irrigation.

In conclusion, to be efficient, breeding program and the implementation of new field practices should consider some economic factors linked to rice cultivation. However, the optimal combination of factors of production depends on the economic system in relation to which the analysis is conducted and specific problems

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