UNIVERSITÀ DEGLI STUDI DI MILANO

Dottorato di Ricerca in Agricoltura, Ambiente e Bioenergia

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IN SILICO IDEOTYPING:

Definition and evaluation of rice ideotypes improved for resistance/tolerance traits to biotic and abiotic stressors under climate change scenarios

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Ph.D. in Agriculture, Environment and Bioenergy
XXIX Cycle, Academic year 2016/17

ACKNOWLEDGMENTS

I would like to apologize to English speakers for writing this part in Italian.

Ringrazio il mio tutor, Prof. Roberto Confalonieri, per avermi guidato e spronato in questo progetto di dottorato e per avermi insegnato, più di ogni altra cosa, l’approccio e il metodo necessari a incanalare la mia passione per questo lavoro. Ringrazio i miei colleghi del gruppo Cassandra lab, in particolare Valentina Pagani, per aver condiviso con me questo percorso di dottorato, ed Ermes Movedi, per aver contribuito direttamente a molti degli studi qui presentati. Ringrazio infine la mia famiglia, per l'incredibile entusiasmo con cui si fanno coinvolgere in tutto ciò che faccio.

ABSTRACT

Paleari, L., 2016. IN SILICO IDEOTYPING: Definition and evaluation of rice ideotypes improved for resistance/tolerance traits to biotic and abiotic stressors under climate change scenarios. Ph.D. Thesis, University of Milan, Italy, 150 pp., 17 figures, 8 tables, 279 references.

Ecophysiological crop models, coupled with advanced computational (e.g., sensitivity analysis, SA) and IT techniques, can be profitably used to analyze G×E×M interactions and, in turn, to support breeding programs through the design of ideotypes suitable for specific growing conditions.

However, the exploitation of the potential of simulation technologies for breeding purposes is limited by the uncertainty in the distribution of values for the *in silico* representation of traits for available germplasms and by the partial suitability of the models themselves. Indeed, although to a different extent, available modelling approaches lack a clear relationship between model parameters and plant traits and, in most cases, they miss algorithms for processes involved with resistance/tolerance, which are often priority aspects within breeding programs. This, as well as technological barriers, prevented the modelling and breeding communities from interacting.

This research addressed these issues focusing on rice – given its role as staple food for more than half of the world’s population – and targeting both current conditions and future climate projections, to support the definition of breeding strategies in the medium-long term.

A new procedure to identify parameters whose uncertainty in distributions would affect ideotype design was developed and district-specific ideotypes improved for resistance/tolerance traits were defined by means of variance-based SA techniques or by mimicking introgression of traits from donor genotypes. A paradigm shift towards a new generation of

models explicitly built around traits for which breeding programs are ongoing was proposed, with a case study presenting a new model for salt tolerance used for ideotyping purposes in two different environments. A modelling platform for district-specific ideotyping was also designed and developed by targeting breeders as final users, highlighting the role of an interdisciplinary approach to increase the usefulness of simulation technologies for supporting breeding programs.

Reference to the contents of Chapters II, IV, and VI should be made by citing the original publications.

Page

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Since their early origins in the late 1960s, crop models have been developed targeting two main objectives (Hammer et al., 2002): (i) assisting crop management and agricultural polices (e.g., Berndt and White, 1976; Wilkerson et al., 1983), and (ii) supporting breeding activities via the *in silico* analysis of (G) genotype × (E) environment × (M) management interactions (e.g., Duncan et al., 1967; Loomis et al., 1979; Hammer and Vanderlip, 1989). Crop models, indeed, have a great potential to identify key traits and design plant types for specific agro-environmental contexts, because of their capability to reproduce crop growth and development as a function of environmental (including management) factors and of model parameters representing genotypic features (Hammer et al., 1996; Boote et al., 2013; Ramirez-Villegas et al., 2015).

However, while there are a number of examples proving the role of crop models for cropping systems management (e.g., Jones et al., 2003; Confalonieri et al., 2006; Lehmann et al., 2013), their use to analyze G×E×M interactions has received much less attention, thus making this area one of the most promising for future model development and contributions (Hammer et al., 2002; Tardieu, 2010; Boote et al., 2013). A consequence of this developmental pathway is that current crop models are only partly suitable to support breeding activities (Yin et al., 2000; Chenu et al., 2008; Bertin et al., 2010; Hammer et al., 2010; Messina et al., 2011). Among the main reasons, a key role is played by the lack of a clear relationship between model parameters and plant traits, which decreases the possibility of realizing *in vivo* the ideotypes defined *in silico* (Boote et al., 2001; Hammer et al., 2002).

However, attempts to use crop models to design plant ideotypes have been carried out in the past 25 years, starting from pioneering studies performed in the 1990s (Dingkhun et al., 1991; Kroppf, 1994a, Aggarwal et al., 1997) until recent examples (e.g., Quilot-Turion et al., 2012; Raza et al., 2013; Singh et al., 2014; Drewry et al., 2014; Tao et al., 2016; Ding et al., 2016). Actually, there is an increasing effort towards the use of crop models to aid ideotype design (Martre et al., 2015a; Rötter et al., 2015). After decades from the first proposal of the *ideotype* concept for crop improvement (Donald, 1968), indeed, the interest in the ideotype-based approach for defining plant breeding strategies has continuously grown (e.g., Kush et al., 2001; Peng et al., 2008; Habash et al., 2009; Kush, 2012; Dingkhun et al., 2015). According to this approach, breeders should select directly for the plant ideotype rather than empirically for grain yield, where the ideotype is defined as “a combination of morphological and physiological traits (or their genetic bases) conferring to a crop a satisfying adaptation to a particular biophysical environment, crop management and end use” (Martre et al., 2015a). “Ideotyping” thus refers to a two-steps process: (i) identifying key traits for the objective/s to be accomplished (e.g., improving quantitative/qualitative aspects of productions and/or use of resources), and (ii) providing indications about their putative value. The analysis should be performed in the given agro-environmental context targeted by the breeding program, in order to take full advantage of the interactions between G, E and M (Hammer et al., 1996). In the challenge of meeting an ever-increasing global food demand (Foley et al., 2011) while yields are stagnating for most of staple crops (Deepak et al., 2012) and weather extremes are expected to increase their frequency and intensity (Teixeira et al., 2013), this approach for adapting crops to specific conditions assumes even more relevance.

Despite their limits and uncertainties, ecophysiological models provide a unique platform for integrative analyses of the impact of traits (single or in combination) on the behavior of the whole plant. This impact is indeed difficult to predict without advanced supporting tools, since plant response to environmental factors is often strongly non-linear (e.g., Hammer et al., 2009; Bertin et al., 2010) and because of complex dynamic interactions between different traits. Moreover, process-based crop models allow exploring the putative value of traits in a wide range of environmental and management conditions (e.g., Casadebaig et al., 2011; Jeuffroy et al., 2012)

− even in entire production districts (Tao et al., 2012; Confalonieri et al., 2013). Exploring the same degree of variability with field trials in experimental stations is practically unfeasible. Using crop models, ideotypes can thus be tailored for specific agro-climatic contexts by effectively exploring the environments targeted by breeding programs (Hammer et al., 2006; Chapman et al., 2008; Chenu et al., 2011). The analysis can also include climate change scenarios (e.g., Semenov and Shewry, 2011; Zheng et al., 2012; Semenov and Stratonovitch, 2013; Tao et al., 2016) to account for the long-term prospective of breeding strategies and the need to develop new varieties better suited to future climate conditions (Tester and Langridge, 2010; Ramirez-Villegas et al., 2015).

From a methodological point of view, model-based ideotyping is carried out by modifying model parameters according to the known genotypic variation of the corresponding trait (e.g., Kropff et al., 1994a; Semenov and Halford, 2009; Hammer et al., 2010) or by exploiting optimization algorithms (e.g., Semenov and Stratonovitch, 2013) using simple or composed objective functions (e.g., Drewry et al., 2014), as well as standard or Bayesian approaches (Van Oijen and Höglind, 2016) for defining parameter values. However, one of the most popular methodologies to evaluate putative traits relies on sensitivity analysis techniques (e.g., Aggarwall et al., 1997; Habekotté et al., 1997; Herdl et al., 2007; Quilot-Turion et al., 2012; Confalonieri et al., 2013; Martre et al., 2015b; Casadebaig et al., 2016), which can be also used to screen traits before running optimization algorithms (Génard et al., 2016; Quilot-Turion et al., 2016). Sensitivity analysis (SA) indeed, allows quantifying the portion of model output variance due to variation in input factors (Tarantola and Saltelli, 2003) and therefore, under the assumption of a close relationship between model parameters and plant traits, it can be used to identify traits breeders should focus on under specific conditions.

Beside these efforts for deriving model-based ideotypes, a wide area of modelling research is focusing on the integration of genetic information on QTLs in crop models, to turn them into effective tools for solving the issue of linking genotype to its corresponding phenotype (the so-called “G-P” problem Cooper et al., 2002). These studies are based on supposed relationships between genes/QTLs and the values of model parameters after calibration using data from phenotyping on multi-environmental trials. The existence of such relationships would allow incorporating in the definition of ideotypes the knowledge on the genetic basis of traits (Chenu et al., 2009). This “gene-based” modelling approach started in the mid-1990s with the seminal work from White and Hoogenboom (1996) and the following researches from Yin et al. (2000), and it continued to be a dynamic research area until today (e.g., Tardieu et al., 2003; Messina et al., 2006; Letort et al., 2008; Quilot-Turion et al., 2016). However, despite its theoretical potential, an accepted methodology to actually link model parameters to genetic information is still missing. From a modelling point of view, main limits for achieving that goal refer to the uncertainty in parameter values estimation due to (i) the limited availability of phenotypic data, needed to incorporate in crop models knowledge on gene-gene and gene-environment interactions, and (ii) multicollinearity issues, i.e., different combinations of parameters leading to the same value for a phenotypic trait.

Another area of concern relies on the way physiological knowledge is currently formalized in crop models, whose adequacy to represent subtle differences among genotypes and phenotypic plasticity remains questionable (Bertin et al., 2010; Hammer et al., 2010; Messina et al., 2011). This drives, again, to the fact that current models are not fully suitable to target such applications as they have been developed for different purposes.

1.2. Key issues

Despite the number of studies involving SA techniques to drive ideotype design, there are sources of uncertainties that should be eliminated – or at least quantified − to improve the reliability of SA results and, in turn, the feasibility of ideotypes. One of the most critical issue is the impact of the uncertainty in parameters ranges/distributions on SA outcomes (Pianosi et al., 2016). Many authors, indeed, demonstrated how variations in parameters ranges/distributions can drastically alter SA results (Wang et al., 2013; Shin et al., 2013). However, robust, standard and reproducible procedures to quantify this impact and provide possible solutions are not available. This issue is relevant regardless of the purpose of the specific study, e.g., identifying parameters to calibrate (van Werkhoven et al., 2009), analyzing model structure (Sieber and Uhlenbrook, 2005) or developing new models (Jakeman et al., 2006), but it becomes crucial when SA is used to suggest breeders promising traits to work on (e.g., Martre et al., 2015b).

Beyond methodological concerns, a key point in modelling studies targeting ideotypes design is the lack of specific algorithms for simulating the impact of biotic (e.g., diseases) and abiotic (e.g., weather extremes) stressors. Despite they are priority in most breeding programs worldwide (e.g., Ballini et al., 2008; Das and Rao, 2015) and key factors for adaptation to climate change (Garret et al., 2006; Howden et al., 2009; Fisher et al., 2012), model-based ideotyping studies have completely ignored traits affecting resistance to disease and tolerance to environmental constraints. Exceptions are tolerance to drought (e.g., Chenu et al., 2009) and few studies on heat stress (e.g. Singh et al., 2014). However, both biotic and abiotic stressors show strong G×E interactions (Robert et al., 2004; Magarey et al; 2005; Tardieu, 2012), which provide crop models a key role to evaluate the potential benefits deriving from improved resistance/tolerance traits (Tardieu and Tuberosa, 2010; Ramirez-Villegas et al., 2015). The main reason for overlooking ideotypes improved for resistance/tolerance traits is the lack of a systematic implementation of approaches for the simulation of abiotic/biotic stressors in most crop models (Donatelli and Confalonieri, 2012; Caubel et al., 2012; Bassu et al., 2014). Another reason is likely related to the unsuitability of the few available approaches for analyzing the effect of changes in the values of related traits. Concerning biotic stressors, many epidemiological models are available, both pathosystem-specific (e.g., Calonnec et al., 2008; Robert et al., 2008) and generic ones (e.g., Audsley et al., 2005; Garin et al., 2014). However, either these models do not allow the estimation of disease-induced yield losses because they consider only the impact of architectural traits on epidemics development (e.g., Robert et al., 2008), or the level of detail in the way knowledge on the underlying processes is formalized do not allow their use for ideotyping applications (e.g., Luo et al., 1995). Taken salt stress as an example of abiotic constraint, models for evaluating salinity-induced yield losses are available (e.g. Ferrer-Alegre et al., 1997), although they were developed to support irrigation management, and this led to a simplified representation of plant traits that prevents their use for ideotyping purposes. Indeed, while breeders are working on a variety of traits involved with salt tolerance (e.g., Ismail et al., 2007; Munns and Tester, 2008; Munns et al., 2012; Roy et al., 2014), available modelling approaches represent the plant response to salt stress via few empirical parameters directly linking yield losses to salt concentration in the soil. These considerations – again – drive to the basic concerns about the suitability of current crop models for actually representing phenotypic traits of interest for breeding, which may call for specific model improvement (Chenu et al., 2008; Bertin et al., 2010; Hammer et al., 2010; Messina et al., 2011; Boote et al., 2013).

Model parameters do not always have a biophysical meaning and, even when they have, a clear link to traits involved in breeding programs is often missing. A direct representation of the physiological and genetic basis of traits via model parameters acting at organ or crop level is surely hard to derive, for the uncertainty related with the integration across levels of biological organization (Sinclair et al., 2004; Yin and Struik, 2010) and the dependencies on epistatic and pleiotropic interactions (Cooper and Podlich, 2002; Cooper et al., 2009). However, a bridge between model parameters and genotypic features scored by breeders needs to be established in order to avoid discrepancies between the hypothesis tested *in silico* and their in vivo realization (Boote et al., 2001; Hammer et al., 2002; Tardieu et al., 2003; Hammer et al., 2010; Ramirez-Villegas et al., 2015). Higher model complexity and physiological detail, *per se*, are neither needed (Hammer et al., 2006) nor they represent a guarantee of deriving models with explicit and direct relationships between model parameters and plant traits. This because the same knowledge can be formalized by using different modelling structures and model complexity is not always a guarantee of higher adherence to biological processes (Confalonieri et al., 2016).

While this paradigm shift in the development of crop models would be hard to put in place, it would be crucial to overcome the dualism between the interpretation of the ideotype concept by modellers (a combination of model parameters) and plant breeders (a combination of phenotypic traits) (Andrivon et al., 2013).

Such dualism may also derive from the lack of modelling tools to be used directly by breeders themselves. Pioneering attempts to use mathematical models in crop breeding is provided by the modelling platform QU-GENE (Podlich and Cooper, 1998) which can be used in conjunction with the APSIM biophysical models to evaluate different breeding strategies (Chapman et al., 2003). However, even in this case, advanced IT and crop modelling expertise are required, thus making crop models still far from being routinely used for supporting the definition of breeding strategies.

As argued by different authors (e.g., Shorter et al., 1991; Tardieu et al., 2003; Boote et al., 2013; Andrivon et al., 2013), a collaborative effort from geneticists, physiologists, crop modelers, breeders and end-users is likely the only solution to increase the suitability of crop modelling for supporting breeding programs.

1.3. Objectives and organization of the research

This research covered a variety of issues crucial for the use of mathematical models for breeding purposes that are – to a large extent – ignored or overlooked by the modelling community. In particular, the aim of this research was to increase the adequacy of crop models for ideotyping purposes through:

i) the improvement of methodologies already in use,

ii) the inclusion of traits involved with resistance/tolerance in ideotyping studies,
iii) the proposal of a paradigm shift for the development of crop models explicitly targeting ideotyping applications (trait-based development), and
iv) the development of software tools to encourage the integration of expertise from the modelling and breeding communities.

Both current conditions and climate change scenarios were considered, given time horizons targeted by breeding programs and the impact of climate change on the way promising traits affect crop yields.

The focus was on rice (*Oryza sativa* L.) for its role as a staple food for more than half of the world's population and as a pioneering crop in the adoption of the ideotype perspective in plant breeding.

1.4. Outline of the research

The uncertainty in parameter distributions can markedly affects SA results, thus misleading the identification of putative traits for improving qualitative/quantitative aspects of crop production. **Chapter 2** presents the first procedure – actually a sensitivity analysis of a sensitivity analysis – to identify parameters whose uncertainty in distribution can alter SA results, i.e., parameters whose distributions need to be defined with more caution. The procedure would allow to reduce one of the main sources of uncertainty in SA-based ideotyping studies, as shown in **Chapter 3**. In this Chapter, indeed, global SA is applied to evaluate potential benefits deriving from increasing resistance to fungal pathogens and tolerance to abiotic stressors as compared to those coming by improving phenotypic traits affecting potential yield (i.e., light interception, photosynthetic efficiency) and grain quality. A dedicated SA-based index to design district-specific ideotypes is also proposed and evaluated.

Improving resistance/tolerance to diseases and abiotic constraints is indeed priority among breeding goals to derive new varieties better adapted to specific agro-environmental contexts. However, understand which trait/s will be key ones is hampered by strong G×E×M interactions. Given the potential of ecophysiological modelling to address this issue, **Chapter 4** was focused on the model-based definition of ideotypes improved for resistance to fungal pathogens and tolerance to abiotic stressors. The analysis was conducted at district level, to account for characteristics of available germplasm and spatial heterogeneity among and within production districts. As results show, these aspects can markedly affect ideotype features.

However, despite their potential, ecophysiological models are still far from being completely suitable for ideotyping applications. The main limit concerns the lack of clear correspondence between model parameters and plant traits breeders are working on, which could lead to discrepancies between *in silico* ideotypes and their in vivo realizations. A strategy to reduce this risk is to conduct ideotyping studies involving only parameters with a close link to phenotypic traits targeted by breeding programs, as suggested in **Chapter 3** and **Chapter 4**. However, to fully overcome this issue, new models specifically developed to target ideotyping studies (i.e., built around actual plant traits) are needed. This paradigm shift in crop models development is presented in **Chapter 5**, taking rice and salt stress as a case study.

Beside the improvement (or re-design) of crop models to increase their suitability for ideotyping purposes, the availability of modelling tools usable by breeders without specific simulation or IT skills could represent another path to promote an effective integration of crop modelling within breeding activities. **Chapter 6** presents the first ideotyping platform (ISIde, *In Silico* Ideotyping platform) specifically developed targeting ideotyping studies at district level and breeders as final users.

Notes

Chapter 2, Chapter 4 and Chapter 6 have been published on, respectively, Ecological Modelling, Climatic Change and Computers and Electronics in Agriculture. Chapter 3 has been re-submitted after revisions to Global Change Biology (under review). Chapter 5 has been submitted for publication to Scientific Reports (under review). The reference lists from these individual papers were combined into one list at the end of the thesis. I would like to acknowledge the editorial boards of Ecological Modelling, Climatic Change and Computers and Electronics in Agriculture for their permission to include the papers in this thesis.

CHAPTER 2

SENSITIVITY ANALYSIS OF A SENSITIVITY ANALYSIS: WE ARE LIKELY OVERLOOKING THE IMPACT OF DISTRIBUTIONAL ASSUMPTIONS

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Published on
Ecological Modelling (2016), 340, 57-63.

2.1. Abstract

Although uncertainty in input factor distributions is known to affect sensitivity analysis (SA) results, a standard procedure to quantify its impact is not available. We addressed this problem by performing a SA (generating sample of parameter distributions) of a SA (generating samples of parameters for each generated distribution) of the WARM rice model using the Sobol' method. The sample of distributions was generated using distributions of jackknife statistics calculated on literature values. This allowed mimicking the differences in distributions that could derive from different selection of literature sources. Despite the very low plasticity of WARM, the ranks of the two most relevant parameters was overturned in 22% of the cases and, in general, differed from what achieved in earlier SAs performed on the same model. SA results were mainly affected by uncertainty in distribution of parameters involved in non-linear effects or interacting with others. The procedure identified parameters whose uncertainty in distribution can alter SA results, i.e., parameters whose distributions could need to be refined.

Keywords: Global sensitivity analysis, ideotyping, Morris method, parameter distribution, Sobol' method, WARM rice model.

2.2. Introduction

Sensitivity analysis (SA) is increasingly used to develop, understand, improve and use environmental simulation models through the analysis of the impact of uncertain input factors on the variability in model outputs (Tarantola and Saltelli, 2003; Jakeman et al., 2006; Confalonieri et al., 2010a; Pianosi et al., 2016). Among the main purpose of SA, indeed, a key role is played by the identification of parameters to calibrate (Asseng et al., 2002), the improvement of models through reduction or simplification processes (Ratto et al., 2001), the support to model development (Jakeman et al., 2006), and the evaluation of models (Confalonieri et al., 2012). Under the assumption of relationships between model parameters and plant traits, SA was recently used also in ideotyping studies to identify plant traits on which breeders should focus on to increase quantitative and qualitative aspects of productions (Martre et al., 2015b; Casadebaig et al., 2016).

A variety of SA techniques were proposed, each characterized by pros and cons that make them suitable for specific purposes or conditions. Among the most popular, the method of Morris (1991) is often used to screen parameters in case of models with many parameters or demanding in terms of computational time (Campolongo et al., 2007). The variance-based methods of Sobol’ (Sobol’, 1993) is instead considered as a reference technique for its capability of decomposing the output variance into terms of increasing dimension, representing the contribution to output uncertainty of each input factor and of pairs, triplets, etc. However, it is very expensive in terms of model executions and – to reduce the computational time – it is often used to estimate the total sensitivity index (Homma and Saltelli, 1996), i.e., the overall contribution of each input factor, considering all possible interactions with others. Even in this case, the computational cost of Sobol’ led to propose other methods based on the Fourier series expansion of the model output to reduce the number of model executions in the approximation of variance-based indices, like Fourier Amplitude Sensitivity Test (FAST; Cukier et al., 1973) and extended FAST (E-FAST; Saltelli et al., 1999). Extensive reviews of SA methods were recently proposed by different authors (e.g., Saltelli et al., 2005; Pianosi et al., 2016). In these reviews, the authors proposed effective criteria to select the SA method according to model assumptions, complexity and computational time per run, and they outlined ongoing development and research priorities.

Like many powerful tools, SA techniques need to be applied by carefully considering all the aspects that can affect their functioning. Results of SA are influenced by the conditions explored (Confalonieri et al., 2010b; Martre et al., 2015b; Casadebaig et al., 2016; Cerasuolo et al., 2015), i.e., by the set of model inputs that are not investigated by the SA but define the simulation scenario. This pushed Stearns (1992) to the point of stating that sensitivity is *situational*. The influence of the conditions explored on SA results can be large and its extent varies in accordance with the model plasticity, defined as the aptitude of a model to change the sensitivity to its parameters while changing the conditions explored (Confalonieri et al., 2012). The mathematical expression proposed for the quantification of plasticity is $L = TDCC \cdot e^{\sigma_{SAM}-1}$, where $TDCC$ is the top-down concordance coefficient (Iman and Conover, 1987) and σ_{SAM} is the standard deviation of a normalized agrometeorological indicator (Confalonieri et al., 2012). L ranges from 0 to about 1.51, with highest plasticity at 0. Despite their capability of quantifying the impact of uncertain input factors on model outputs, SA methods themselves can be affected by uncertainty in their own parameters. Indeed, all SA methods require some settings to be specified, at least the size of the sample of combinations of input factors (number of executions). Some methods need a seed for sample generation, e.g., Morris, FAST/E-FAST methods and some of the regression-based approaches (e.g., Latin hypercube sampling, random). The Morris method requires also the number of levels to define the parameter hyperspace. Confalonieri et al. (2010a) analyzed changes in SA results originated by changes in the parameters of the methods, and in many cases the variations they obtained were not negligible. Recent studies on the convergence of SA methods presented effective procedures to define optimum sample size according to the specific simulation exercise (Nossent et al., 2011; Wang et al., 2013;

Sarrazin et al., 2016), thus partly reducing the uncertainty related with SA method parameterization.

One of the most critical steps in SA is to define ranges – and possibly distributions – for parameters (Pianosi et al., 2016), and this is particularly true for variance-based methods. Many authors, indeed, demonstrated how different definitions of parameter ranges/distributions can drastically alter SA results. Shin et al. (2013) altered the range of two parameters of two hydrological models by arbitrarily changing their original upper-bound values by ±50%, obtaining relevant changes in SA results with the Morris (1991) and Sobol’ (Sobol’, 1993) methods. Wang et al. (2013) run a sensitivity analysis of the crop model WOFOST (van Keulen and Wolf, 1986) using the E-FAST method (Saltelli et al., 1999) and two ranges from the parameters for maize: one was obtained by arbitrarily perturbing the default value for the species by ±10%, the other was derived by Ceglar et al. (2011) from observations and values found in literature. Also in this case, SA results changed drastically while changing parameter ranges. However, the impact of the uncertainty in parameter distributions on the variability in model output and on sensitivity analysis results was never quantified using robust, standard and reproducible procedures.

The aims of this study were (i) to propose and evaluate a procedure for quantifying the sensitivity of a SA method to the uncertainty in the parameter distributions, and (ii) to evaluate it with a case study using the Sobol’ method (Sobol’, 1993) and the WARM model for rice simulations (Confalonieri et al., 2009a).

2.3. Materials and methods

As a case study to illustrate the procedure, the WARM model for rice growth and development (e.g., Confalonieri et al., 2009a, 2010a; Pagani et al., 2014) was used. The model is fully described in the seminal literature and at the model web page (www.cassandralab.com/applications/2). The simulation scenario (one growing season) is the same described by Confalonieri et al. (2010a), with rice scatter seeded on 24 May 2004 in northern Italy (45° 23’ N, 9° 13’ E) and grown under flooded conditions under unlimiting nutrient conditions and absence of weeds, pests and diseases. The target output was aboveground biomass at maturity (AGB).

Fig. 1 shows the flowchart of the procedure proposed to quantify the impact of the uncertainties in parameters and parameter distributions on model behavior. When performing SA using methods requiring distributions, a rigorous way to retrieve the distribution for each ith model parameter is based on: (i) literature search to find out a set $X_i = \{x_{i1}, \ldots, x_{ij}, \ldots, x_{in}\}$of measured or estimated values for the parameter (Fig. 1, step 1) (Pianosi et al., 2016), (ii) checking normality of distribution for X_i (or test alternate distributions), (iii) estimating distribution parameters (e.g., mean and standard deviation in case of normality) for X_i. The cardinality of the various X_i can be different, depending on the number of values retrieved from literature. For the 11 parameters of WARM involved with rice growth, the sets of observations used in this study – always normally distributed according to the Shapiro and Wilk (1965) test (p>0.05) – are presented in Table 1.

The uncertainty in the definition of parameter distributions – deriving from possible different selections of x_{ij} values from literature – was here mimicked using the jackknife technique (Quenouille, 1949), with $K_i = \{k_{i1}, \ldots, k_{ij}, \ldots, k_{in}\}$ being the vector of the jackknife samples of X_i, with each sample k_{ij} including all elements of X_i but x_{ij} (Fig. 1, step 2). After calculating the mean $\overline{k_{ij}}$ of each sample k_{ij} for each i^{th} parameter (step 3), the normality of the distributions of the vectors of the means $\overline{k_{ij}}$ was checked (step 4), and their mean ($\mu_i^* = \sum_{j=1}^{n} \overline{k_{ij}}/n$, equal to $\bar{X}_i$, since k_{ij} are jackknife samples of X_i) and standard deviation ($\sigma_i^* = \sqrt{\sum_{j=1}^{n} \left(\overline{\overline{k_{ij}} - \bar{X}_i}\right)^2 / n}$) were estimated (step 5).

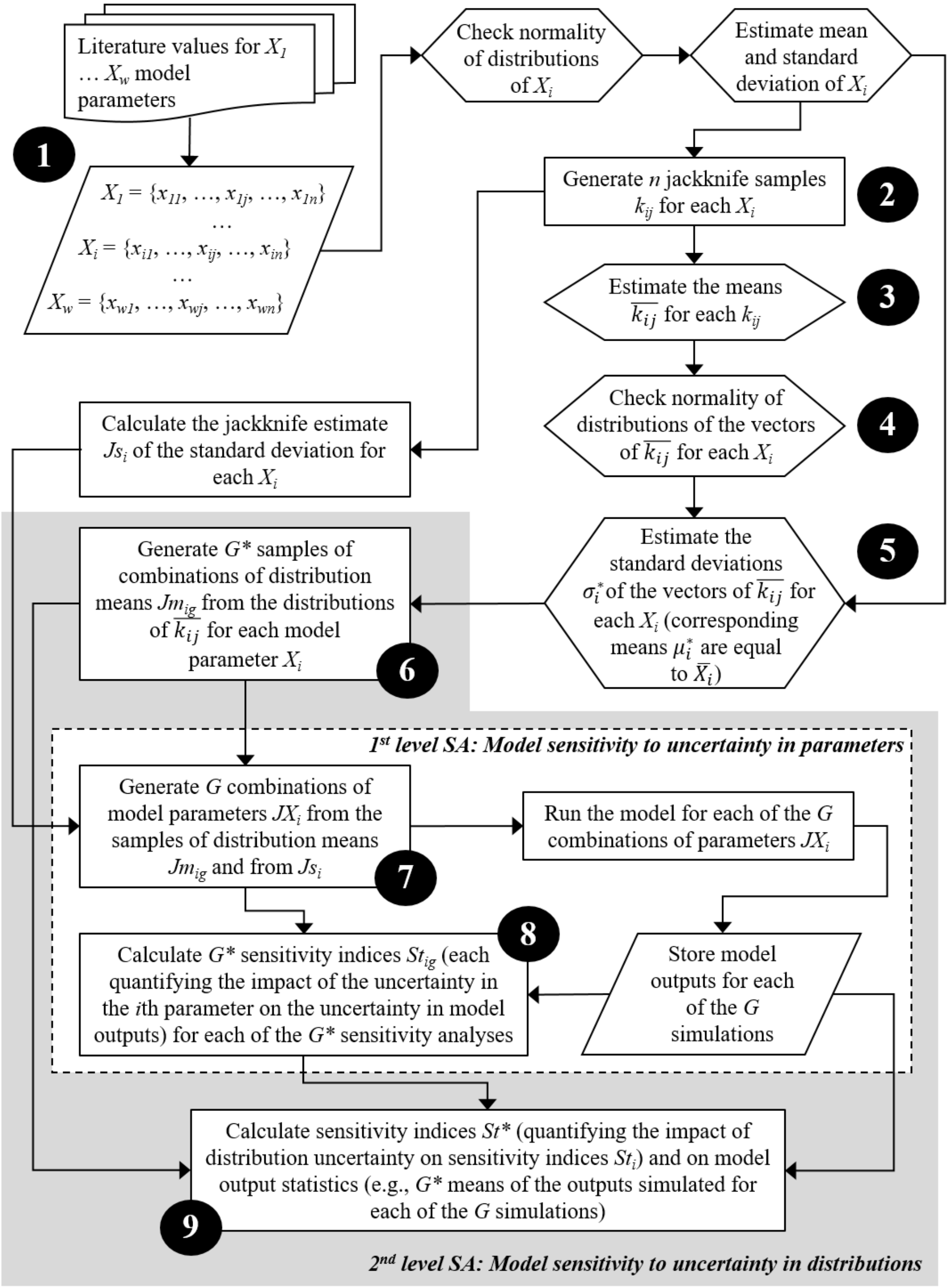

Figure 1. Flowchart of the procedure proposed to analyze the impact of the uncertainty in parameters and parameter distributions on model behavior. Black circles with numbers indicate the key steps in the procedure (described in detail in the text).

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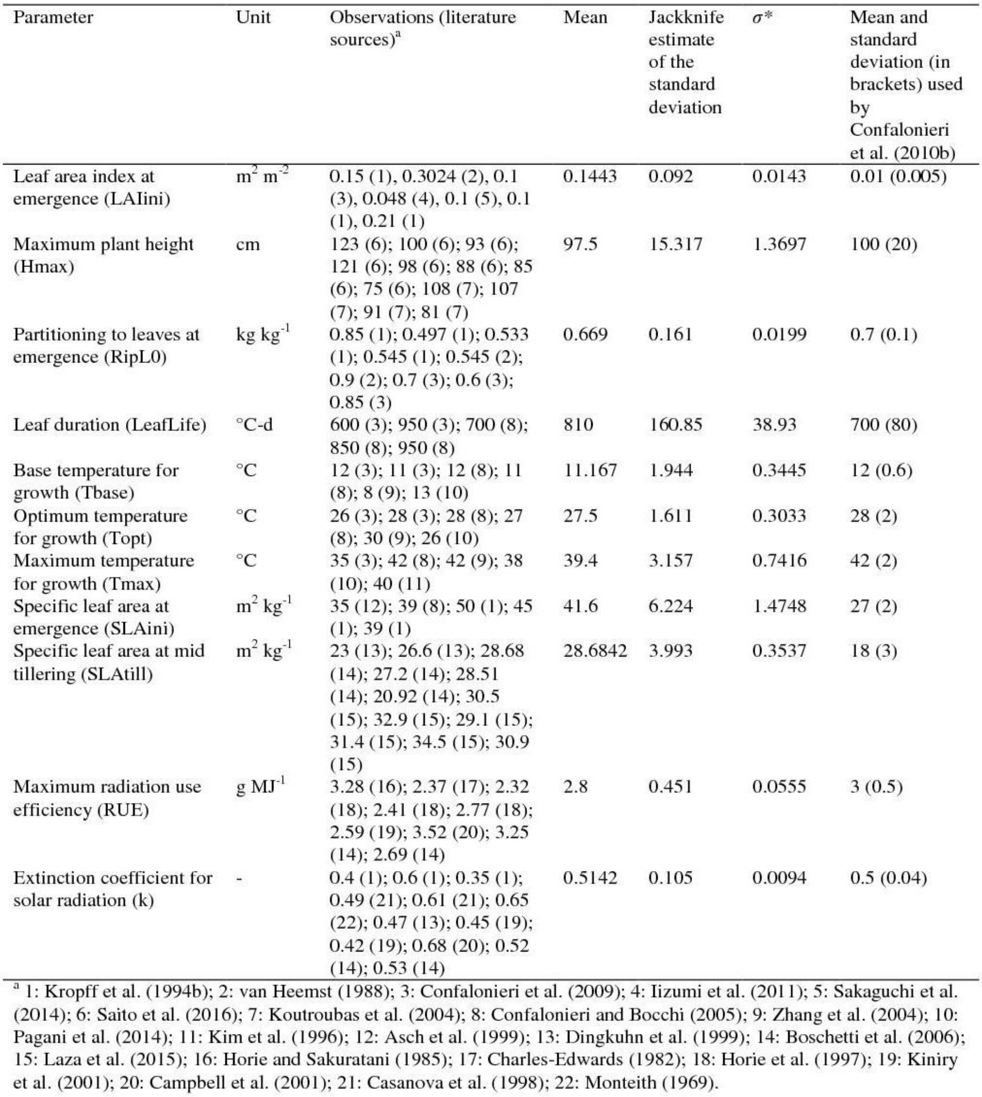
The values of $\mu_i^*$and μ_i^*were then used to generate G^* samples of combinations of distribution means Jm_{ig} for the WARM parameters (step 6), with the latter used – together with the jackknife estimate of the standard deviation for each parameter (Table 1) – to generate combinations of parameter values for each combination of generated means (step 7). The jackknife estimate Js_i of the standard deviation for the ith parameter is $\sqrt{\frac{n-1}{n}\sum_{j=1}^{n}\left(\overline{k_{ij}}-\bar{X}_i\right)^2}$.

This allowed to perform G (sample size for the sensitivity analyses) 1st level SA (estimating the impact of the uncertainty in parameters given a certain distribution, using Jm_{ig} and Js_i, step 8) and a 2nd level SA (estimating the impact of the uncertainty in parameter distributions) of the G 1st level SA. For the latter, the output variable considered was AGB, whereas for the 2nd level SA we used the sensitivity indices (total order effects) derived from the 1st level SA, and AGB statistics (mean, standard deviation, coefficient of variation (CV)) derived from AGB values simulated during each 1st level SA. The variance-based Sobol' sensitivity analysis method (Sobol', 1993) was used for all the sensitivity analyses performed in this study. In both cases, the sample size for the combinations (of distributions and of parameter values) was the lowest value of $G \mid G > (\gamma \cdot n)$, with $G = 2^{(q+3)} \cdot (2n + 2)$, q={1, 2 , 3, …, Q}, γ is the number of model runs for each parameter (500 for Sobol' method according to Confalonieri et al. (2010a), and n is the number of parameters. In the current study, G assumed the value of 6144.

Table 1. WARM parameters involved with rice growth, literature sources, mean of observations, jackknife estimate of the standard deviation, standard deviation of the means of the jackknife subsamples (σ*, see text for details).



Parameter	Unit	Observations (literature sources)[a]	Mean	Jackknife estimate of the standard deviation	$\sigma*$	Mean and standard deviation (in brackets) used by Confalonieri et al. (2010b)
Leaf area index at emergence (LAIini)	$m^2 m^{-2}$	0.15 (1), 0.3024 (2), 0.1 (3), 0.048 (4), 0.1 (5), 0.1 (1), 0.21 (1)	0.1443	0.092	0.0143	0.01 (0.005)
Maximum plant height (Hmax)	cm	123 (6); 100 (6); 93 (6); 121 (6); 98 (6); 88 (6); 85 (6); 75 (6); 108 (7); 107 (7); 91 (7); 81 (7)	97.5	15.317	1.3697	100 (20)
Partitioning to leaves at emergence (RipL0)	$kg\ kg^{-1}$	0.85 (1); 0.497 (1); 0.533 (1); 0.545 (1); 0.545 (2); 0.9 (2); 0.7 (3); 0.6 (3); 0.85 (3)	0.669	0.161	0.0199	0.7 (0.1)
Leaf duration (LeafLife)	°C-d	600 (3); 950 (3); 700 (8); 850 (8); 950 (8)	810	160.85	38.93	700 (80)
Base temperature for growth (Tbase)	°C	12 (3); 11 (3); 12 (8); 11 (8); 8 (9); 13 (10)	11.167	1.944	0.3445	12 (0.6)
Optimum temperature for growth (Topt)	°C	26 (3); 28 (3); 28 (8); 27 (8); 30 (9); 26 (10)	27.5	1.611	0.3033	28 (2)
Maximum temperature for growth (Tmax)	°C	35 (3); 42 (8); 42 (9); 38 (10); 40 (11)	39.4	3.157	0.7416	42 (2)
Specific leaf area at emergence (SLAini)	$m^2 kg^{-1}$	35 (12); 39 (8); 50 (1); 45 (1); 39 (1)	41.6	6.224	1.4748	27 (2)
Specific leaf area at mid tillering (SLAtill)	$m^2 kg^{-1}$	23 (13); 26.6 (13); 28.68 (14); 27.2 (14); 28.51 (14); 20.92 (14); 30.5 (15); 32.9 (15); 29.1 (15); 31.4 (15); 34.5 (15); 30.9 (15)	28.6842	3.993	0.3537	18 (3)
Maximum radiation use efficiency (RUE)	$g\ MJ^{-1}$	3.28 (16); 2.37 (17); 2.32 (18); 2.41 (18); 2.77 (18); 2.59 (19); 3.52 (20); 3.25 (14); 2.69 (14)	2.8	0.451	0.0555	3 (0.5)
Extinction coefficient for solar radiation (k)	-	0.4 (1); 0.6 (1); 0.35 (1); 0.49 (21); 0.61 (21); 0.65 (22); 0.47 (13); 0.45 (19); 0.42 (19); 0.68 (20); 0.52 (14); 0.53 (14)	0.5142	0.105	0.0094	0.5 (0.04)

[a] 1: Kropff et al. (1994b); 2: van Heemst (1988); 3: Confalonieri et al. (2009); 4: Iizumi et al. (2011); 5: Sakaguchi et al. (2014); 6: Saito et al. (2016); 7: Koutroubas et al. (2004); 8: Confalonieri and Bocchi (2005); 9: Zhang et al. (2004); 10: Pagani et al. (2014); 11: Kim et al. (1996); 12: Asch et al. (1999); 13: Dingkuhn et al. (1999); 14: Boschetti et al. (2006); 15: Laza et al. (2015); 16: Horie and Sakuratani (1985); 17: Charles-Edwards (1982); 18: Horie et al. (1997); 19: Kiniry et al. (2001); 20: Campbell et al. (2001); 21: Casanova et al. (1998); 22: Monteith (1969).

2.4. Results and Discussion

Fig. 2 shows the results of the 6144 1^{st} level SA. On average, the parameters LAIini, RUE and Topt were those with the largest influence on model output, in agreement with what achieved by Confalonieri et al. (2010b) with the same model. However, the ranks for the three parameters calculated in this study were different: LAIini was ranked first instead of third, RUE second instead of first, and Topt third instead of second. Considering the very similar agronomic and environmental conditions for the two studies and the same parameterization of the SA method, differences in results can be explained only by the differences in parameter distributions (Table 1). This is a first demonstration of how crucial distributions are in SA, given that differences in the distributions used in the two studies led to different rankings for most relevant parameters, whereas the same ranks were observed by Confalonieri et al. (2010b) for different continentality regimes. The importance of distributions in affecting SA results is confirmed by the results achieved in this study. Indeed, despite the large difference in the mean values of the Sobol' total order effect (St) for LAIini and RUE, their rank was overturned in almost 22% of the 1^{st} level SA because of differences in the generated means (Jm_{ig}), in turn used to generate the samples of parameter combinations.

Besides the three most relevant parameters – i.e., LAIini (responsible on average for 46% of the output variance), RUE (31%) and Topt (10%) – RipL0 and Tbase explained, on average, 3.5% and 2.9% of the total variability in model output, whereas k and Tmax explained only 1.7% and 1.1%. The other parameters achieved St values close to zero and are not further discussed.

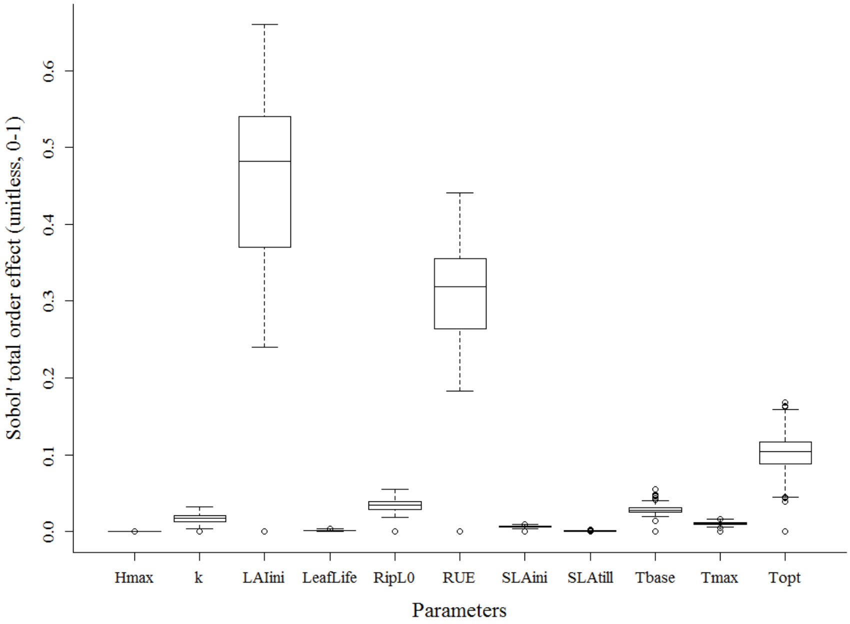


Figure 2. Box plots of the Sobol' total order effects (St) for model parameters obtained for the 6144 1st level sensitivity analyses. Variability results from the use of different distributions (generated) for model parameters.

Fig. 3 shows the results of the 2nd level SA (impact of different parameter distributions). The uncertainty in the distribution of LAIini (on average ranked first in 1st level SA) explained a large part of the variability in SA results, i.e., in the St values obtained for the different parameters during the 1st level SA. In particular, it explained almost all the variability in the St obtained for LAIini (99%, Fig. 3.a), RUE (98%, Fig. 3.f), Topt (89%, Fig. 3.d) and k (83%, Fig. 3.g). It explained a smaller part of the variability in St for RipL0 (Fig. 3.b) and Tbase (Fig. 3.c), whereas it was not relevant for explaining the variability in St for Tmax (Fig. 3.e). Contrarily, the uncertainty in the distribution of RUE (ranked 2nd in 1st level SA) did not affect SA results: regardless of the parameter (including RUE itself), changes in its distribution never explained more than 3% of the variability in St values.

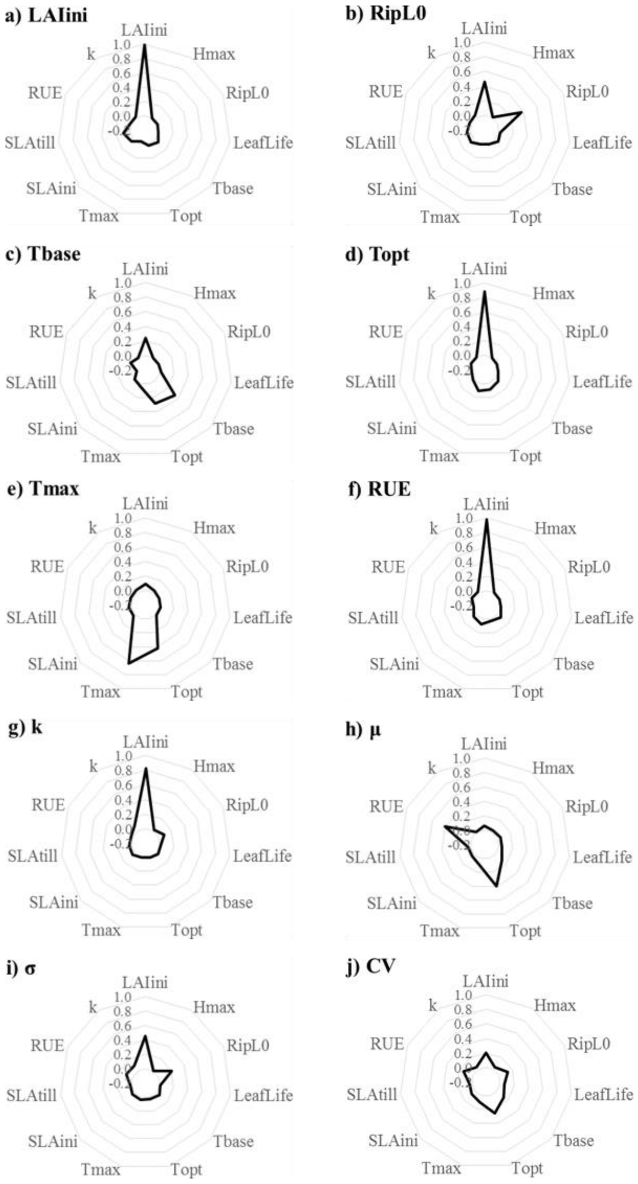


Figure 3. Sobol’ total order effects (St*) calculated for the 2nd level sensitivity analysis (SA) (impact of uncertainty in parameter distributions) using different outputs from the 6144 1st level SA (uncertainty in parameters). a to g: outputs considered were the 1st level SA St for model parameters; h to j: outputs considered were statistics (μ: mean, σ: standard deviation, CV: coefficient of variation) calculated on the 6144 aboveground biomass (AGB) values simulated for each 1st level.

This can be explained by the pronounced linear effect of RUE on AGB accumulation. Indeed, for each model parameter, all generated means Jm_{ig} were used for the 1st level SA with the same value of standard deviation (jackknife estimate of the standard deviation Js_i). Given the role of RUE on the daily rate of biomass accumulation (Warren Wilson, 1967), this led to RUE distributions for the 1st level SA that did not alter considerably the relative role of parameter uncertainties in affecting AGB.

The uncertainty in the distribution of Topt (on average ranked third during the 1st level SA) explained 42% and 28% of the variability in the St values obtained for Tmax (Fig. 3.e) and Tbase (Fig. 3.c), respectively. This made this parameter the one whose uncertainty affected SA results more markedly after LAIini. The reason is likely due to the strong interaction of this parameter with Tbase and Tmax, since they are simultaneously used to define the beta function adopted for thermal limitation to photosynthesis (Yin and Kropff, 1996).

The values of St* for the 2nd level SA calculated on AGB statistics (in turn deriving from the 1st level SA) showed a different picture in terms of relative role of parameter distributions. The variability of the means of the AGB values simulated for each 1st level SA (Fig. 3.h) is largely explained by the uncertainty in the distributions of RUE and Topt (both explaining 40% of the total variance in AGB means). The parameter ranked third in terms of relevance of distribution uncertainty on AGB means is Tbase, whereas the uncertainty in the distribution of LAIini (on average ranked first by 1st level SA) explained only 6% of the variability in AGB means, although it explained 45% of the variability in AGB standard deviations (Fig. 3.i). This can be partly explained by the variability (CV = 64%, with CV for the other parameters ranging between 6% and 24%) in the values for this parameter originally retrieved from the literature (Table 1). The uncertainty in the distribution of RipL0 (on average ranked fourth in the 1st level SA) usually did not affect mean AGB values, although it was ranked second for the impact on the standard deviation of the AGB values simulated during the 1st level SA (Fig. 3.i). Like for LAIini, this is likely explained by the variability (CV = 24%) in literature values for this parameter.

Considering the coefficient of variation as output for the computation of the St values for the 2nd level SA (Fig. 3.j), the uncertainty in the distribution of Topt explained more than 25% of the variability, whereas LAIini explained 20% and RipL0, RUE and Tbase explained about 13%.

2.5. Conclusions

The procedure proposed – actually a sensitivity analysis of a sensitivity analysis – demonstrated its capability of quantifying the impact of the uncertainty in parameter distributions on sensitivity analysis results. Indeed, it proved to be able to identify parameters for which uncertainty in distribution has the largest impact on sensitivity analysis results. Besides the conceptual relevance, the procedure has practical implications for scientists interested in using sensitivity analysis techniques. As an example, it would allow – before running the analysis – to better investigate the distributions for the parameters whose uncertainty is more relevant, e.g., by searching more data to refine the distributions or by screening literature data according to the experimental conditions or to the method used for their estimate. To give an idea of the practical implications that could derive from uncertainty in distributions, we can assume that the simulation experiments presented in the case study would have targeted plant traits identification for ideotyping purposes. In this case, the overturn of LAIini and RUE as top-ranked parameter due to different distributional assumptions (Fig. 2) would have led to plan breeding programs focusing on traits dealing with photosynthetic efficiency (Zhu et al., 2010) instead of traits involved with coleoptile leaf size and initial leaf expansion, and with the effect of environment and management on emergence dynamics (Ogiwara and Terashima, 2001).

However, these considerations are valid regardless of the purpose of the specific study, e.g., identifying parameters to calibrate (van Werkhoven et al., 2009) or traits breeders should focus on under specific conditions

(Martre et al., 2015b), analyzing model structure (Sieber and Uhlenbrook, 2005) or developing new models (Jakeman et al., 2006).

In general, the procedure suggests being very careful when defining distributions: especially for parameters with a non-linear effect on model outputs and for which interactions with other parameters are expected, the uncertainty in distribution may markedly alter the results of the analysis, even overturning the ranks for most relevant parameters.

Moreover, the model used in this study, i.e., WARM, demonstrated in past studies (Confalonieri et al., 2010b, 2012) its low plasticity compared to other crop models (e.g., WOFOST, van Keulen and Wolf, 1986; CropSyst, Stöckle et al., 1994). It is largely expected that the uncertainty in parameter distributions would have an even stronger impact on sensitivity analysis results in case of models with more parameters playing a key and homogeneous role in explaining the variability of model outputs.

Acknowledgments

This research received funding from the European Community's Seven Framework Programme-FP7 (KBBE.2013.1.4-09) under Grant Agreement No. 613817, 2013-2016, MODelling vegetation response to EXTREMe Events (MODEXTREME, modextreme.org).

SURFING PARAMETER HYPERSPACES UNDER CLIMATE CHANGE SCENARIOS TO DESIGN FUTURE RICE IDEOTYPES

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Re-submitted after revisions to *Global Change Biology*

3.1. Abstract

Growing food crops to meet global demand and the search for more sustainable cropping systems are increasing the need for new cultivars in key production areas. This study presents the identification of rice traits putatively producing the largest yield benefits in five areas that markedly differ in terms of environmental conditions in the Philippines, India, China, Japan and Italy. The ecophysiological model WARM and sensitivity analysis techniques were used to evaluate phenotypic traits involved with light interception, photosynthetic efficiency, tolerance to abiotic stressors, resistance to fungal pathogens and grain quality. The analysis involved only model parameters that have a close relationship with phenotypic traits breeders are working on, to increase the in vivo realizability of selected ideotypes. Current climate and future projections were considered, in light of the resources required by breeding programs and of the role of weather variables in the identification of promising traits. Results suggest that breeding for traits involved with disease resistance and tolerance to cold- and heat-induced spikelet sterility could provide benefits similar to those obtained from the improvement of traits involved with canopy structure and photosynthetic efficiency. In contrast, potential benefits deriving from improved grain quality traits are restricted by weather variability and markedly affected by G×E interactions. For this reason, district-specific ideotypes were identified using a new index accounting for both their productivity and feasibility.

Keywords: Blast, canopy structure, chalkiness, head rice, photosynthetic efficiency, sensitivity analysis, spikelet sterility, WARM.

3.2. Introduction

An ever-increasing world population is generating a need for more efficient cropping systems (Foley et al., 2011). This can be achieved via more sustainable and effective management practices and via the development of genotypes that are more efficient under changing climate conditions (Habash et al., 2009; Ziska et al., 2012).

During the Green Revolution, yield potential was increased mainly by breeding for greater photosynthate partitioning to harvested organs and by changing canopy structure to increase the efficiency of light interception (Drewry et al., 2014). According to many authors, several yield related traits are close to their biological limits (e.g. Long et al., 2006) and this highlights the need to target a wider number of traits that determine crop productivity (Drewry et al., 2014). There is undoubtedly room for further improvements to both the efficiency with which light is captured by crop canopies (Long et al. 2006) and photosynthetic efficiency, for which there are still wide theoretical margins for improvement (Zhu et al., 2010). Some desirable traits reduce the yield gap (Espe et al., 2016) by conferring increased resistance/tolerance to biotic and abiotic stressors (Kush, 2001; Oerke, 2006; Paleari et al., 2015). Improving tolerance to extreme climatic events is key to facing future challenges dealing with food security (Battisti & Naylor, 2009; Semenov & Stratonovitch, 2013). Another factor that is expected to play a key role under climate change conditions is the quality of products, especially for cereals (Porter & Semenov, 2005; Martre et al., 2011).

Ecophysiological models are increasingly used to support breeding programs via the putative identification of desirable traits (e.g. Tardieu, 2003; Dingkuhn et al., 2007; Herndl et al., 2007; Messina et al., 2011; Martre et al., 2015a; Casadebaig et al., 2016), or quantifying the performance of improved genotypes under a wide range of edaphic and climatic conditions (Jeuffroy et al., 2013). This can be done by using single crop models or model ensembles, like in the study from Tao et al. (2017), where eight models were used to design climate-resilient barley ideotypes for Boreal and Mediterranean climatic zones in Europe.

An effective way to identify traits to improve yield performance is by conducting sensitivity analysis using ecophysiological models (Martre et al., 2015a). Sensitivity analysis, indeed, quantifies the portion of model output variance explained by changes in the values of input factors (Tarantola & Saltelli, 2003). The underlying assumption, of course, is the existence of a close relationship between model parameters and plant traits (Semenov & Stratonovitch, 2013; Casadebaig et al., 2016), which does not always hold. As an example, most rice models among those analysed by Li et al. (2015) have a parameter for leaf area index at emergence, which has nothing to do with a trait but it is simply needed to allow the interception of radiation at the first time step after emergence. The reason, of course, is the lack of explicit representation of the processes involved with germination and with heterotrophic growth of rice seedlings. In addition, some plant breeders focus on traits (e.g., stay-green; Thomas and Ougham, 2014) for which most models do not have corresponding parameters (Fig. 1).

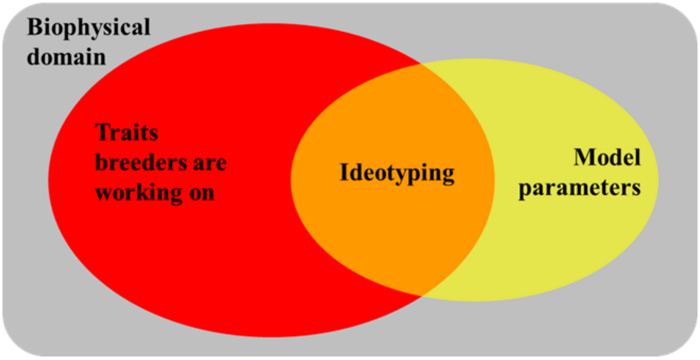

Figure 1. Diagram showing how model-based ideotyping only deals with a fraction of plant traits and model parameters, given the lack of relationships between some plant traits and model parameters.

The complex interactions between genotype (G), environment (E) and management (M), as well as the large impact of explored conditions on sensitivity analysis results (Confalonieri et al., 2012), strongly suggest there is value in performing this kind of analysis at a sub-region level (Paleari et al., 2015). The objective of this study is the identification of rice traits that putatively produce the largest yield increase in five areas that markedly differ in terms of environmental conditions in the Philippines, India, China, Japan, and Italy. Phenotypic traits that were analysed impact light interception and photosynthesis, tolerance to abiotic stressors, resistance to diseases and grain quality. Given the time and resources required to develop improved cultivars (Brennan & Martin, 2007; Heffner et al., 2010) and the impact of promising traits as affected by environmental variables (Zheng et al., 2012), the study was performed under both current (1986-2005 baseline) and projected climate conditions. In particular, 20-year series centred on 2030 and 2050 were derived for IPCC AR5 (IPCC, 2013) representative concentration pathways (RCP) 2.6 and 8.5 and two general circulation models using a stochastic weather generator.

Although ecophysiological models are far from being completely suitable for ideotyping, they are increasingly structured to reproduce the interaction between plant physiology and genetics (Luquet et al., 2016). We attempted to reduce discrepancies between in silico ideotypes and in vivo realizations by selecting model parameters that have a direct link with specific phenotypic traits (orange area in Fig. 1). This allowed us to test in silico strategic breeding, while avoiding the need to conduct sensitivity analysis on all model parameters.

3.3. Materials and methods

3.3.1. The modelling solution

An hourly-time step modelling solution based on WARM (Confalonieri et al., 2009; Pagani et al., 2014) was used. A micrometeorological model (Confalonieri et al., 2005) allows using temperatures at the meristematic apex for phenological development and spikelet sterility induced by thermal shocks, whereas mid-canopy temperature is used for photosynthesis, leaf senescence and leaf blast infection. The micrometeorological model is based on the solution of the surface energy balance equation at different depths into the canopy; the same approach is used for floodwater temperature, with an additional term representing the heat stored into the water (function of net radiation and water depth). In particular, temperature at the meristematic apex is assumed the same as the water before panicle initiation, whereas later as the canopy layer corresponding to the panicle height. Net photosynthesis is quantified through the concept of radiation use efficiency (RUE), with the latter modulated by temperature, senescence, enzymatic chains saturation to light, and rice blast disease, *Magnaporthe oryzae* B. Couch (Confalonieri et al., 2009). Photosynthate partitioning to plant organs is based on a set of parabolic and beta functions, with leaf area index (LAI) increase derived by daily leaf mass production and a dynamic specific leaf area (SLA). Leaf senescence is simulated based on thermal time accumulated by each daily-emitted unit of leaf area. Cold-induced spikelet sterility is estimated from the difference between a threshold temperature and hourly temperatures at the meristematic apex during stem elongation (microsporogenesis; Hayashi et al., 2006) and flowering (development of the anthers as well as the dehiscence, ripening, shedding and germination of pollen grains; Sanchez et al., 2014). The heterogeneity in development between plants and between tillers of the same plant (time interval between the emergence of successive tillers) is taken into account by weighting daily stress values (in turn derived by cumulating hourly ones) by using bell-shaped functions (Confalonieri et al., 2009). Heat-induced sterility is estimated in a similar fashion around flowering. The day of the disease onset is estimated based on hydrothermal time accumulation according to Arai & Yoshino (1987) and Kim (2000) as a function of hourly air temperature for hours with relative humidity exceeding 93%, whereas the daily rice blast infection efficiency is computed according to Magarey et al. (2005). The simulation of the length of the latency, incubation and infectious periods is based on hourly air temperature. Damages to leaf tissues are incorporated using a compartmental susceptible-infected-removal model, with decrease in photosynthate accumulation based on the estimated loss in photosynthetic leaf area due to blast lesions. Reduction of grain yield is reproduced by reducing the daily fraction of assimilates partitioned to the panicles by the rice growth model after the panicle initiation stage (Bregaglio et al., 2016).

Simulated grain quality variables are the percentage of kernels that are chalky or opaque and head rice yield (relative weight of largely intact kernels, also referred to as whole grain, after milling), both of which are key factors in determining rice market price at global level. The percentage of chalky kernels is simulated depending on the growing degrees daily accumulated above a critical temperature post-heading, modulated according to cultivar-specific susceptibility and seeding density (Nagahata et al., 2006). Potential head rice yield is simulated as function of hourly night-time air temperature during grain ripening, and is then decreased when rainfall, wind speed and temperature exceed critical thresholds for starch synthesis during the sensitive period after full flowering (Cappelli et al., 2016). Differences among varieties for susceptibility to chalkiness is represented by different values of the threshold temperatures inducing the damage; a threshold temperature is also used to reproduce the cultivar-specific susceptibility to grain breakage.

3.3.2. The ideotyping experiments

The analysis was conducted for the sites described in Table 1. The first four sites (Los Baños, Ludhiana, Nanjing, and Shizukuishi) were described by Li et al. (2015), who provided detailed data on management and on other information used to run simulations. The Milan site was added to expand the climatic conditions included in the analysis, with management information for the simulations provided by Confalonieri et al. (2010). For each site, sensitivity analyses were conducted for current climate conditions (baseline, 1986-2005) and for two projected 20-year time frames centred on 2030 and 2050. Climate change scenarios were generated for the IPCC AR5 (IPCC, 2013) RCP 2.6 (emissions peak in 2010-2020, decline later) and 8.5 (emissions continue to rise), to account for the largest variability in future projections. For the same reason, two general circulation models (GCMs) were used: HadGEM2 (Hadley Centre, UK; Collins et al., 2011) and GISS-ES (NASA; Schmidt et al., 2006). For each time frame × RCP × GCM, synthetic weather series were generated using the CLIMAK weather generator (Danuso, 2002; Confalonieri, 2012).

Table 1. Study sites and characterization.

Site	Los Baños	Ludhiana	Nanjing	Shizukuishi	Milan
Country	Philippines	India	China	Japan	Italy
Coordinates	121°9'E, 14°6'N	75°48'E, 30°54'N	118°59'E, 32°56'N	140°57'E, 39°41'N	8°41'E, 45°4'N
Climate type	Tropical, humid	Subtropical, semiarid	Subtropical, semihumid	Cool temperate, humid	Temperate, semiarid
Mean Tmax[a] (°C)	30.2	29.3	20.3	13.7	18.2
Mean Tmin[b] (°C)	23.2	16.8	12.0	5.1	8.6
Mean Rad[c] (MJ m^{-2} d^{-1})	15.9	18.7	14.1	12.1	14.6
Yearly rainfall (mm)	2060	703	1076	1557	698
Emberger continentality[d]	11.0 (oceanic insular)	31.8 (semi-continental)	32.3 (semi-continental)	33.1 (semi-continental)	31.1 (semi-continental)
SAM Aridity index[e]	0.13	-0.39	-0.20	-0.01	-0.36

[a] Mean of maximum daily air temperature.

[b] Mean of minimum daily air temperature.

c Mean of daily global solar radiation.

[d] Difference between mean daily maximum temperature of the warmest month and of mean daily minimum temperature of the coldest month.

[e] (ET0-Rain)/(ED0+Rain), with ET0 and Rain being cumulated reference evapotranspiration and rainfall, respectively.

Parameters on which sensitivity analyses were conducted are listed in Table 2, with references supporting their key role in breeding programs, the distribution used to sample the parameters hyperspace, and the sources of information used to produce the distributions. The sampling of incoherent values from the distributions was avoided using a 0.05 truncation.

For T-ColdSter, two distributions were used to account for marked differences comparing genotypes grown under temperate and tropical environments.

The study was performed using the variance-based global sensitivity analysis method of Sobol' (1993), considered as a reference for sensitivity analysis (Saltelli & Sobol', 1995), with the variance of model output decomposed in terms of increasing dimension (partial variances) representing the contribution of each parameter, pair of parameters, triplets, etc. to the overall output uncertainty. Monte Carlo sampling is used to explore the parameter hyperspace. Statistical estimators of partial variances are calculated through multi-dimensional integrals. In this study, we focused on the total order effect (S), providing a synthetic representation of the effect of each parameter, including possible interactions with others (Homma & Saltelli, 1996).

The number of simulations for each experiment was the lowest value of $M|M > (\gamma \cdot n)$, with $M = 2^{q+3}(2n + 2)$, $M = 2^{q+3}(2n + 2)$, γ is the number of model runs for each parameters (Confalonieri et al., 2010), and n is the number of parameters in the sensitivity analysis. The number of combination of parameters varied for the simulations was 5120.

The variable analyzed was:

$$Y_L \cdot V - Y_L[(1 - HR) + C] \cdot \frac{V}{2}$$

where Y_L (t ha^{-1}) is grain yield, V (€ t^{-1}) is the value of whole and non-chalky grains, HR (unitless, 0-1) is the head rice yield, C (unitless, 0-1) is the chalkiness. This simultaneously accounts for both production (t ha^{-1}) and the value of broken and chalky grains being about half of the value of non-chalky whole grains. The mean of the outputs simulated over the 20 years was used, to account for the seasonal variability in weather data.

The total number of simulations was in excess of 6.6 millions.

Parameters were ranked using the Sobol' total order sensitivity index and the agreement between rankings from different districts and under multiple climate scenarios within the same location was evaluated using the top-down coefficient of concordance (TDCC; Helton et al., 2005). Values for TDCC close to one indicate a high level of consistency between the rankings of parameters.

Table 2. Parameters on which the sensitivity analyses were performed, relevance for breeding of the corresponding traits, parameters distribution and source of information.

Parameter	Relevance for breeding[a]	Distribution[b]	Source
Radiation use efficiency (RUE; g MJ^{-1})	Peng et al., 2008; Dingkhun et al., 2015	Normal (*m* 2.7; *s* 0.1)	Kiniry et al., 2001; Boschetti et al., 2006
Light extinction coefficient (k; -)	Peng et al., 2008; Sheehy et al., 2013	Normal (*m* 0.47; *s* 0.04)	Casanova et al., 1998; Dingkhun et al., 1999; Kiniry et al., 2001; Boschetti et al., 2006
SLA at emergence (SLAini; m^2 kg^{-1})	Peng et al., 2008; Kush , 2012	Normal (*m* 41.6; *s* 5.9)	Kropff et al., 1994b; Ash et al., 1999; Confalonieri & Bocchi, 2005
SLA at tillering (SLAtill; m^2 kg^{-1})	Kush, 2001; Peng et al., 2008	Normal (*m* 28.7; *s* 3.9)	Laza et al., 2015; Boschetti et al., 2006
Threshold T for cold sterility (T-ColdSter; °C)	Suh et al., 2010; Andaya & Mackill, 2003	Normal (temp[c] *m* 13.5; *s* 1.4) (trop[d] *m* 17.2; *s* 0.8)	Satake, 1987; Da Cruz et al., 2006; Farrel et al., 2006; Gothandam et al., 2007; Thakur et al., 2010; Dreni et al., 2012; National Rice Authority
Threshold T for heat sterility (T-HeatSter; °C)	Matsui et al., 1997; Jagadish et al., 2010	Normal (*m* 34.4; *s* 1.5)	Yoshida, 1981; Satake, 1995; Matsui, 1997; Ishimaru et al., 2010; Jagadish et al., 2010; Shah et al., 2011; Maruyama et al., 2013 Satake and Yoshida 1978.
Blast resistance (BlastRes; -; 1 to 3)	Fukoka et al., 2009, Fisher et al., 2012	Discrete (1, 2, 3)	National Rice Authority
Threshold T for chalkiness (T-Chalkiness; °C)	Yamakawa et al., 2007; Usui et al., 2014	Normal (*m* 26.4; *s* 0.9)	Wakamatsu et al., 2007; Yamakawa et al., 2007; Morita, 2008; Madan et al., 2012; Usui et al., 2014; Matsutomi et al., 2015
Threshold T for grain breakage (T-HeadRIce; °C)	Siebenmorgen et al., 2013; Sreenivasulu et al., 2015	Normal (*m* 23.9; *s* 2.1)	Ambardekar et al., 2011; Okada et al., 2011; Siebenmorgen et al., 2013

[a] Sample references among those available.
[b] In case of normal distribution, *m* is the mean and *s* is the standard deviation; truncation = 0.1.
[c] Distribution used for temperate environments.
[d] Distribution used for tropical environments.

Sensitivity analysis can be used not only to provide indications about traits with the highest impact on yield but also to explore putative ideotypes (Suriharn et al., 2011). The combination of parameter values derived by sampling the parameters hyperspace, indeed, can be regarded as hypothetical genotypes, which can be evaluated based on productivity. However, yield is not the only criteria to consider for this purpose. In order to derive a comprehensive evaluation of the ideotypes, a synthetic index was also developed (Ideotype score, I_{score}, unitless):

$$I_{score} = \left[\sum_{i=1}^{n}\left(\left(\frac{|x_i - m_i|}{m_i} \cdot 100\right) \cdot \frac{1}{\sqrt{S_i}}\right) \cdot \frac{1}{n}\right] \cdot \left(1 - \frac{Y_v}{Y_{vmax}}\right)$$

where n is the number of parameters defining the ideotype, x_i is the value of the ith parameter, m_i is the distribution mean of the ith parameter, S_i is the Sobol' total order index for the ith parameter, and $Y_v/Y_{v\,max}$ is the production of the ideotype (expressed as € ha^{-1}) normalized to the maximum of all ideotypes under evaluation. This index takes into account (i) how much a trait should be improved as compared to the population mean and (ii) its relative importance for yield via the value of S (i.e., what is the effect of the improvement in terms of productivity). This allows scoring for traits for which even small improvement has an effect on yield. For each site and time frame × RCP × GCM combination, the I_{score} was calculated for each of the 5120 combinations of parameters, and the district-specific ideotype was derived by averaging the parameter values of the 1% combinations top-ranked according to I_{score}. This allows identifying ideotypes that are less affected by the presence of local minima in the hyperspace and to avoid providing breeders with putatively superior ideotypes that have a low probability of being reproducible *in vivo*.

3.4. Results

Results are presented and discussed only for the extreme climate scenarios that were tested: RCP8.5-HadGEM2 (the warmest) and RCP2.6-GISS-ES (the mildest). Moreover, only results for the 2030 time frame are discussed in detail, due to relevant differences not arising comparing the two time frames.

Regardless of the site and climate scenario, parameters involved with photosynthetic efficiency and light interception had a high impact on yield. For the latter, SLA_{till} (involved with leaf area expansion) and k (canopy light extinction coefficient, involved with canopy structure) always explained a large part of the output variability (Fig. 2), whereas the relevance of SLA_{ini}

(leaf area expansion during early vegetative growth) was markedly influenced by the interaction with environmental conditions. Indeed, SLA_{ini} achieved high values for the sensitivity metric only in Shizukuishi (Fig. 2j, k, l), i.e., the coldest site (Table 1) among those studied. This is largely explained by considering that the thermal limitation to photosynthesis is partly compensated for by rapid canopy development. For the same reason, changes in the sensitivity metric under different environments were obtained also for k, for which the highest values for S were obtained for the site where the average radiation was lowest (Shizukuishi) and vice versa (Table 1).

However, the largest variability in SA among sites and climate scenarios was obtained for parameters involved with resistance/tolerance to biotic and abiotic stressors. The parameter representing blast resistance had the highest relevance in Milan under both current climate and future projections. Although to a lesser extent, the same was achieved for Nanjing and Ludhiana, whereas very low sensitivity metrics were estimated in Los Baños and Shizukuishi. It is important to note that low model sensitivity to a parameter does not mean that the impact of the process involved is negligible. It just means that changes in the parameter value explain only a small portion of the total variance in model outputs. Indeed, the mean impact of blast disease on yield simulated for Los Baños and Shizukuishi was, under current climate, equal to 12.1% and 11.7%, respectively.

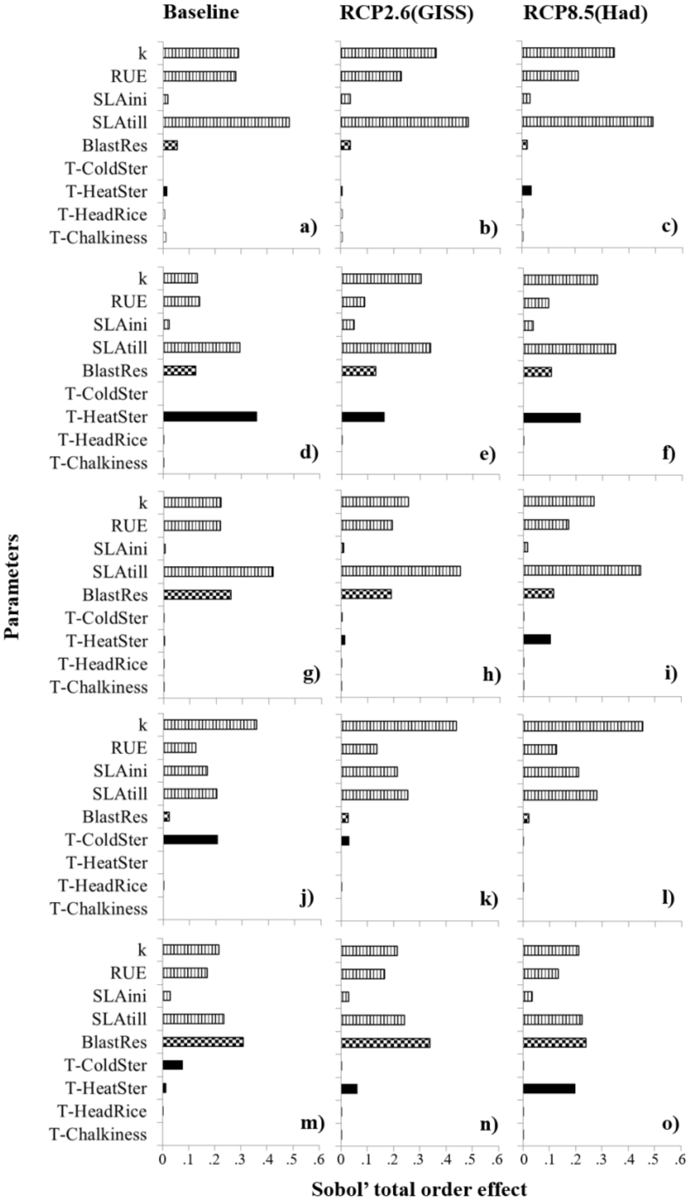

Figure 2. Sensitivity analysis results for current climate (left column) and for the 2030 time frame: RCP2.6-GISS-ES (central column), RCP8.5-HadGEM2 (right column). a, b, c: Los Baños; d, e, f: Ludhiana; g, h, i: Nanjing; j, k, l: Shizukuishi; m, n, o: Milan. *Stripped, chequered, black and white bars* refer to parameters involved with interception and photosynthesis, blast resistance, tolerance to temperature shocks, grain quality.

As expected, strong G×E interactions affected the relevance of parameters involved with tolerance to heat- (T-HeatSter) and cold-induced (T-ColdSter) spikelet sterility. Under current climate conditions, the model was markedly sensitive to T-HeatSter only in Ludhiana (Fig. 2d), where the average maximum temperature during the season is the highest. The relevance of this parameter was slightly lower under future climate projections, despite the general temperature increase. This was not due to changes in simulated phenological development that shifted the time window when the crop is susceptible but to the overall increase in the frequency and intensity of the events, that increased the mean impact of heat-induced sterility while decreasing, regardless of the parameter value, the variability in related outputs. For the Milan and Nanjing sites, the projected temperature increase resulted in an increased impact of T-HeatSter, which was more pronounced for the warmer scenario (RCP8.5 HadGEM2). The model was sensitive to T-ColdSter only in the two coldest sites of Shizukuishi and Milan and only under the current climate; the projected warming of climate, indeed, is expected to reduce the relevance of this parameter (Paleari et al., 2015).

Changes in the values of parameters involved with grain quality did not affect model output (i.e., V, € t-1), despite the overall relevance of the processes involved with head rice, e.g., broken rice under current climate ranged between 17.5% (Los Baños) and 19.7% (Shizukuishi). This suggests that, under the conditions explored, the influence of environmental factors is larger than the genotype effect.

The marked heterogeneity in the climate conditions and the G×E×M interactions affecting the importance of parameters representing resistance/tolerance traits led to poor concordance of parameter rankings among sites (TDCC values ranging from 0.52 to 0.75), thus confirming the need to perform ideotyping studies at a sub-region level. In contrast, concordance between rankings obtained within the same location under different climate change scenarios (RCP2.6-GISS-ES and RCP8.5-HadGEM2) was higher, with TDCC always exceeding 0.98. This proves the independence of the relevance of the key traits identified via sensitivity analysis from the uncertainty in climate change projections. Instead, the inclusion of current climate (baseline) in the evaluation of the concordance among parameter rankings within site led to lower values for TDCC (ranging from 0.84 to 0.98). This demonstrates the importance of testing ideotypes under both current climate and future projection to define medium-term breeding strategies.

The improvement suggested for the traits under evaluation – expressed as percentage variation compared to the parameter distribution mean – is shown in Fig. 3. In general, ideotypes presented higher values for parameters representing traits involved with canopy architecture and photosynthetic efficiency (SLA_{till}, k, RUE), with the exception of SLA_{ini}. In this case, the pathways of improvement followed both directions based on the environment: higher than the distribution mean for cold sites (e.g., Shizukuishi, Fig. 3d) and lower for warm sites, especially for HadGEM-RCP8.5. Indeed, while sensitivity analysis, alone, led to identifying key traits, its use within I_{score} allowed the extent and the direction for improvement to be better defined. The reduction in SLA_{ini} under warm conditions is explained by considering its role in determining the increase in LAI during early stages. Indeed, the higher the rate of increase of LAI during early growth, the faster the decrease of green LAI due to senescence when those LAI units die, after they have accumulated a given thermal time. In warm environments, the thermal time threshold is reached earlier and the simulated decrease in green LAI is more rapid. In case of high SLA_{ini}, the LAI units that start dying at the beginning of senescence are larger and the photosynthetic rates in mid-late stages are lower, due to less radiation being intercepted.

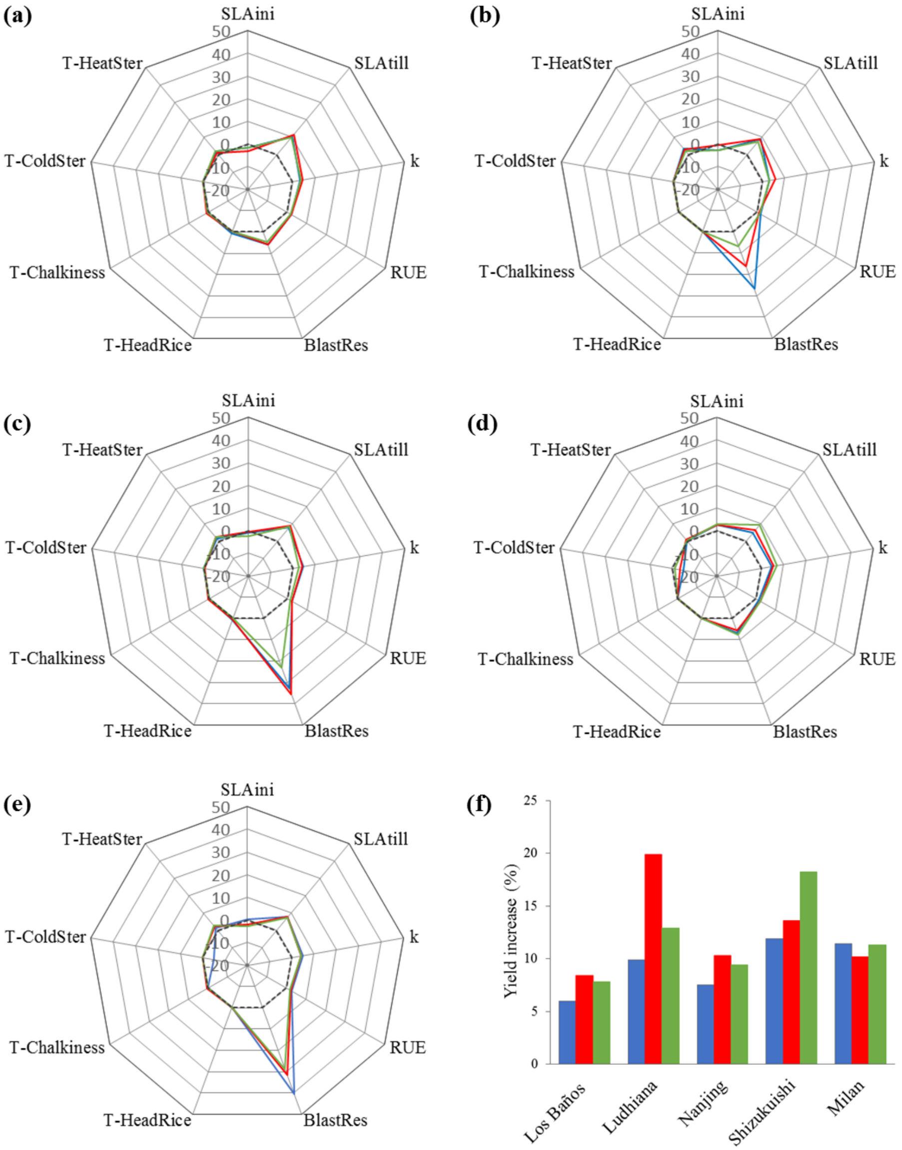

Figure 3. Profile of the ideotypes identified for the five districts. a: Los Baños; b: Ludhiana; c: Nanjing; d: Shizukuishi; e: Milan. Traits improvement is expressed as percentage variation of the values of related parameter compared to the distribution mean (*dashed lines*). f: percentage yield increases compared to the genotype defined by parameter distribution means. *Blue series* refers to current climate, *red series* to a 2030 climate change projection derived using RCP2.6 and GISS-ES, *green series* to a 2030 climate change projection using RCP8.5 and HadGEM2.

Regarding the traits involved with resistance/tolerance to biotic/abiotic stressors, district-specific ideotypes showed marked heterogeneity. Improvement for blast resistance was particularly relevant in Milan (Fig. 3e), Nanjing (Fig. 3c) and Ludhiana (Fig. 3b) (from +40.4% to +26.9% in parameter value under current climate), whereas it is more limited in Los Baños (Fig. 3a) and Shizukuishi (Fig. 3d) (lower than +10%). The importance of improvements for blast resistance also changed according to the climate scenario, decreasing for the warmest and driest projection (HadGEM-RCP8.5), i.e., for conditions less favourable for blast disease epidemics. However, the evolutionary potential of the pathogen in the medium-long term was not considered in this study, since information is currently not available to predict the development of virulent strains.

Heterogeneity across sites and scenarios characterized ideotypes in terms of tolerance to heat- and cold-induced spikelet sterility, as already shown by the sensitivity analysis. Despite the high value for the related sensitivity metrics, the extent of the improvement required for the ideotypes was quite limited, never exceeding 5%. This is because the high model sensitivity to changes in the values of the parameters produced significant yield benefits even with small variations in parameter values. Once more, this highlighted the importance of I_{score} for identifying possible breeding pathways.

In general, although the approach proposed led to quite conservative ideotypes (lacking extreme deviations for parameter values compared to the distribution means), the simulated yield increase ranged from 6% to 20% (Fig. 3f). Using broader distributions would have led to larger variations in parameters/traits and to greater increases in ideotypes' productive performance, but would have very likely increased doubts about the possibility of producing *in vivo* the resulting plant types. However, in case of availability of data coming from dedicated field measurements carried out on breeding populations, the ideotypes can be further refined to exploit the available genetic variability.

3.5. Discussion

We performed a global sensitivity analysis involving parameters dealing with canopy architecture and photosynthetic efficiency, resistance/tolerance to biotic and abiotic stressors and grain quality in five contrasting rice production areas, under current weather and climate change scenarios to identify future rice ideotypes. These parameters are related with traits currently regarded as priority for breeding (Peng et al., 2008; Dingkuhn et al., 2015), but a comparative evaluation of their relative importance under different conditions was not available. The analysis was performed only on parameters with a close link to traits breeders are working on. This would allow an increase in the in vivo realizability of ideotypes and to consider the effect of the interactions among target parameters (traits). Such interactions – quantified by total-order sensitivity index – are indeed specific for the set of parameters analysed, thus preventing the inclusion of the effect of interactions of target parameters with others without clear relationships with traits. Although breeding for CO2 responsiveness is increasingly being considered as a promising strategy for C3 crops (Ziska et al., 2012), this was not considered in this study. Reasons are the non-systematic relationships between CO2 responsiveness and specific plant features (in turn generating the absence – at the present – of specific models), and the difficulties in up-scaling knowledge on related biological processes from single leaf/plant to canopy level (Ziska et al., 2012). Concerning climate scenarios, CO2 concentration was here considered as affecting only photosynthetic rate. However, the modelling community should start developing tools suitable for supporting breeding for CO2 responsiveness, especially considering the interactions between rising CO2 and key processes and traits for which breeding programs are on-going (Ziska et al., 2012), like, e.g., those involved with grain quality (Taub et al., 2008), resistance to diseases (Lake and Wade, 2009), tolerance to water stress (Lin and Wang, 2002), and leaf morphological features (Ishizaki et al., 2003).

Results we obtained suggest that breeding for traits involved with disease resistance and tolerance to sterility induced by thermal shocks could provide benefits similar to those obtained from improving canopy structure and photosynthetic efficiency. However, breeding pathways should account for heterogeneity both in space (production areas) and time (climate change scenarios), given its key role in modulating ideotypes features. Resistance to blast disease is one of the traits with the largest impact on yields in Milan – in agreement with the priority given to this trait in Italian rice breeding programs (Faivre-Rampant et al., 2011) – but tolerance to heat stress also is expected to play a key role in the coming decades. The impact of climate change on ideotype features was reported also by Semenov & Shewry (2011), who suggested that heat stress could become the main constraint for wheat production in Europe in the future. Another important finding of the current study is that environmental factors may hide the potential benefits obtained by developing improved genotypes, as in case of traits involved with grain quality. This is in agreement with the large variability in the percentage of broken kernels because of seasonal variability during ripening (Rossel & Marco, 2008; Sreenivasulu et al., 2015).

Of course, this kind of analysis was built on the assumptions of a close relationship between model parameters and plant traits (Martre et al., 2015a) and of a proper formalization in models of the knowledge on physiological feedbacks and interactions between phenotypic features. Although to a different extent, both these assumptions hold only partially in available crop models (e.g., Messina et al., 2011), including the one used in this study. To alleviate potential undesired impacts of partial model unsuitability, ensemble modelling is becoming increasingly used within the scientific community (e.g., Rotter et al., 2012; Martre et al., 2015c; Li et al., 2015), and recent studies are available where multi-model ideotyping was suggested (Rötter et al., 2015) or performed (Tao et al., 2017). However, one of the key novelties of the current study was designing ideotypes by including in the analysis traits involved with resistance to pathogens and grain quality variables, and no other rice models are currently available that include both these aspects. This prevented us from performing the study using ensemble modelling techniques. Moreover, epistatic or pleiotropic interactions were not taken into account. Indeed, correlations between traits (due to gene-gene interactions or close linkage or genes affecting multiple traits) could limit the number of the potential ideotypes under evaluation. However, accounting for them would have required (i) correlation coefficients between plant traits and (ii) quantitative relationships between plant traits and model parameters. Moreover, correlation coefficients should be carefully derived via multi-environmental trials in order to avoid including any G×E effect in the relationships, and this is currently unavailable (Jahn et al., 2011). For these reasons, despite explicitly considering correlations between traits would likely increase the in vivo realizability of ideotypes (Yin et al., 2016), they are not included in current model-based ideotyping studies (e.g., Casadebaig et al., 2016; Quilot-Turion et al., 2016). Another potential criticism of our study is that, despite this likely being the first sensitivity analysis-based ideotyping study incorporating plant-pathogen interactions, we did not considered the evolutionary potential of the pathogen in response to changing climate and the potential for development of strains more adapted to local conditions (Chakraborty, 2013).

Despite the potential areas of improvements just discussed, one of the main strength of this study is related to the collective breadth of conditions explored and with the number of processes considered. Indeed, the analysis was performed on traits involved with light interception and photosynthesis as well as with tolerance to abiotic stressors, resistance to fungal pathogens and grain quality. Moreover, the use of multiple climate change scenarios allowed uncertainty associated with climate projections to be addressed and provide directions for the medium-long term for breeding programs. These elements make the current study an effective screening of traits that could represent a basis for further studies, which – starting from these findings – could refine the analysis through a deep involvement of breeders and, possibly seed companies, also to better tailor distributional assumptions on specific germplasm.

Sensitivity analysis confirmed its suitability to identify key traits under different agro-environmental contexts. The index we developed (I_{score}) moves a step forward, leading to the identification of district-specific ideotypes in light of both their productivity and their feasibility, by implicitly considering the probability of high-potential ideotypes to be successfully realized in vivo. The index is calculated from total-order effects and from the outputs of each simulation performed within the sensitivity analysis. Thus, I_{score} allowed deriving ideotypes directly from sensitivity analysis, without further steps. Other approaches, instead, use sensitivity analysis only to identify promising traits that should be considered by rice breeders (e.g., Martre et al., 2015a; Casadebaig et al., 2016), since the optimal values for those traits under target conditions (in turn defining the ideotype) are derived using optimization algorithms (e.g., Semenov and Stratonovitch, 2013; Drewry et al., 2014; Quilot-Turion et al., 2016). The approach we propose allows use of a single computational procedure for both purposes, since the optimization of trait values is performed by exploiting the capability of the sensitivity analysis sampling techniques to effectively explore the parameter hyperspace. In case quantitative information on correlated traits will be available, our approach would allow for accounting for pleiotropy and epistasis, since advanced methods for sensitivity analysis allow sampling parameter hyperspaces by explicitly considering correlations (Mara and Tarantola, 2012).

Acknowledgments

The authors declare that there are no conflict of interest.

DISTRICT-SPECIFIC *IN SILICO* EVALUATION OF RICE IDEOTYPES IMPROVED FOR RESISTANCE/TOLERANCE TRAITS TO BIOTIC/ABIOTIC STRESSORS UNDER CLIMATE CHANGE SCENARIOS

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Published on
Climatic Change (2015), 132, 661-675.

4.1. Abstract

Using crop models as supporting tools for analyzing the interaction between genotype and environment represents an opportunity to identify priorities within breeding programs. This study represents the first attempt to use simulation models to define rice ideotypes improved for their resistance to biotic stressors (i.e., diseases); moreover, it extends approaches for evaluating the impact of changes in traits for tolerance to abiotic constraints (temperature shocks inducing sterility). The analysis – targeting the improvement of 34 varieties in six Italian rice districts – was focused on the impact of blast disease, and of pre-flowering cold- and heat-induced spikelet sterility. *In silico* ideotypes were tested at 5 km spatial resolution under current conditions and climate change scenarios centered on 2020, 2050 and 2085, derived according to the projections of two general circulation models – Hadley and NCAR – for two IPCC emission scenarios– A1B and B1. The study was performed using a dedicated simulation platform, i.e., ISIde, explicitly developed for ideotyping studies. The ideotypes improved for blast resistance obtained clear yield increases for all the combinations GCM × emission scenario × time horizon, i.e., 12.1% average yield increase under current climate, although slightly decreasing for time windows approaching the end of the century and with a marked spatial heterogeneity in responses across districts. Concerning abiotic stressors, increasing tolerance to cold-induced sterility would lead to a substantial yield increase (+9.8%) only for *Indica*-type varieties under current climate, whereas no increases are expected under future conditions and, in general, for *Japonica*-type varieties. Given the process-based logic behind the models used – supporting coherence of model responses under future scenarios – this study provides useful information for rice breeding programs to be realized in the medium-long term.

Keywords: Blast; genotype × environment; spikelet sterility;
Oryza sativa L.; WARM.

4.2. Introduction

The development of crop models has been driven since the end of the 1960s by two main objectives: increasing the efficiency of agricultural production systems to maximize yields while reducing costs, and supporting breeding programs via *in silico* analyses of the genotype × environment interaction (Donatelli and Confalonieri, 2011). Both these goals are crucial to successfully facing the challenges deriving from the need for increasing the economic and environmental sustainability of agricultural systems. However, most modelling studies focused on the first one, and a large number of applications targeting the development of tools for optimizing management practices were developed. Instead, the use of crop models for designing plant ideotypes – second goal – is still in its infancy (Tardieu, 2010).

The development of process-based modelling frameworks integrating the available knowledge on the genotype × environment interaction – and on how such interaction is modulated by management practices – could effectively support breeding programs (Boote et al., 2001), that usually require 10 to 15 years and sizable resources to gain effective results, although marker-assisted breeding could reduce this time by three-six years (Alpuerto et al., 2009). Genetic improvement can be emulated via changes in the values of the model parameters describing specific morphological and physiological plant traits (Duncan et al., 1967; Semenov and Stratonovitch, 2013; Confalonieri et al., 2013). This allows one to develop *in silico* ideotypes that can be used (i) *a priori*, to identify the complex of genes on which breeders should focus (Herndl et al., 2007) or (ii) *a posteriori*, to efficiently test the performances of the modified genotypes under different agro-environmental contexts and over long term periods (Hammer et al., 2002). Interesting attempts have been made along these lines for different crops: Aggarwal et al. (1997) used ORYZA1 to estimate the impact of changes in vegetative and reproductive rice traits in tropical environments. Raza et al. (2013) used the crop model CropSyst to analyze the relationship of alfalfa cultivar traits with soil water dynamics to derive an ideotype for temperate, semi-arid conditions. Drewry et al. (2014) successfully demonstrated how changes in canopy structural traits could allow to simultaneously improve productivity and water use efficiency in modern cultivar of soybean. The main limits in the use of crop models for this kind of studies lie in the absence of explicit algorithmic formalizations representing the genetics behind morphological and physiological traits, and thus in possible discrepancies between *in silico*-improved varieties and their *in vivo* realizations (Hammer et al., 2002). However, a model can be considered suitable to reproduce the behavior of a given genotype when it implements parameter-driven response functions reflecting the way the plant actually reacts to agro-environmental conditions (Tardieu, 2003). Model parameters must have a biophysical meaning, and changes in their values should impact on multiple physiological processes, thus reproducing the feedback mechanisms of gene expression (Boote et al., 2001). Finally, a conservative definition of *in silico* ideotypes can be achieved only by changing parameter values within the range of their known genetic variability.

“Stressors” are defined as environmental factors assuming values – or evolving with dynamics – that prevent the plant to complete its cycle or achieve an acceptable yield. Despite the key role of biotic (e.g. diseases) and abiotic (e.g. lodging) stresses in determining actual crop production levels (e.g., Oerke, 2006), model-based ideotyping studies have completely ignored traits related to resistance to diseases and – except for drought and only few studies about heat stress (e.g. Singh et al., 2014) – to tolerance to critical environmental conditions. A reason for this is the absence of specific algorithms for biotic and abiotic stressors in most of the available crop models (Donatelli and Confalonieri, 2011). The inclusion of these factors in *in silico* ideotyping studies could instead greatly support the design of medium and long term breeding programs, since expected changes in climate (IPCC, 2007) would likely lead to shifts in breeding priorities, making the integrated, a priori evaluation of the performances of new cultivars a pre-requisite for defining effective adaptation strategies

(Semenov and Stratonovitch, 2013). Extreme events are expected to increase their frequency, undermining future global food security (Battisti and Naylor, 2009). Breeders should thus know which traits will be the key ones in the coming 15-25 years to derive the most suitable varieties in this challenging context. As an example, increasing drought tolerance in wheat could appear as a priority under current climate in Europe whereas, according to future projections, heat stress would likely represent the major constraint to wheat production in this area (Semenov and Shewry, 2011).

Rice (*Oryza sativa* L.) is the staple food for more than a half of the world population (FAOSTAT, http://faostat.fao.org/) and should be considered as a key crop in this context. Rice production is indeed heavily affected by biotic stressors, with blast (*Magnaporthe oryzae* B. Couch) being one of the most severe threats to rice yields in the main production districts worldwide. Each year, blast is indeed responsible for losses in the global rice production that ranges from 10 to 30%. Farmers are thus forced to apply chemical treatments to prevent blast epidemics, increasing the economic cost and the environmental impact of rice-based cropping systems. The development of varieties resistant to *M. oryzae*, is therefore the primary objective of any rice breeding program worldwide (Ballini et al., 2008). Concerning abiotic factors affecting rice production, critical temperatures during panicle differentiation and flowering stage inducing spikelet sterility play a major role (Suh et al., 2010; Sanchez et al., 2014).

This study presents the results of the first *in silico* evaluation of the performances of ideotypes derived from the introgression of traits for resistance to biotic stressors in available rice genotypes. Moreover, ideotypes improved for tolerance to abiotic factors (temperature shocks inducing sterility) were also evaluated. Simulation results are analyzed in light of the different responses achieved in different production districts and under different climate scenarios.

4.3. Materials and methods

4.3.1. The study area

Italy is responsible for more than a half of the total European rice production, with 1,500,000 tons produced on 246,500 ha in 2011 (FAOSTAT, http://faostat.fao.org/). Rice plays an important role in the country, because of economic, social and cultural reasons, and it is mainly grown under flooded conditions in six districts (Fig. S1) characterized by markedly heterogeneous conditions. The Lombardo-Piemontese is the widest, accounting for 90% of the Italian rice cropped area (National Rice Authority, www.enterisi.it), followed by the Emiliano (9,965 ha), Veneto (5,992 ha), Oristanese (3,524 ha), Sibari (565 ha) and Toscano districts (279 ha). Climate conditions are nearly continental in the northern districts (i.e. Lombardo-Piemontese and Veneto) becoming typical of lowland littoral areas while moving to the southern part of the country (i.e. Sibari and Oristanese districts). Southern areas are also characterized by heat waves and drier weather, especially during the growing period of rice (i.e. from May to September).

The high latitudes of the main three districts (around 45° N) expose the crop to cold air irruptions from the Alps that – when occurring between panicle initiation and heading – could induce spikelet sterility. The frequency of this event is about one every four years, and the intensity could lead to yield losses larger than 30% for the most susceptible varieties. Concerning fungal diseases, they affect rice production regardless from the district; blast represents the most serious and widespread pathogen (Faivre-Rampant et al., 2011), requiring the application of chemical treatments on more than 75% of the Italian rice acreage.

4.3.2. Data used for the ideotyping experiment

Daily maximum and minimum air temperatures, global solar radiation, rainfall, wind speed and reference evapotranspiration needed for the baseline climate were extracted from the MARS database (http://mars.jrc.ec.europa.eu/mars/Web-Tools) at 25 km × 25 km spatial resolution. For the generation of climate change scenarios, the uncertainty related to the future economic, demographic and technological development was handled by using the projections of two emission scenarios (A1B and B1; IPCC, 2007) as provided by two general circulation models (GCM): Hadley3 (UKMO-HadCM3, Gordon et al., 2000) and NCAR (NCAR-CCSM3, Collins et al., 2004). A1B is a conservative scenario, based on a balanced use of fossil and non-fossil energy resources, whereas for B1 the implementation of mitigation strategies to reduce carbon emissions is assumed. Climate change projections associated with these emission scenarios are thus considered to be, respectively, at the high and low range of current forecast (IPCC, 2007). Four 20-year time horizons were considered: 1991-2010 (baseline), 2020-2040, 2050-2070, and 2085-2105. The generation of synthetic weather series was carried out using the CLIMAK weather generator (Confalonieri, 2012), which has proved its reliability under a variety of conditions, also in comparative studies (e.g. Acutis et al., 1998). For each combination emission scenario × GCM × time frame × MARS grid cell, monthly mean temperature and precipitation anomalies were applied to the CLIMAK parameters describing baseline rainfall and temperature patterns. In particular, CLIMAK parameters on which deltas were applied are monthly α and β of the gamma distribution for rainfall amount and the four coefficients (annual mean maximum and minimum temperatures for dry and rainy days) of the second order Fourier series for temperature. Future climate change scenarios used to test the performance of rice ideotypes are described in Table S1. In agreement with Coppola and Giorgi (2010) – who assessed climate change projections provided by 19 GCMs over the Italian peninsula – the greatest temperature increase is realized under the A1B emission scenario, whereas the B1 leads to the lowest. GCMs also affected climate projections, with Hadley3 providing warmer scenarios than NCAR, in agreement with findings of Covey et al. (2003). Projected rainfall varies across scenarios without displaying clear patterns; however, the combination GCM × emission scenario Hadley-A1B leads to a “drier” climate during the rice growing season as compared to NCAR-B1. Spatial heterogeneity in climate change projections is expected, with northern districts showing greater temperature increase than the southern ones (e.g., +4.5 °C and +2.5 °C for the Lombardo-Piemontese and Oristanese districts, respectively, under the Hadley-A1B 2085 scenario).

Since most of the biophysical processes – e.g., crop growth and development, plant-pathogen interaction, spikelet sterility, floodwater effect on vertical thermal profile – were simulated using a hourly time step, hourly weather data were estimated at runtime using generators implemented in the CLIMA software library (http://agsys.cra-cin.it/tools/clima/help/).

Rice distribution maps – as well as sowing dates – were derived from an integrated analysis of available thematic cartography (European Corine Land Cover for the year 2006; http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-clc2006-100-m-version-12-2009) and remote sensing data. Ten years (2002-2011) of MODIS 8-Day composites images – MOD09A1 product of surface reflectance at 500 m spatial resolution – were processed in order to derive a rice distribution database. The processing method combines information on the status of vegetation and on the presence of floodwater to detect rice areas and derive information on rice development (Boschetti et al., 2009). Remote sensed information – 10 years median – were aggregated at 5 km × 5 km spatial resolution, identified as elementary spatial unit for the simulations in light of (i) the spatial resolution of the different information layers and (ii) the need for finding a compromise between the level of detail to represent the spatial heterogeneity among and within rice districts and the computational cost of the simulations.

To account for the heterogeneity of rice varieties commonly grown in Italy (Russo, 1994), thirty four varieties were selected as the basis for the ideotyping study according to their representativeness in each of the six Italian rice districts. The criterion for the selection was a relative presence higher than 1% in the district in at least three years during the period 2006-2010 (National Rice Authority, www.enterisi.it).

4.3.3. The simulation environment

4.3.3.1. The modelling solution

Simulations were performed using the WARM model (Confalonieri et al., 2009b), adopted since 2005 by the European Commission for rice monitoring and yield forecasting in Europe. In the configuration used in this study, crop growth and development are reproduced using a hourly time step, in both cases with a curvilinear response function to temperature. Floodwater effect on vertical thermal profile is simulated by using the TRIS micrometeorological model (Confalonieri et al., 2009b), based on the solution of the surface energy balance equation for each 10 cm canopy layer and for air-water interface, for the latter assuming the heat accumulation into the water as storage term. TRIS, coupled with a model for plant height, allows using temperature at the meristematic apex for the simulation of development and thermal shock around flowering, and mid-canopy temperature for photosynthesis. Biomass accumulation is simulated with a net-photosynthesis approach based on radiation use efficiency (RUE), the latter modulated by temperature, senescence, atmospheric CO2 concentration, and light-saturation of the enzymatic chains. For the latter, a quadratic function decreasing RUE for global solar radiation higher than 25 MJ m^{-2} day^{-1} is used (Choudhury, 2001). Photosynthates partitioning to the different plant organs, as well as translocation to grains during grain filling, are simulated using a set of beta and parabolic functions driven by development stage and by partitioning to leaves at emergence. Daily increase in leaf area index (LAI) is derived from leaf biomass rate and a development-driven specific leaf area, whereas leaf senescence is simulated by killing LAI units that reached a thermal time threshold. WARM parameters for crop growth and development are shown in Table S2.

Concerning the simulation of abiotic damages, the impact of cold-induced spikelet sterility is simulated by reducing the amount of assimilates daily partitioned to the grains according to the stress factor ($Sterility_F$, 0-1, unitless) shown in Eq. 1 (Confalonieri et al., 2009b):

$$Sterility_F = \begin{cases} \sum_{i=d_{1.6}}^{d_{1.9}} \left\{ bellF_i \cdot \sum_{h=i}^{24} \left(T_{TC} - T_{i,h} \right) \right\} & 1.6 < DVS < 1.9 \cap T_{TC} > T_{i,h} \\ 0 & elsewhere \end{cases} \tag{1}$$

where $d_{1.6}$ and $d_{1.9}$ are the days corresponding to panicle initiation and heading, respectively, with 1.6 and 1.9 being the values assumed in those stages by a development stage numerical code (*DVS*; 0: sowing; 1: emergence; 2: flowering; 3: physiological maturity; 4: harvestable) obtained by standardizing the cumulated thermal time; T_{TC} (°C) is the threshold temperature inducing sterility; $T_{i,h}$ is the temperature of day i at hour h; *bellFi* – representing the different susceptibility in the period between panicle initiation and heading – is derived from a normal distribution function (Eq. 2):

$$bellF_i = \frac{\delta}{\gamma\sqrt{2\pi}} \cdot \exp - \left[\frac{\left(DVS_i - 1.8 \right)^2}{2\gamma^2} \right] \tag{2}$$

where DVS_i is the *DVS* of the day i; γ and δ are empirical coefficients set, respectively, to 0.1 and 0.25. The threshold temperature was set, in this study, to 12°C and 13°C for current *Japonica*-type and *Indica*-type varieties, respectively. The impact of heat-induced spikelet sterility is simulated using the same approach but with ($T_{i,h}$-T_{TH}) used instead of (T_{TC}-$T_{i,h}$) in Eq. 1, being T_{TH} (°C) the threshold temperature for heat induced sterility (i.e., 35°C for *Japonica*-type and 35.5°C for *Indica*-type varieties).

From panicle initiation to heading, photosynthates daily partitioned to panicles are reduced as a function of the integral of $Sterility_F$. From flowering to the end of grain filling phase, the value of $Sterility_F$ cumulated till heading (*DVS* 1.9) is used.

The simulation of blast impact on rice growth was carried out by using the set of models of the Diseases software library (http://www.cassandralab.com/components/), implementing approaches for the simulation of (i) the phases of infection, latency, infectiousness, sporulation, spores dispersal, and (ii) the impact on plants.

The whole epidemic development is simulated as a function of agro-environmental variables – e.g., leaf wetness, air relative humidity, air temperature – and variety resistance. The models implemented in the library were successfully evaluated for blast against a large set of data from field experiments in northern Italy (Bregaglio et al., 2016). Parameters describing the specific responses of *M. oryzae* to environmental conditions are given in Table S3. According to Bastiaans (1991), the impact of blast epidemic on the crop is simulated by considering the reduction of green leaf area and the decrease in the photosynthetic activity of remaining green leaf tissues. The first coupling point between the disease and the crop model is represented by the dynamic removal of the fraction of diseased leaf area using a compartmental susceptible-exposed-infected-removed model (Bregaglio and Donatelli, 2015; http://www.cassandralab.com/components/). This allows to take into account the reduction in the amount of light absorbed because of the lesions. A second coupling point concerns the decrease in the photosynthetic rate in the remaining green leaf area via a linear function relating disease severity and rice radiation use efficiency (Bingham and Topp, 2009).

The WARM modelling solution was here implemented in a dedicated platform (ISIde; *In Silico* Ideotyping platform), based on the BioMA architecture (Donatelli and Rizzoli, 2008), specifically developed for district-specific ideotyping studies and targeting breeders as final users. ISIde is currently available for the six rice districts in Italy, although its software architecture allows its extension to other contexts or crops. The complete documentation of all the models and software components implemented in the modelling solution is available at http://www.cassandralab.com/components.

4.3.3.2. Parameterization of the solution for current genotypes

Adapting the modelling solution to the 34 selected varieties required the definition of the values of parameters describing morphological and physiological plant features, and of those involved with the simulation of biotic and abiotic stressors.

Five phenological groups were identified by applying the k-means clustering method (Dulli et al., 2009) to the length (days) of the vegetative and reproductive phases reported for each variety by the National Rice Authority (www.enterisi.it). This agency indeed, carried out extensive field trials in different sites and seasons to provide a detailed description of morphological and phenological characteristics of the main Italian rice cultivars. Starting from available parameter sets for Italian *Indica*- and *Japonica*-type varieties obtained by calibrating model parameters using data from field experiments (Confalonieri et al., 2009b), new parameter sets were thus developed by combining information on the ecotypes with those derived from the cycle lengths clusters (Table S2).

Parameters involved with blast resistance (Table S4) were derived by screening the databases and reports of both the National Rice Authority (www.enterisi.it) and the Regional Agency for Agricultural and Forest Services (www.ersaf.lombardia.it). These agencies performed extensive multi-site and multi-year trials from 2000 to 2011 where the resistance of each cultivar to fungal pathogens was determined. These data were analyzed and integrated with information coming from interviews to a panel of Italian experts in rice diseases. This led to identify three blast resistance levels (low, moderate, intermediate), in agreement with Faivre-Rampant et al. (2011), who defined Italian rice varieties as having a low to intermediate resistance to leaf blast after extensive analyses performed under greenhouse conditions. The resistance levels were then translated into parameter values by increasing the length of the latency period and by reducing the infectiousness duration and the infection/sporulation efficiencies in case of higher resistance (Table S3).

Temperature thresholds applied on hourly basis for cold- and heat-induced spikelet sterility for *Japonica*- and *Indica*-type varieties (12°C and 13°C, 35 °C and 35.5°C, respectively; Table S4) were retrieved from literature (Andaya et al.,2003; Dreni et al., 2012; Sanchez et al., 2014) and consultation with local experts. This allowed to account for peculiarities of Italian rice cultivars, selected in recent decades to cope with the conditions explored in the main Italian rice district, considered extreme for rice because of the proximity to the Alps (Russo, 1994; Confalonieri et al., 2005). Information available did not allow to further discriminate among threshold values within each group of varieties.

4.3.3.3. Definition of the new ideotypes

The evaluation of the impact of potential improvement for resistance/tolerance level was carried out by changing values of model parameters representing these traits within the range of their known genetic variability. Ideotypes were thus created by assigning to resistance/tolerance parameters the values defined for resistant/tolerant varieties; this was considered as the level of improvement potentially achievable. Assumptions concern the absence of relationships between the traits involved with the processes analyzed and those related with other plant characteristics (e.g., Singh et al., 2012 for resistance to pathogens). Resistant varieties – used as sources of effective resistance/tolerance traits – are often traditional genotypes selected after long adaptation to the specific Italian agro-climatic context (Faivre-Rampant et al., 2011; Russo, 1994). For instance, the variety Gigante Vercelli, released in 1946 and has not been cultivated for decades, was already selected as a donor of blast resistance in breeding programs because of the resistance to main Italian strains of the pathogen (Roumen et al., 1997; Faivre-Rampant et al., 2011). It presents an intermediate resistance to blast disease. This value was thus used to set blast resistance parameters for the improved ideotypes. The temperature threshold below which cold sterility occurs was reduced – for the ideotypes – by 0.5°C for *Japonica*-type and by 1°C for *Indica*-type varieties. This led to set a lowest temperature threshold of 11.5°C, which reflects the response of the cold tolerant varieties currently grown in the Italian district. The smaller magnitude of the improvement simulated for *Japonica*-type varieties is in line with their better adaptation to temperate conditions (Russo, 1994; Suh et al., 2010). They present indeed slightly lower thermal requirements for both growth and development (Sanchez et al., 2014), and a lower threshold triggering sterility damages compared to *Indica*-type varieties (temperature thresholds applied on hourly basis are 12°C versus 13°C, Table S4). The opposite criterion was followed for the quantification of the possible genetic improvement for heat-induced sterility (increase in threshold temperature for heat induce spikelet sterility was 0.5°C for *Indica*-type and 1°C for *Japonica*-type varieties), since *Indica*-type varieties are generally more suited to tropical environments (Sanchez et al., 2014). A higher variability is reported for tolerance to heat stress in rice (Matsui et al., 2001) but Italian rice varieties have not been selected for this traits since they are rarely exposed to this damage.

The information available on the potential incidence of the different damages led to evaluate the ideotypes for tolerance to cold-induced sterility in the districts Lombardo-Piemontese, Veneto, Emiliano and Toscano. For the same districts and in the Oristanese one, ideotypes were defined for blast resistance. Improvement involved with the tolerance to heat-induced sterility was instead tested for the districts Oristanese and Sibari.

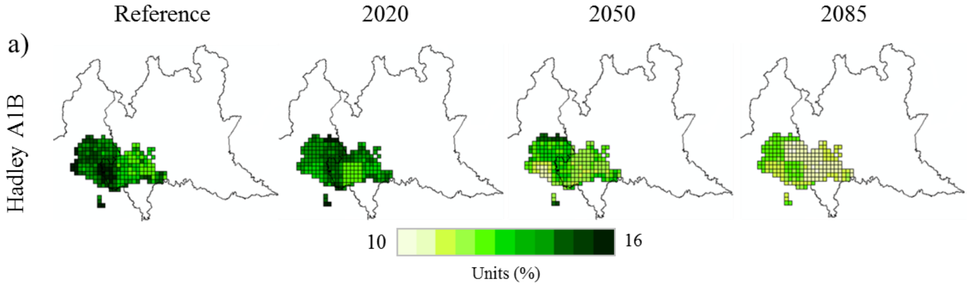
For each climate scenario and elementary spatial unit, simulations were run twice for each variety: the first using the parameterizations for the current genotype and the second reproducing the behavior of the corresponding improved ideotype. Three production levels were considered: blast-, cold sterility- and heat sterility-limited. For each production level, the percentage yield increase (Δ_y, %) was quantified.

The total number of simulations was 19,910,400, given by the combination of 34 rice varieties, three production levels, two general circulation models, two emission scenarios, four time horizons, 20 years for each time horizons, two genotypes simulated – one for the current variety and one for the corresponding ideotype – and by 305 elementary simulations units.

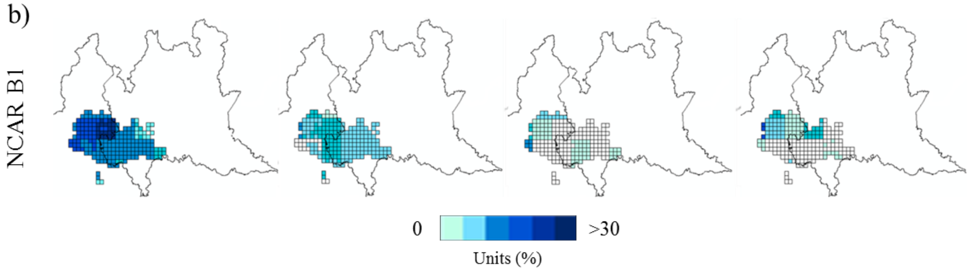
4.4. Results and discussion

The high spatial resolution used to perform the simulations allowed an in depth analysis of the performances of improved ideotypes. Fig. 1 presents sample results for the main Italian rice district for (a) an ideotype derived by increasing blast resistance of the variety Volano (Hadley-A1B projections), and for (b) an ideotype derived by increasing the tolerance to cold-induced sterility for Thaibonnet (NCAR-B1). The largest increases for the blast resistance-improved ideotypes were achieved in the central-western part of the district when the current climate was considered, whereas they were achieved in north-western areas while moving forward the time horizon (Fig. 1.a). In general the eastern part of the district appears to be less affected by possible breeding programs in the long term (2085 time horizon). Fig. 1.b shows that a marked heterogeneity in the yield increase extent was simulated. The general north-south gradient is due to the colder climate in the northern part of the district, whereas the pattern characterized by a high level of granularity is – to a large extent – driven by differences in management practices. The use of different sowing dates, indeed, leads to shifts in crop phenology and, thus, in the time windows when the crop is susceptible, i.e., from panicle initiation to heading. Although benefits decrease while moving forward the climate projection because of the raising temperature, spot areas markedly affected can be observed for the 2085 time horizon. The reason is the shortening of the crop cycle due to the higher temperatures, which exposes the crop to the damage in an earlier moment during the season, when the frequency of cold air irruptions is higher.

The mean percentage yield increase achieved with the ideotypes improved for resistance/tolerance traits compared to the 34 varieties they derive from are shown in Fig. 2 and Fig. 3 for each combination general circulation model × time horizon × emission scenario. No results are presented and discussed for Sibari since the only stressor potentially present in the district – heat-induced sterility – never affected the crop, regardless of the climate scenario.



Average yield increase for an ideotype derived by improving cv. Volano for resistance to blast disease



Average yield increase for an ideotype derived by improving cv. Thaibonnet for cold-induced sterility

Figure 1. Percentage yield increase in the Lombardo-Piemontese district due to the increased (a) resistance to blast disease for the variety Volano and (b) tolerance to cold-induced sterility for the variety Thaibonnet. For these sample results, the Hadley-A1B and the NCAR-B1 climate change scenarios were used, respectively, for blast and sterility. Simulations were performed at 5 km × 5 km spatial resolution. For both examples, a marked spatial heterogeneity was observed, and a decrease of the yield increase extent while proceeding along the time horizons

In general, improving genotypes for their resistance to *M. oryzae* led to remarkable potential yield increases for all the varieties, regardless of the district and the time horizon (Fig. 2). The average yield increase was 11.87%, with a small variability among districts and climate scenarios (coefficient of variation = 11.6%). However, future climate projections revealed that the extent of increases could experience a modest decline in some of the districts (Toscano and Veneto). The climate scenarios derived from the NCAR general circulation model and the B1 emission scenario led to the largest yield increases in most of the combinations district × time horizon, whereas the lowest were usually achieved with the combination Hadley × A1B. This is explained by considering the thermal requirements of the pathogen (Table S3) and the temperature increases in the climate change projections.

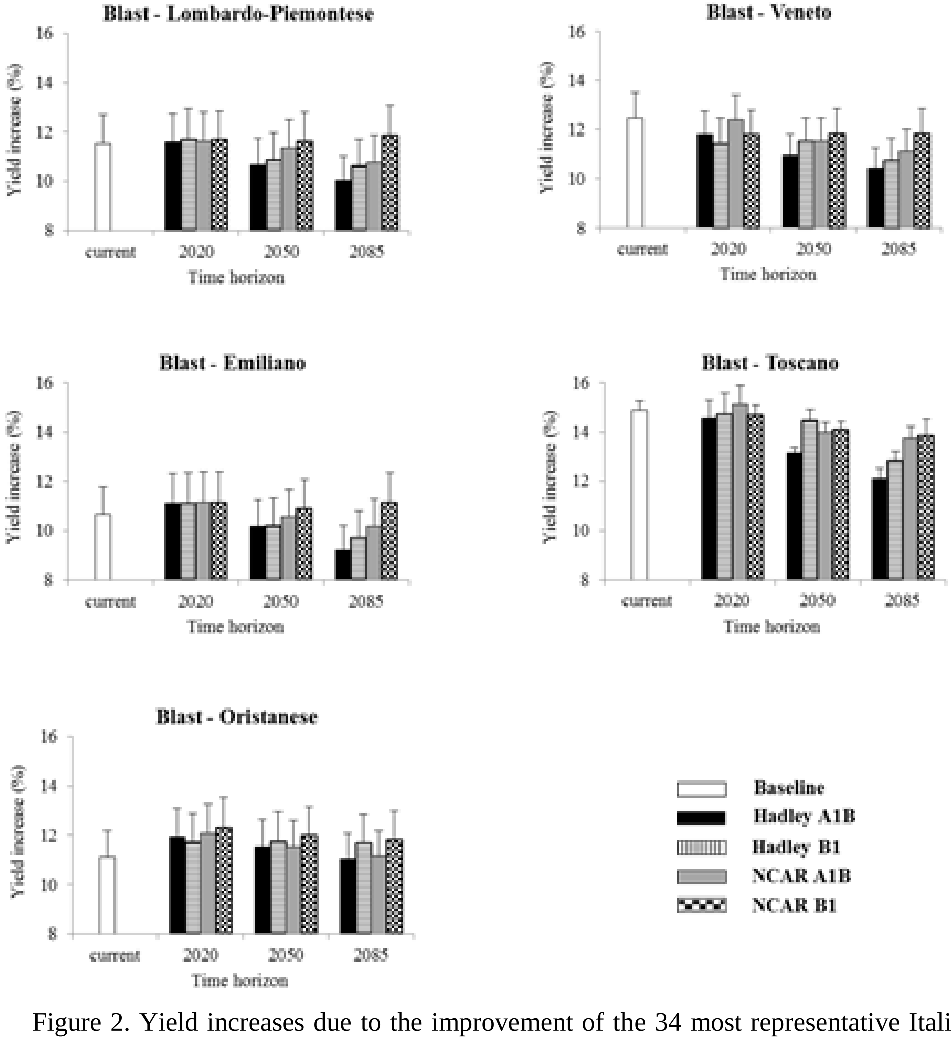


Figure 2. Yield increases due to the improvement of the 34 most representative Italian rice varieties for resistance to blast (average value of the twenty-year period). *White bars* refer to results achieved under current climate, *black and grey bars* to the Hadley-A1B and NCAR-A1B realizations, respectively, *striped and checkered bars* to the Hadley-B1 and NCAR-B1 projections. Relevant benefits from possible breeding activities are expected for resistance to blast, regardless of the district and climate scenario.

Under the conditions explored, indeed, the climate generated using the A1B emission scenario and the Hadley circulation model is characterized by large increases in temperature, in turns generating conditions less favourable for the pathogen, with temperatures during the crop cycle frequently exceeding the optimum for the pathogen.

The differences in yield increase among districts – coefficient of variation ranging from 11% to 16% according to the time horizon considered – were due to the different suitability of the climate conditions to blast epidemics. The Toscano district presented the highest yield increases, because of the large number of rice varieties with a low resistance to blast.

This study was carried out without considering the evolutionary potential of the pathogen – that could rapidly develop new races more adapted to changing climate conditions (Chakraborty, 2013) – since no quantitative information is available on this issue.

Concerning ideotypes improved for the tolerance to cold-induced spikelet sterility (Fig. 3), *Indica*-type genotypes achieved larger yield increases with respect to *Japonica*-type ones in all the districts and for all the combinations general circulation model × emission scenario. For *Japonica*-type varieties, indeed, the increased tolerance did not lead to relevant benefits, especially under climate change scenarios (data not shown). However – even for *Indica* ideotypes – yield increases are comparable to those simulated for blast resistance-improved ideotypes only under current climate conditions. In effect, simulations performed using future climate projections revealed that their performances are expected to become increasingly similar to those of the current varieties, in turn raising doubts about the usefulness of breeding programs focusing on this trait in the medium/long term. The heterogeneity in the responses across districts was decidedly more pronounced compared to that discussed for the ideotypes improved for blast resistance: coefficients of variation calculated on the yield increases range from 30% to 87% for the 2020 and 2050 time horizons, respectively. However, a large part of this variability is due to the low impact of cold-induced sterility on the yields simulated in the Emiliano district because of its warmer climate.

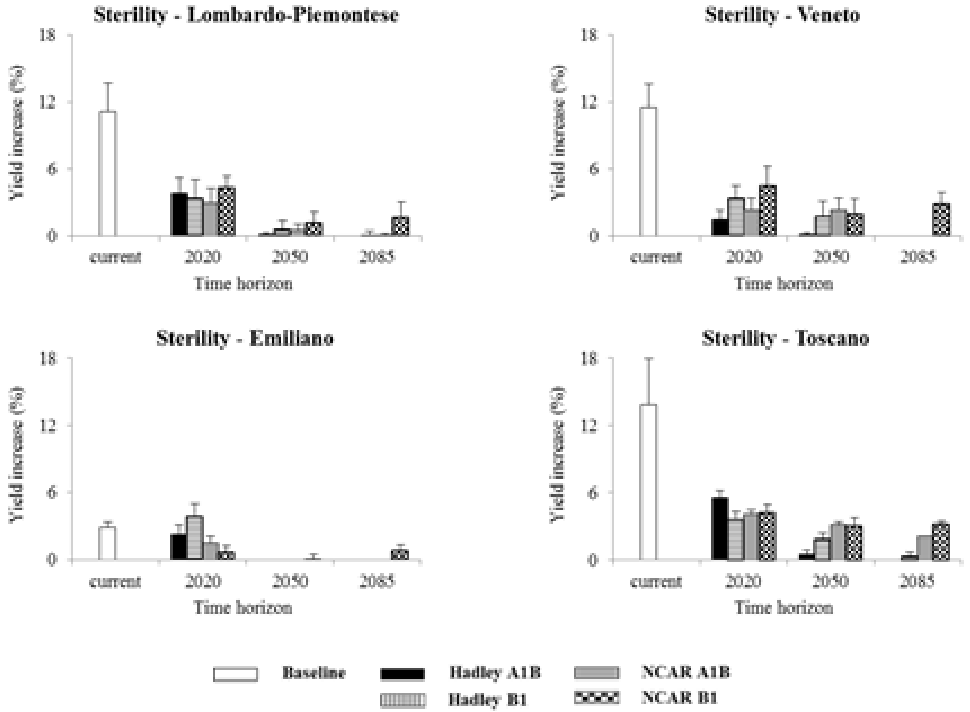


Figure 3. Yield increases deriving from improving tolerance to pre-flowering cold-induced spikelet sterility in the *Indica*-type varieties studied (average values of the years in which the damage occurs). Results refer to yield increases achieved under: the current climate (*white bars*), the Hadley-A1B and NCAR-A1B climate scenarios (*black and grey bars*, respectively) and the Hadley-B1 and NCAR-B1 projections (*striped and checkered bars*). Under the conditions explored, a marked decrease of the impact of cold induced spikelet sterility is expected.

Contrarily to yield losses caused by blast, cold-induced sterility is driven by events – i.e., cold air irruptions during the period between panicle initiation and heading – that do not occur during all seasons and, even when they occur, present a different intensity. Under current climate, the frequency of these events is about one out of five years. Lowering the threshold temperature inducing the damage created a marked reduction of the average number of events under future climate projections, although with differences among general circulation models and emission scenarios.

The model did not simulate impacts of heat-induced sterility – for both current varieties and improved ideotypes – in the two districts potentially interested by this phenomenon (Sibari and Oristanese), regardless of the climate scenario and time horizon considered. This clearly indicates that rice in Italy would not be affected by this phenomenon even under conditions warmer than the current ones. Despite A1B already provides high-impact scenarios, further studies might involve the use of more severe emission scenarios (e.g., RCP 8.5, IPCC 2013) to test the performance of improved ideotypes under a wider range of climate conditions.

4.5. Conclusions

The development of new varieties better adapted to future climate is one of the most effective strategies to alleviate the impact of climate change on agriculture and related food-security issues. In this context, identifying key traits is crucial since relatively few breeding cycle are exploitable in the forthcoming decades (Lobell et al., 2011).

We performed here for the first time an *in silico* ideotyping study targeting the improvement of current genotypes for resistance traits to biotic stressors at district level. Traits related with tolerance to abiotic constraints were also evaluated. The analysis was carried out for rice in Italy by varying parameters referred to resistance/tolerance traits according to their known variability among Italian rice varieties. This allowed to define ideotypes accounting for the level of improvement potentially achievable for these traits. Simulations were performed under current climate conditions and using future climate projections, to provide indications able to properly cope with the time resources needed by breeding programs. Our results clearly demonstrate that – under the conditions explored – breeders should focus on increasing resistance to blast disease, since *M. oryzae* appears as the main factor limiting rice productions in Italy regardless of the district and the climate scenario. On the other hand, increasing tolerance to pre-flowering cold shocks inducing spikelet sterility would lead to sizeable benefits only for *Indica*-type cultivars under current climate conditions in three out of six districts (Lombardo-Piemontese, Veneto, Toscano), whereas no relevant yield increases are expected after 2020, despite the general circulation model and emission scenario. The effect of increasing CO_2 on photosynthetic rate and changes in phenological development were explicitly considered, thus providing a comprehensive evaluation of ideotypes. Despite the assumptions behind the study – i.e., (i) absence of interactions between resistance/tolerance traits and others, (ii) no adaptation strategies considered (e.g., alternative sowing date), and (iii) lack of pathogen potential evolution in response to long-term climate variations and increased host resistance – we demonstrated the usefulness of simulation models as tools for supporting breeding programs via the a priori evaluation of the suitability of different ideotypes for specific conditions (i.e., the districts).

Acknowledgments

This study has been partially funded under the project RISINNOVA (Integrated genetic and genomic approaches for new Italian rice breeding strategies), funded by Ager (http://risinnova.entecra.it/), and under the EU FP7 project MODEXTREME (Grant Agreement No. 613817).

Supplementary figures

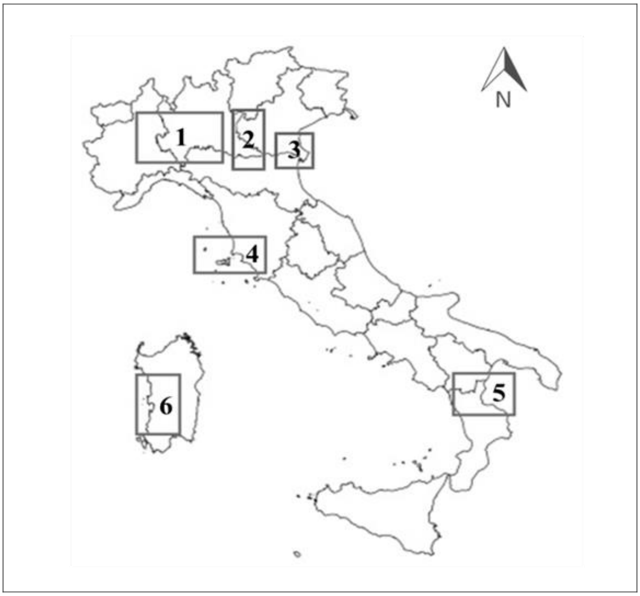


Figure S1. Main rice districts in Italy.
1. Lombardo-Piemontese; 2. Veneto; 3. Emiliano; 4.Toscano; 5. Sibari; 6. Oristanese.

Supplementary tables

Table S1. Characteristics of the twelve climate scenarios used in this study. They derived from the projections of two General Circulation Models (GCM, Hadley3 and NCAR) for two CO2 emission scenarios (A1B and B1, IPCC 2007) referred to three timeframes (2020/2050/2085). Average increase of mean-temperature for the growing season (i.e. May to October) as compared to the reference period (1991-2010) is reported.

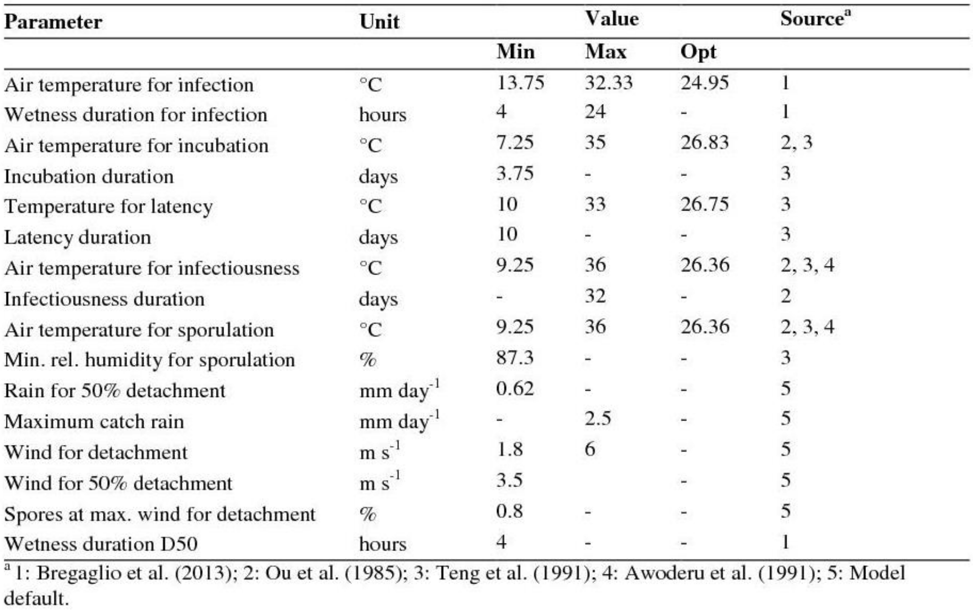
General Circulation Model	**Emission scenario**	**CO_2 changes (ppm) 2020 / 2050 / 2085**	**Temperature increase (°C) 2020 / 2050 / 2085**
Hadley3	A1B	447 / 522 / 639	0.6 / 2.3 / 3.9
NCAR	A1B	447 / 522 / 639	0.6 / 1.8 / 2.4
Hadley3	B1	432 / 482 / 530	0.3 /1.4 / 2.4
NCAR	B1	432 / 482 / 530	0.6 / 1.0 / 0.9

Table S2. WARM parameters describing traits involved with crop growth and development (JE, JM, JL: *Japonica*-type early, medium and late varieties; IE, IL: *Indica*-type early and late varieties).

Parameter	**Units**	**Value**					**Description**	**Source**
		JE	**JM**	**JL**	**IE**	**IL**		
$T_{base}D$	°C	12					Base temperature for development	C
$T_{opt}D$	°C	28					Opt. temperature for development	C
$T_{max}D$	°C	42					Max. temperature for development	C
GDD_{em}	°C-days	120			100		GDD from sowing to emergence	C
$GDD_{em\text{-}fl}$	°C-days	890	910	900	980	980	GDD from emergence to flowering	T
$GDD_{fl\text{-}mat}$	°C-days	580	580	630	540	660	GDD from flowering to maturity	T
RUE	$g\ MJ^{-1}$	2.6			3.2		Radiation use efficiency	C
K	-	0.50			0.55		Extinction coefficient for solar radiation	C
$T_{base}G$	°C	12					Base temperature for growth	C
$T_{opt}G$	°C	26			28		Opt. temperature for growth	C
$T_{max}G$	°C	35					Max. temperature for growth	C
SLA_{ini}	m^2kg^{-1}	28			29		Specific leaf area at emergence	C
SLA_{till}	m^2kg^{-1}	18			19		Specific leaf area at tillering	C
RipL0	-	0.7			0.6		Partitioning to leaves at emergence	C
LeafLife	°C-days	600					Leaf duration	C
ApexHeight	cm	100			80		Maximum panicle height	C
Kc	-	1.05					Crop coefficient at full canopy	C

C: Confalonieri et al. 2009b.
T: This study.

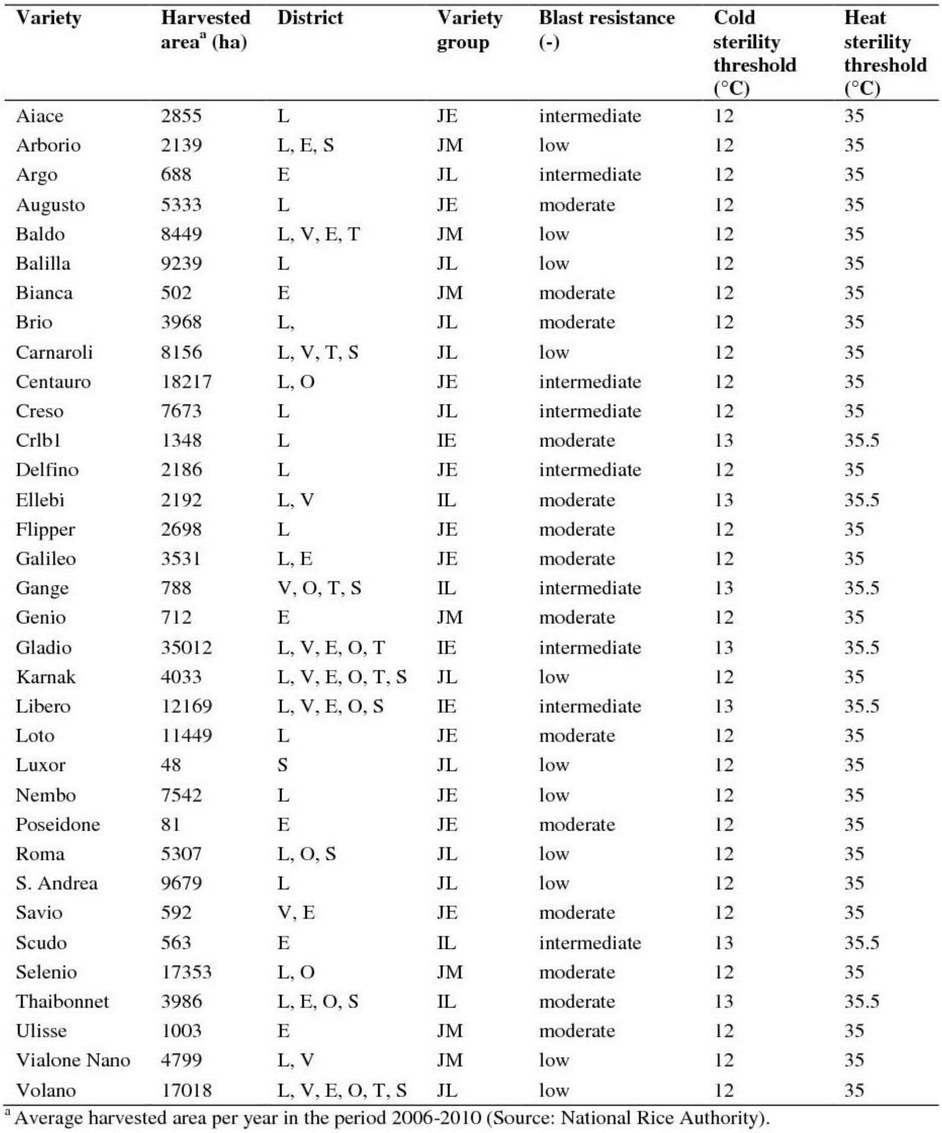
Table S3. Parameters of the disease model for *Magnaporthe oryzae* B. Couch, and sources of information.



Parameter	Unit	Value			Source[a]
		Min	Max	Opt	
Air temperature for infection	°C	13.75	32.33	24.95	1
Wetness duration for infection	hours	4	24	-	1
Air temperature for incubation	°C	7.25	35	26.83	2, 3
Incubation duration	days	3.75	-	-	3
Temperature for latency	°C	10	33	26.75	3
Latency duration	days	10	-	-	3
Air temperature for infectiousness	°C	9.25	36	26.36	2, 3, 4
Infectiousness duration	days	-	32	-	2
Air temperature for sporulation	°C	9.25	36	26.36	2, 3, 4
Min. rel. humidity for sporulation	%	87.3	-	-	3
Rain for 50% detachment	mm day^{-1}	0.62	-	-	5
Maximum catch rain	mm day^{-1}	-	2.5	-	5
Wind for detachment	m s^{-1}	1.8	6	-	5
Wind for 50% detachment	m s^{-1}	3.5		-	5
Spores at max. wind for detachment	%	0.8	-	-	5
Wetness duration D50	hours	4	-	-	1

[a] 1: Bregaglio et al. (2013); 2: Ou et al. (1985); 3: Teng et al. (1991); 4: Awoderu et al. (1991); 5: Model default.

Table S4. Parameters of the disease model for *Magnaporthe oryzae* B. Couch, and sources of information.



Variety	Harvested area[a] (ha)	District	Variety group	Blast resistance (-)	Cold sterility threshold (°C)	Heat sterility threshold (°C)
Aiace	2855	L	JE	intermediate	12	35
Arborio	2139	L, E, S	JM	low	12	35
Argo	688	E	JL	intermediate	12	35
Augusto	5333	L	JE	moderate	12	35
Baldo	8449	L, V, E, T	JM	low	12	35
Balilla	9239	L	JL	low	12	35
Bianca	502	E	JM	moderate	12	35
Brio	3968	L,	JL	moderate	12	35
Carnaroli	8156	L, V, T, S	JL	low	12	35
Centauro	18217	L, O	JE	intermediate	12	35
Creso	7673	L	JL	intermediate	12	35
Crlb1	1348	L	IE	moderate	13	35.5
Delfino	2186	L	JE	intermediate	12	35
Ellebi	2192	L, V	IL	moderate	13	35.5
Flipper	2698	L	JE	moderate	12	35
Galileo	3531	L, E	JE	moderate	12	35
Gange	788	V, O, T, S	IL	intermediate	13	35.5
Genio	712	E	JM	moderate	12	35
Gladio	35012	L, V, E, O, T	IE	intermediate	13	35.5
Karnak	4033	L, V, E, O, T, S	JL	low	12	35
Libero	12169	L, V, E, O, S	IE	intermediate	13	35.5
Loto	11449	L	JE	moderate	12	35
Luxor	48	S	JL	low	12	35
Nembo	7542	L	JE	low	12	35
Poseidone	81	E	JE	moderate	12	35
Roma	5307	L, O, S	JL	low	12	35
S. Andrea	9679	L	JL	low	12	35
Savio	592	V, E	JE	moderate	12	35
Scudo	563	E	IL	intermediate	13	35.5
Selenio	17353	L, O	JM	moderate	12	35
Thaibonnet	3986	L, E, O, S	IL	moderate	13	35.5
Ulisse	1003	E	JM	moderate	12	35
Vialone Nano	4799	L, V	JM	low	12	35
Volano	17018	L, V, E, O, T, S	JL	low	12	35

[a] Average harvested area per year in the period 2006-2010 (Source: National Rice Authority).

TRAIT-BASED MODELLING FOR DESIGNING IDEOTYPES. A CASE STUDY ON SALT TOLERANCE AND RICE

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Scientific Reports (under review)

5.1. Abstract

Eco-physiological models are increasingly used to analyze G×E×M interactions to support breeding programs via the design of ideotypes for specific contexts. However, available crop models are only partly suitable for this purpose, since they often lack clear relationships between parameters and traits breeders are working on. Taking salt stress tolerance and rice as a case study, we propose a paradigm shift towards the building of ideotyping-specific models explicitly around traits involved in breeding programs. Salt tolerance is a complex trait relying on different physiological processes that can be alternatively selected to improve the overall crop tolerance. By developing a new model explicitly accounting for these traits, we were able to show how an increase in the overall tolerance can derive from completely different physiological mechanisms according to soil/water salinity dynamics. The study demonstrated that a trait-based approach can increase the usefulness of mathematical models for supporting breeding programs.

Keywords: Ideotyping; rice; salinity; salt stress; sensitivity analysis; breeding.

5.2. Introduction

One of the key steps in the planning of breeding programs is the definition of ideotypes able to assure high and stable yields in target areas (Martre et al., 2015a; Dingkhun et al., 2015). An ideotype is a combination of traits that makes a crop suited to the edaphic, climatic and management factors defining a specific agronomic context. However, exploring in vivo the deep interaction between those factors and plant genotypes is expensive and time-consuming, being genotype responses nonlinear and the heterogeneity in the environmental and management factors huge. Moreover, one of the priorities for the analysis is trying to account for future climate conditions, but understanding how genotypes would behave in a changing climate remains a challenge (Newton et al., 2011).

Given their capability of interpreting genotype (G) × environment (E) × management (M) interactions, crop models are increasingly considered as powerful tools to support breeding activities (Martre et al., 2015b; Hammer et al., 2016). Representing genotype features via model parameters, indeed, crop models can be used to answer the “what if” question when the potential impact of varying one or more plant traits is under evaluation (Casadebaig et al., 2016). This kind of analysis can involve current conditions and climate change scenarios as well as entire production districts, thus allowing to effectively exploring both spatial and temporal heterogeneity (Paleari et al., 2015). Moreover, physiologically sound crop models have the potential to integrate the effect of genes or QTLs across different hierarchical levels of organization of biological systems, thus providing insight into their impact at crop scale (Chenu et al., 2009; Hammer et al., 2016).

Despite this potential, model development in last decades has been mainly driven by the need of defining management strategies and agricultural policies, and this limited – although to a different extent – their suitability for ideotyping studies (Boote et al., 2001; Tardieu, 2010). Model parameters do not always have a biological meaning and, even when they have, relationships between model parameters and plant traits are often unclear. This could make the model-based definition of putative ideotypes a speculative exercise (Hammer et al. 2002).

Dissection and modelling of physiological processes explicitly targeting specific traits of interest within ongoing breeding programs could represent a solution to overcome these limitations. In this way, the overall performance of modelled genotypes would be a consequence of dynamics modulated by variations in the values of parameters that would directly represent plant traits breeders are working on. The resulting *in silico* ideotypes would thus provide clear indications about putative traits for crop improvement, especially when context-specific dependencies play a key role in determining the optimal value of traits contributing to complex plant responses, like in case of tolerance to abiotic stressors (Tardieu and Tuberosa 2010).

With more than 830 million hectares of salt-affected soils, salinity is one of the major environmental stress limiting agricultural production worldwide (Martinez-Beltran and Mazur, 2005). Moreover, soil salinization is further increasing (Rengasamy et al., 2006) because of both human activities (e.g., inappropriate irrigation practices) and natural causes (e.g., tsunamis), with the latter being exacerbated by climate change (IPCC, 2013; Dasgupta et al., 2015). Despite rice is one of the most sensitive crop to salt stress (Munns and Tester, 2008; Al-Tamimi et al., 2016), its frequent cultivation in coastal areas and river deltas increases it exposure because of recurrent flooding and seawater intrusion (Ismail et al., 2007). For these reasons, ongoing rice breeding activities target different tolerance traits: (i) reduction of shoot sodium (Na^+) uptake (Ren et al., 2005; Faiyue et al., 2012); (ii) sequestration of Na^+ into structural tissues (Costafits et al., 2012; Suzuki et al., 2016); (iii) compartmentation of Na^+ into senescent leaves (Fukuda et al., 2004), (iv) higher leaf tissue tolerance via sequestration of toxic ions into the vacuole and synthesis of osmoprotectants (Kader and Lindberg, 2005), (v) and higher tolerance to salt-induced sterility (Hossain et al., 2015). These traits rely on different genetic basis (Munns and Tester,

2008) and can be combined in the same genotype or singularly introduced in commercial varieties even via non-GM technologies (i.e., using marker-assisted selection) (Munns et al., 2012). Since the effectiveness of these traits varies according to the environmental context, breeding programs should target these "component traits" and not the overall performance at crop level Ismail et al., 2007; Roy et al., 2014).

Although models reproducing crop response to salt stress are available (e.g., Ferrer Alegre et al., 1997; Karlberg et al., 2006), they mainly focus on the effect of salinity on soil osmotic potential, without explicitly considering the toxic effect of Na^+ in plant tissues which, instead, is a key component of salt stress (Munns and Tester 2008; Faiyue et al., 2012). In these approaches, plant tolerance is accounted for via few empirical parameters directly linking plant response (in terms of yield or overall growth rate) to soil salinity, without taking into account the physiological traits at the basis of such response. Therefore, these models cannot be considered as suitable to design ideotypes actually relying on the real tolerance traits identified by breeders.

The objectives of this study were (i) building a new model for the toxic effect of Na^+ on rice by explicitly taking into account the tolerance traits breeders are working on, and (ii) presenting a case study on ideotype design targeting production districts in California and Greece.

5.3. Methods

5.3.1. The growth chamber experiments

Two rice (*Oryza sativa* L. spp. japonica) cultivars differing in their level of salt tolerance, i.e., Baldo and Vialone Nano, were grown in dedicated hydroponics growth chamber experiments. Caryopses were sterilized with 50% (v/v) $Ca(ClO)_2$ for 30 min, thoroughly rinsed with deionized water and placed on wet filter paper at 26 °C in the dark for four days. Seven days old seedlings were then transferred to black plastic boxes containing the following complete nutrient solution (Nocito et al., 2011): 1.5 mM KNO_3, 1 mM $Ca(NO_3)_2$, 500 µM $MgSO_4$, 250 µM $NH_4H_2PO_4$, 25 µM Fe-tartrate, 46 µM H_3BO_3, 9 µM $MnCl_2$, 0.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$, 30µM Na_2O_3Si (pH 6.5). Floating polystyrene foam sheets were used to hold seedlings and to allow renewing solutions without touching the plants to avoid any potential damage to the roots (which would make Na^+ entering directly from root lesions). Growing conditions were set to 14 h photoperiod; photosynthetically active radiation was supplied by fluorescent lamps (400 mmol m^{-2} s^{-1}); day/night temperatures were 26 °C/18 °C; relative humidity ranged between 58 and 92%. Five NaCl treatments were applied from three weeks after sowing until maturity: 0 mM, 10 mM, 25 mM, 35 mM and 50 mM. In order to maintain NaCl concentrations nearly constant, solutions were renewed each three days.

At late heading (BBCH code 51) and maturity (BBCH code 92) three plants for each combination cultivar × treatment were harvested and divided into stems, panicles and leaves. The latter were further separated into apical, medium and senescent leaves (referring respectively to the two youngest leaves, others green leaves and dead ones) to detect potential variation in Na^+ accumulation among leaves of different ages. Plant height, number of tillers and dry biomass of each organ were measured. Dry biomass samples were ground to a fine powder and digested by concentrated HNO_3 (10 mM) in a microwave digester (ETHOS D, milestone, Italy) at 100°C (Amari et al., 2014). The mineralized material was dissolved in 5 mL 0.1 M HNO_3 and Na^+ content was measured by inductively coupled plasma mass spectrometry (Bruker Aurora M90 ICP-MS, ICP Mass Spectrometer). Na^+ content and dry biomass of different plant organs were then used to calculate corresponding Na^+ concentrations. Immediately before the first sampling event (late heading), the impact of salt stress on net photosynthetic rate, stomatal conductance and transpiration rate was measured on the youngest fully expanded leaf using a CIRAS-3 Portable Photosynthesis System (PP Systems, Amsbury, MA, USA). Apical and medium leaves were then scanned to determine plant leaf area and SLA, the latter calculated as ratio between leaf area and leaf dry biomass. At harvest, spikelet sterility was determined.

5.3.2. The ideotyping study

5.3.2.1. Case studies and simulation scenarios

An ideotyping study was carried out to using the salt stress model to identify the traits breeders should focus on in two production areas differing for the salinity seasonal dynamics (Fig. 2). Colusa is one of the six counties at the north of Sacramento where the production of rice in California – the second largest rice-producing state in the US – is concentrated. While most of the irrigation water has a low salt content, water holding periods for herbicide distribution and high temperatures promote evapo-concentration of salt in rice fields (Scardaci et al., 2002; Linquist et al., 2015). This leads salinity in field water during the first part of the crop cycle to increase up to 3.5 dS m^{-1}, and to decrease rapidly once the flow of fresh water is restored. Although the mean seasonal salinity is not high, the rice susceptibility during early phenological phases can lead yield losses to exceed 10% (Grattan et al., 2002). The second scenario targets the southeastern region of Axios River plain, near Thessaloniki, one of the key regions for rice production in Greece (Ntanos et al., 2001). Ninety percent of the soils in the area are saline, causing increases in the salts content of irrigation water during infiltration. Evapo-concentration of salts due to high temperatures also contributes to increase salinity, which progressively reaches values of 2.5 dS $m^{-1}$from mid-season to harvest. Dynamics of salinity in field water and information on management practices used for the two case studies were derived from Scardaci et al. (2002) and Linquist et al. (2015) (California), and from Lekakis et al. (2015) (Greece).

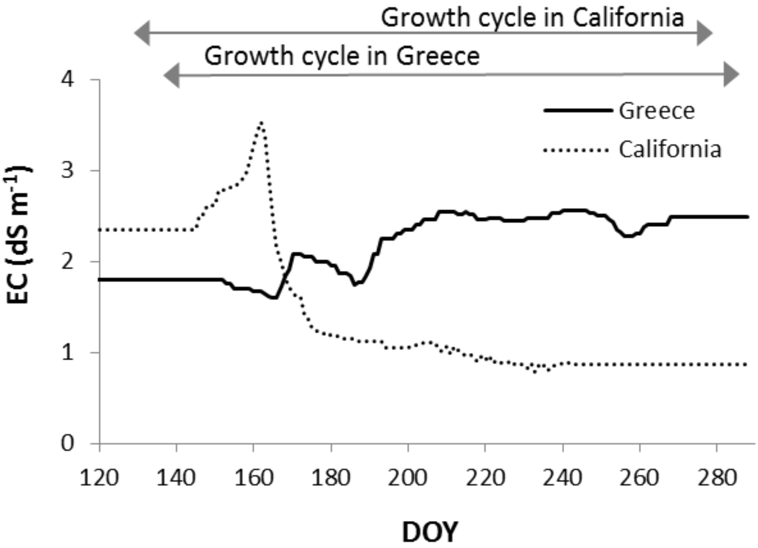


Figure 2. Seasonal electrical conductivity (dS m^{-1}) of field water for the two scenarios used for the ideotyping study, derived from Scardaci et al. (2002) (California) and Lekakis et al. (2015) (Greece). Salinity dynamics follow different patterns: in the Californian site the highest level of salinity is reached in the first part of the crop cycle whereas in the Greek one salt concentration increases gradually until mid-season and remains constant afterwards.

Simulations were performed for the cultivar Thaibonnet (also known as L202), developed by the California Co-operate Rice Research Foundation and currently representing an important variety in Greece (Ntanos et al., 2001).

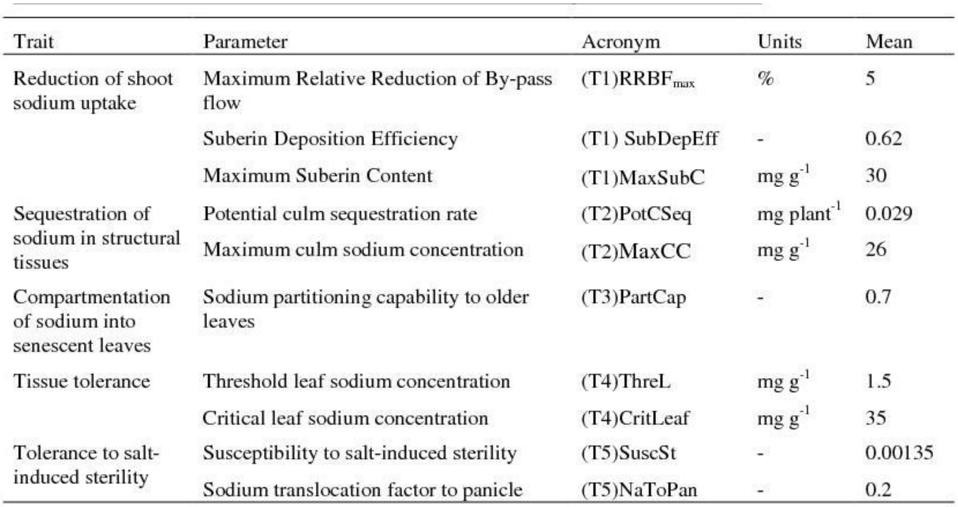
5.3.2.2. Sensitivity analysis

The ideotyping study was performed using global sensitivity analysis (SA) techniques (e.g., Martre et al., 2015b; Casadebaig et al., 2016). In particular, the variance-based method of Sobol' (Sobol', 1993) – considered as a references for SA (Saltelli and Sobol', 1995) – was used, targeting yield as reference output. The analysis focused on first- and total-order effects, accounting, respectively, for the effects of variations in each parameter on simulated yield, and for the effects of variations in parameter including possible interactions with others. The sample size for the combinations of parameters was 5632, i.e., the lowest value of $M|M > (\gamma \cdot n)$, with $M = 2^{q+3}(2n + 2)$, $q = \{1, 2, 3, \dots, Q\}$, γ is the suggested number of model runs for each parameter, and n is the number of parameters in the sensitivity analysis. In this study, γ was set to 500 (Confalonieri et al., 2010a).

Parameterization of the crop model WOFOST-GT2 for Tropical Japonica rice varieties was derived from Stella et al. (2014). Concerning the parameters of the salt stress model, the values derived from the growth chamber experiments were used as means (Table 1), with the exception of a correction factor applied to the maximum suberin content to account for differences in root development between hydroponic and soil conditions (Kotula et al., 2009). Distributions for the SA were assumed as normal, and standard deviations were set to 5% of the mean values for the parameters (Richter et al., 2010).

In order to avoid the risk of including in SA results the effect of a specific meteorological season, simulations for both sites were performed on 20-year series of weather data (1995-2015) retrieved from the European Centre for Medium-Range Weather Forecasts (ECMWF; ERA-Interim database; www.ecmwf.int.

Table 1. Plant traits, corresponding model parameters, and distribution means. Distributions were assumed as normal and standard deviations were set to 5% of the mean values (Richter et al., 2010).



Trait	Parameter	Acronym	Units	Mean
Reduction of shoot sodium uptake	Maximum Relative Reduction of By-pass flow	(T1)$RRBF_{max}$	%	5
	Suberin Deposition Efficiency	(T1) SubDepEff	-	0.62
	Maximum Suberin Content	(T1)MaxSubC	mg g^{-1}	30
Sequestration of sodium in structural tissues	Potential culm sequestration rate	(T2)PotCSeq	mg $plant^{-1}$	0.029
	Maximum culm sodium concentration	(T2)MaxCC	mg g^{-1}	26
Compartmentation of sodium into senescent leaves	Sodium partitioning capability to older leaves	(T3)PartCap	-	0.7
Tissue tolerance	Threshold leaf sodium concentration	(T4)ThreL	mg g^{-1}	1.5
	Critical leaf sodium concentration	(T4)CritLeaf	mg g^{-1}	35
Tolerance to salt-induced sterility	Susceptibility to salt-induced sterility	(T5)SuscSt	-	0.00135
	Sodium translocation factor to panicle	(T5)NaToPan	-	0.2

5.4. Results

5.4.1. A new model for salt stress built around actual plant traits

A new model for the salt stress on rice was developed by directly targeting tolerance traits breeders are working on (Fig. 1). In the following equations, terms with the prefix (T-) refer to traits.

5.4.1.1. Plant Na^+ uptake

The plant capability to reduce the shoot Na^+ uptake relies on a "root filter" (Ash et al., 1997) that prevents Na^+ from entering the roots and getting translocated, via the xylematic stream, to the shoot (Roy et al., 2014). The root filter can be more or less pronounced, leading to the identification of "excluder" genotypes as opposite to varieties less effective in preventing Na^+ from entering the xylem stream (Faiyue et al., 2012).

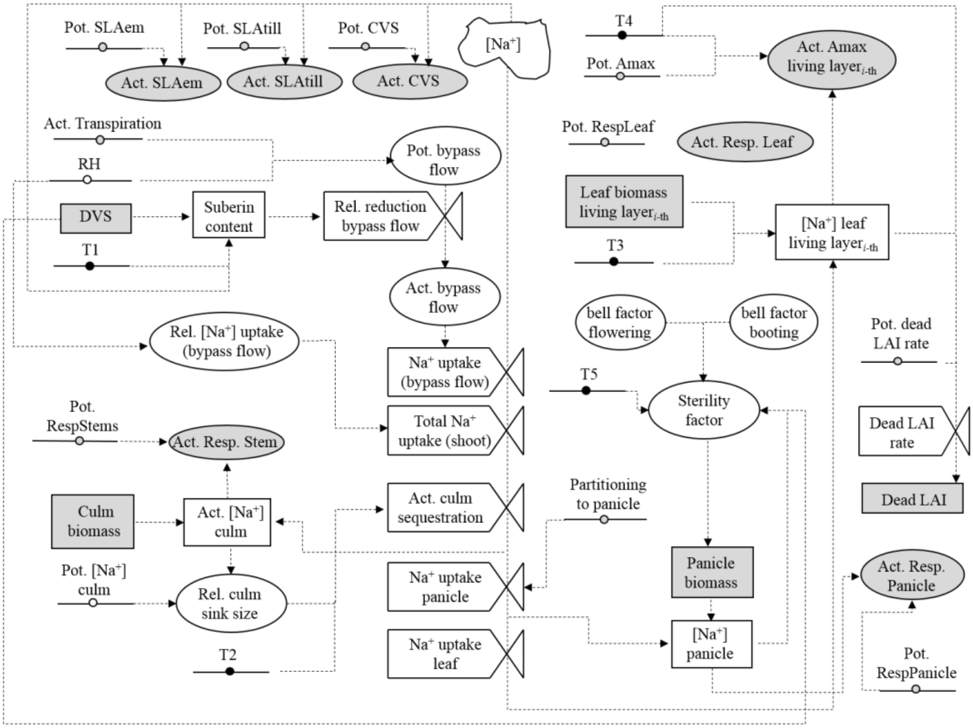


Figure 1. Flowchart of the new model for the impact of salt stress on rice growth. *T1, T2, T3, T4,* and *T5* refer, respectively, to the traits involved with the capability limiting shoot Na^+ uptake, sequestrating Na^+ into the culm base, storing toxic ions in senescent leaves, decreasing the toxicity of Na^+ in photosynthetically active leaves, and reducing salt-induced spikelet sterility. Grey items represent coupling points between the salinity model and the crop simulator.

From a physiological standpoint, the root filter is made of two components: morphological barriers reducing the apoplastic entry of Na^+ (bypass flow), and channels in the plasma membrane of root epidermal/cortical cells that mediate the selective and non-selective transport of Na^+ (Tester and Davenport, 2003; Ren et al., 2005). Although by-pass flow accounts for 22-35% of the total Na^+ uptake in rice (Faiyue et al., 2010; Faiyue et al., 2012), it is a key responsible for the differences in the degree of salt tolerance in rice genotypes. This makes the reduction of bypass flow a promising trait for increasing salt tolerance in rice

(Senadheera et al., 2009; Faiyue et al., 2012). The amount of water potentially flowing through the apoplastic pathway (JvB, mm day-1) is calculated according to the following equation:

$$Jv_B = \frac{Tr_{act} \cdot RJv_B}{100} \tag{1}$$

where Tr_{act} (mm day^{-1}) is the actual transpiration and RJv_B (%) is the percentage of water uptake through the apoplastic pathway, estimated as a function of air relative humidity (RH; %) according to Steudle et al. (2000) and Faiyue et al. (2010):

$$RJv_b = -0.0275 \cdot RH + 3.92 \tag{2}$$

The development of Casparian bands and the deposition of suberin lamellae in the root exo- and endodermis reduce the water transport through the apoplast and thus the bypass flow-Na$^+$ uptake (Krishnamurthy et al., 2009). The relative reduction of bypass flow (*RRBF*, unitless, 0-1) is thus calculated as a function of the root suberin content (Krishnamurthy, 2011):

$$RRBF = \frac{1}{100} \cdot (T1)RRBF_{max}^{\frac{SC - SC_{min}}{(T1)MaxSubC - SC_{min}}} \tag{3}$$

where $(T1)RRBF_{max}$ (%) is the reduction of bypass-flow when the suberin content is maximum; SC (mg g^{-1}) is the root suberin content; $(T1)MaxSubC$ (mg g^{-1}) is the maximum suberin content; SC_{min} (mg g^{-1}) is the minimum root suberin concentration at which bypass flow start to be reduced. The root suberin content is derived as a function of plant age (equation 4) and of the genotype sensitivity to salinity (equation 5) (Krishnamurty et al, 2009; Zhou et al., 2011):

$$SC = (T1)MaxSubC \, \frac{DVS \cdot (0.5 \cdot (1 - F_{sc}) + 1)}{(1 - F_{sc}) + DVS} \tag{4}$$

$$F_{sc} = \frac{[Na^+]_{ext}}{Max\,[Na^+]_{ext}} \cdot \frac{1}{(1 - (T1)SubDepEff)} \tag{5}$$

where DVS (unitless; 0-2) is a SUCROS-type development stage code (0: emergence; 1: anthesis; 2: maturity); F_{sc} (unitless; 0-1) is deposition of suberin in response to salinity; $(T1)SubDepEff$ (unitless; 0-1) is the suberin deposition efficiency (genotype specific); $[Na^+]_{ext}$ and $Max[Na^+]_{ext}$ (mM) are, respectively, the actual and maximum (at which the suberin deposition is maximum) Na^+ concentrations in the external medium. Therefore, the amount of Na^+ actually delivered to the shoot via bypass-flow ($NaUptake_{AP}$, mg ha^{-1}) is:

$$NaUptake_{AP} = Jv_b \cdot RRBF \cdot [Na^+]_{ext} \tag{6}$$

The fraction of bypass Na^+ on the total Na^+ uptake ($RNaUptake_{AP}$; %) is derived analogously to equation 2 (Faiyue et al., 2010). Finally, the total amount of Na^+ daily entering the shoot (*NaUptake,* mg ha^{-1}) is derived according to equation 7:

$$NaUptake = NaUptake_{AP} \frac{100}{RNaUptake_{AP}} \tag{7}$$

5.4.1.2. Sequestration of Na^+ into structural/senescent organs

To reduce the amount of Na^+ reaching the leaves, plants have developed mechanisms to accumulate toxic ions in the tissues of culm base and leaf sheath (Costafits et al., 2012; Suzuki et al., 2016). The former is estimated considering genotypic differences and feedback mechanisms triggered by the amount of Na^+ already stored in culms (Asch et al., 1997). The actual culm sequestration rate (*ActCulmSeqRate*; mg ha^{-1}) is derived as:

$$ActCulmSeqRate = (T2)PotCSeq \cdot D \cdot \{1 - [0.08 \cdot ((RelSinkSize + 0.1)^{-1.13} - 0.08)]\} \tag{8}$$

where $(T2)PotCSeq$ (mg $plant^{-1}$) is the potential capability of the genotype to sequestrate Na^+ in culms; D is the plant density (plant ha^{-1}); *RelSinkSize* (unitless, 0-1) is a dynamic sink capacity of culm for Na^+ sequestration accounting for feedback mechanisms (equation 9), with $(T2)MaxCC$ and $[Na^+]culmAct$ (mg g^{-1}) being the maximum and actual Na^+ concentration in culms, respectively.

$$RelSinkSize = 1 - \frac{[Na^+]_{culmAct}}{(T2)MaxCC} \tag{9}$$

The amount of Na^+ daily reaching the panicle (*NaPanicle,* mg ha^{-1}) is derived as a function of the Na^+ not sequestrated in culms and of the transport of photosynthates to panicles:

$$NaPanicle = \begin{cases} ParP \cdot (T5)NaToPan \cdot (NaUptake - ActCulmSeqRate) & 0.6 \leq DVS \leq 2 \\ 0 & elsewhere \end{cases} \tag{10}$$

where *ParP* (unitless) is the relative amount of photosynthates daily partitioned to panicles and (*T*5)*NaToPan* (unitless; 0-1) is the factor for Na^+ translocation to panicles.

The amount of Na^+ daily reaching leaves (*NaLeaves*, mg ha^{-1}) is the difference between total Na^+ uptake in shoots and the amounts of Na^+ sequestrated in the culms and partitioned to panicles. Plants tend to accumulate Na^+ in the oldest leaves to preserve photosynthetically active tissues from toxic ions (Tester and Davenport, 2003; Cotsaftis et al., 2012). This is represented using equation 11:

$$NaUptakeL_i = \frac{Na_{leaves}}{\sum_{x=1}^{N}\left[(1-(T3)PartCap)^{\left[\frac{(x-1)}{(N-1)}\right]}\right]} \cdot (1-(T3)PartCap)^{\left[\frac{(x-1)}{(N-1)}\right]} \tag{11}$$

where *NaUptakeL*$_i$ (mg ha^{-1}) is the Na^+ delivered to the *i*th canopy layer; (*T*3)*PartCap* (unitless; 0-1) is the genotype capability of partitioning Na^+ to oldest leaves; *x* is the *i*th canopy layer; *N* is the number of living canopy layers. To account for this heterogeneity in Na^+ accumulation in leaves of different ages, the model for salt stress should be coupled with a crop model providing a multilayer canopy structure.

5.4.1.3. Impact of Na^+ on photosynthesis, leaf senescence and spikelet sterility

The Na^+ effect on photosynthesis (dos Reis et al., 2012) and leaf senescence (Rajendran et al., 2009; Hairmansis et al., 2014) depends on the genotype ability to sequestrate toxic ions in the vacuole and to synthesize osmolytes to counterbalance the osmotic pressure. The stress factor for photosynthesis (*RPn*, unitless 0-1) – also used to increase senescence – is derived using equation 12:

$$RPn = \left[\left(\frac{[Na^+]L_i - [Na^+]_{leaf\,min}}{(T4)ThreL - [Na^+]_{leaf\,min}}\right) \cdot \left(\frac{(T4)CritLeaf - [Na^+]L_i}{(T4)CritLeaf - (T4)ThreL}\right)\right]^{\left(\frac{(T4)CritLeaf - (T4)ThreL}{(T4)ThreL - [Na^+]_{leaf\,min}}\right)^{C}} \tag{12}$$

where $[Na^{+}]L_{i}$ (mg g^{-1}) is the Na^{+} concentration in leaves at the *i*th canopy layer; $[Na^{+}]_{leaf\ min}$ (mg g^{-1}) is the Na^{+} concentration in unstressed leaves; $(T4)ThreL$ (mg g^{-1}) is the Na^{+} concentration above which salt stress starts; $(T4)CritLeaf$ (mg g^{-1}) is the Na^{+} concentration at which photosynthesis becomes null; C is a shaping coefficient.

Na^{+} also increases maintenance respiration due to the high metabolic costs of the processes of ion exclusion, vacuolar compartmentation and synthesis of osmolytes (Jacoby et al., 2011). The factor increasing maintenance respiration in leaves (*MRespF*, unitless, 0-1) is thus:

$$MRespF = \frac{[Na^{+}]_{leaves} - [Na^{+}]_{Thresh}}{[Na^{+}]_{Crit} - [Na^{+}]_{Thresh}} \qquad (13)$$

where $[Na^{+}]_{Crit}$ (set to 3 mg g^{-1}) is the Na^{+} concentration at which respiration is double; $[Na^{+}]_{Thresh}$ (set to 0.5 mg g^{-1}) is the Na^{+} concentration at which maintenance respiration starts to be affected; $[Na^{+}]_{tissue}$ (mg g^{-1}) is the average concentration in leaves (weighted for layers' biomass). The same function is used for the increased maintenance respiration in culms.

Salt stress also affects spikelet sterility (Hossain et al., 2015) according to panicle Na^{+} concentration and plant susceptibility, the latter depending on the genotype and phenological stage (equation 14).

$$SterilityF = \begin{cases} (T5)SuscSt \cdot [Na^{+}]_{panicle} \cdot bellF & 0.6 \leq DVS \leq 1.1 \\ 0 & elsewhere \end{cases} \qquad (14)$$

where *SterilityF* (unitless, 0-1) is the factor reducing spikelet fertility due to salt stress; $(T5)SuscSt$ (unitless; 0-1) represents the genotype susceptibility; *bellF* (unitless; 0-1) is a factor modulating susceptibility according to the within- and between-plant heterogeneity phenological development (Confalonieri et al., 2009b). The *bellF* is calculated considering two phenological stages of maximum susceptibility to abiotic stress-induced sterility: booting (microsporogenesis; DVS=0.8) and flowering (DVS=1). *SterilityF* is then used to reduce the amount of photosynthates daily partitioned to panicles.

The reduction of growth due to the osmotic potential in the external medium (Munns and Tester, 2008) is derived by reducing leaf area expansion (Poorter et al., 2009) (equation 15) and culm growth (Rajendran et al., 2009) (equation 16) because of limitations to cell wall extension caused by a reduced cell water uptake (Confalonieri et al., 2014).

$$SLAstress = -0.0002 \cdot [Na^{+}]_{ext}^{2} + 0.008 \cdot [Na^{+}]_{ext} + 1 \tag{15}$$

$$CVSstressfactor = 0.0002 \cdot [Na^{+}]_{ext}^{2} - 0.024 \cdot [Na^{+}]_{ext} + 1 \tag{16}$$

where $SLAstress$ (unitless, 0-1) is the reduction factor for specific leaf area (SLA; m^2 kg^{-1}); $CVSstress$ (unitless, 0-1) is the reduction factor for culm growth; $[Na^{+}]_{ext}^{2}$.(mM) is the Na^+ concentration in the external medium.

The salinity model was coupled to the WOFOST model as modified by Stella et al. (2014), to benefit from an explicit multi-layer canopy representation. The resulting modelling solution was evaluated using the growth chamber datasets described below and then used for the ideotyping study.

5.4.2. Model evaluation

The agreement between observed and simulated values of aboveground biomass, yield, biomass of culms, leaves and panicles, leaf area index and plant sodium content is shown in Fig. 3. In general, the model showed good performances in reproducing the impact of salt stress on aboveground biomass accumulation and yield (Fig. 3.a), with relative root mean square error (RRMSE; %; 0 to +1, optimum 0) equal to 28.0% and 23.8%, respectively. Good values for these two variables were achieved also for R^2 (0.89 and 0.90) and modelling efficiency (EF; -∞ to +1, optimum +1; Nash and Sutcliffe, 1970): 0.83 and 0.87. The values of coefficient of residual mass (CRM; -∞ to +∞; optimum = 0; Loague and Green, 1991) close to zero highlighted the absence of systematic over- or underestimations.

Good performances were achieved also for the simulation of the biomass of culms, leaves, and panicles (Fig. 3.b), with RRMSE never exceeding 36% and values of R^2 ranging between 0.74 (leaves) and 0.94 (panicles). Regardless of the organ, EF was always above positive. Concerning the simulation of panicles weight, the model showed a slight tendency to underestimate the values at heading, likely because of the observed heterogeneity among tiller development. An opposite behavior (slight overestimation around heading) was instead observed for culm and leaf biomass. In this case, the reason is likely an underestimation of the impact of the osmotic component of salt stress on leaf area expansion and tiller development, which are only implicitly reproduced in the current version of the modelling solution, since the crop model used does not explicitly simulate tillering and leaf size. Future model improvements could thus refer to the implementation of dedicated approaches for the simulation of the Na^+-induced reduction in cell turgor pressure and related decrease in tissues expansion.

The discussed overestimation of leaf biomass explains the similar behavior showed by the model for the simulation of leaf area index (Fig. 3.c) (CRM = -0.33), although the model correctly reproduced the relative reduction in leaf area for increasing Na^+ concentrations (R^2=0.89; EF=0.65). This is considered as particularly important, since leaf area index is a key state variable involved with the amount of water daily transpired and thus with the potential entry of Na^+ through the apoplastic pathway (Faiyue et al., 2012; equations 1-6).

Concerning Na^+ uptake (Fig. 3.d), simulated Na^+ contents showed a good agreement with measured data, although the values of the performance metrics were slightly worse compared to those achieved for other outputs (RRMSE was around 50% and CRM was -0.20). However, the large portion of variance explained (R^2 = 0.72) and the largely positive value of EF (0.66) allows considering also this variable as satisfactorily simulated. Indeed, Na^+ content results from the simulation of both Na^+ dynamics (entry, translocation, sequestration, etc.) and the related effects on crop growth, and also by the uncertainty of the crop model itself. The interaction between the crop model and the salt stress model is deeply involved with the way plant growth drives Na^+ uptake through the simulation of actual transpiration (in turn driven by leaf area index) and Na^+ sink capacity (driven by organs biomass). For this reasons, the general overestimation of plant Na^+ content could be related to the overestimation in leaf area index and culm biomass (Fig. 3.c). However, the model was not able to reproduce the high plant Na^+ content measured at harvest for the cultivar Baldo at the highest Na level (150 mg plant^{-1}). Another aspect able to partly explain the values for the agreement metrics obtained for the simulation of Na^+ content is the Na^+ accumulation for the control treatment (0 mM NaCl). In this case, the model simulates a null Na^+ plant content, whereas small values were measured in real plant tissues.

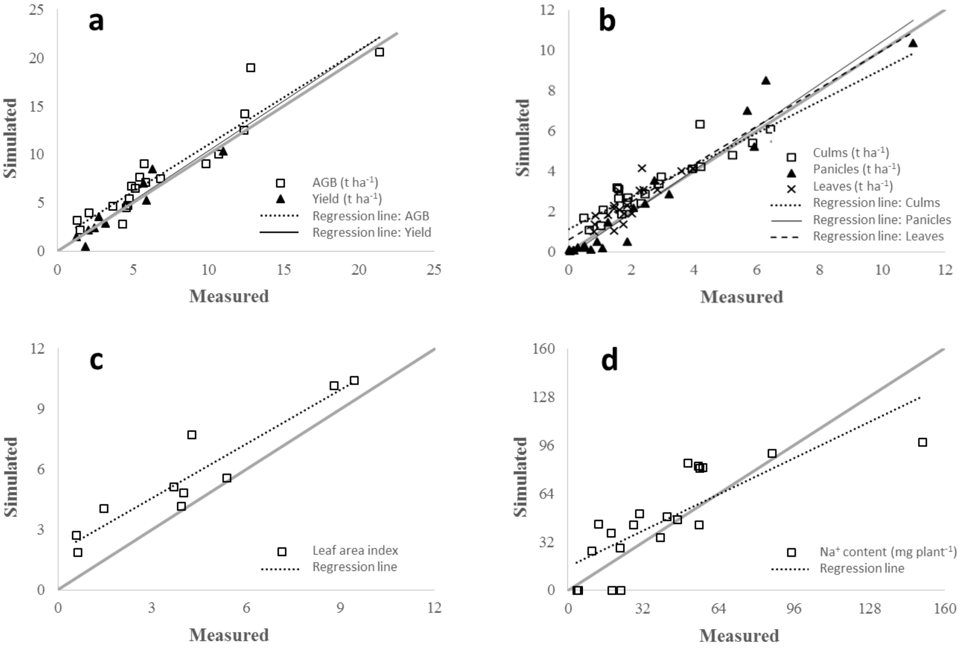


Figure 3. Measured and simulated values of (a) aboveground biomass (AGB) and yield, (b) biomass of culms, leaves and panicles, (c) leaf area index, and (d) plant sodium content. The grey line indicates the 1:1 agreement between measured and simulated data.

5.4.3. Identification of key traits in different scenarios

Simulations performed during the SA led to 14% and 12% average yield losses in California and Greece, respectively, in agreement with the expected yield reduction under salinity levels similar to those explored (Grattan et al., 2002).

Results of the SA (Fig. 4) showed how different scenarios could allow defining contrasting breeding strategies to increase the overall cultivar salt tolerance and, in turn, further demonstrated the potential of trait-based modelling for designing district-specific ideotypes (Paleari et al., 2015). Indeed, simulations revealed that – despite salt tolerance is an issue in both scenarios – different traits would guarantee the highest increase in yields in California and Greece. This is in agreement with Roy et al. (2014), who observed how different traits could be exploited to increase salt tolerance under different salinity levels. Tissue tolerance (T4, indicated in pink in Fig. 4) was the most important trait in California (Fig. 4.b), where high-salinity peaks occurs for short periods in the first part of the season (Fig. 2). Indeed, although the reduction of shoot Na^+ uptake (T1, green in Fig. 4) also played a role (because of the relevance of a parameter involved with Na^+ exclusion at root level via suberin deposition), the sharp increase in salinity in a moment when root barriers are still not developed makes the response at leaf level more important to increase the overall plant tolerance. A similar peak occurring later in the season (with a higher root suberin content) would have led to different results.

In case of prolonged stressful conditions like those characterizing the Greek scenario (Fig. 2), instead, the most important tolerant traits were involved with the plant capability to prevent Na^+ from reaching leaf blades (Fig. 4.d), i.e., reduction of Na^+ uptake (suberin deposition) and − to a lesser extent – higher sequestration in structural tissues (T2, indicated in red in Fig. 4). The trait involved with tissue tolerance were not considered as relevant for this scenario, because Na^+ accumulation in leaf tissues was so fast to rapidly overcome the capability of the plant to segregate toxic ions into the vacuole and to synthesize osmolytes. This is in agreement with Munns et al. (2012), who observed the same relationship between high salinity and the importance of excluding Na^+ at root level for wheat.

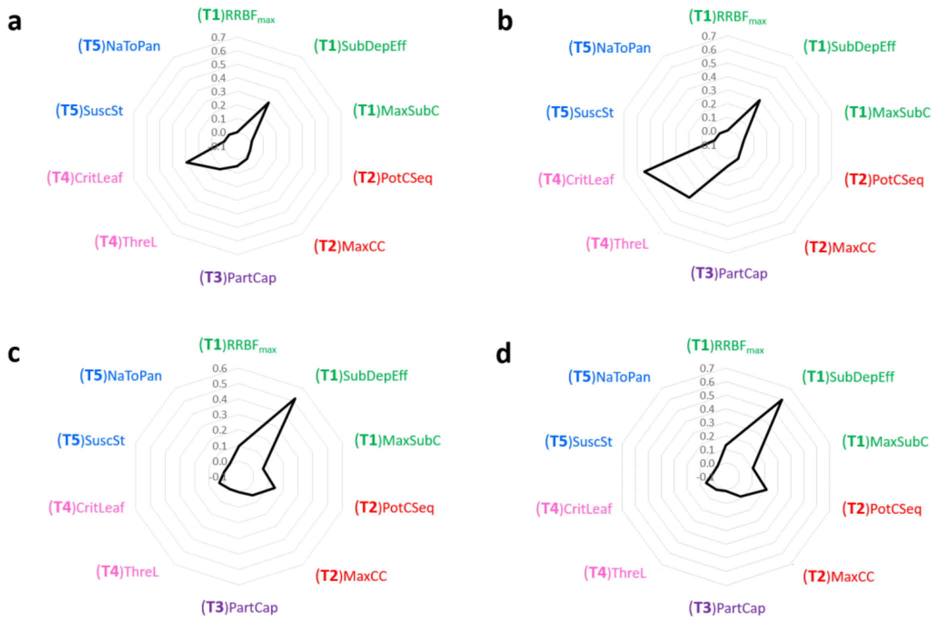


Figure 4. Sobol’ first- (a, c) and total-order (b, d) effects calculated for the sensitivity analysis performed on model parameters representing salt tolerance traits (Table 1). The analysis was carried out for two scenarios, one in California (a, b) and the other in Greece (c, d). The output for which sensitivity metrics were calculated was the final yield.

The comparison of the Sobol’ first- and total-order effects highlighted strong interactions only for the Californian scenario (Figs. 4.a and 4.b) for the two parameters modulating the toxic effect of Na^+ in leaf blades, i.e., (T4)ThreL and (T4)CritLeaf. This is partly due to the fact that these two parameters are involved in the same response function (equation 12), thus they interacted with each other. However, the large differences in the values of the first- and total-order sensitivity metrics suggest that these two parameters interacted also with others, being tissue tolerance strictly depending on the whole chain of processes modulating the sodium uptake at root level and its translocation and accumulation in photosynthetic tissues.

Regardless of the scenario, parameters involved with tolerance to salt-induced spikelet sterility did not resulted important in affecting yields. The reason is that Na^+ concentration in the young panicles was not high enough during the two sensitive stages (i.e., booting and flowering). In California, indeed, the accumulation of Na^+ in the developing panicles before flowering was low because of the reduction of salinity due to fresh water inflow after the herbicide treatments, whereas in Greece Na^+ concentration in field water started to increase too late to generate high contents in the reproductive organs before flowering.

5.5. Discussion

Taking salt stress tolerance and rice as a case study, we showed how the design of ideotypes can benefit from the availability of models explicitly developed starting from traits breeders are working on. Indeed, the model developed demonstrated its suitability for analyzing in depth key G×E×M interactions. Salt tolerance is a complex trait relaying on a variety of physiological mechanisms that can be alternatively selected to improve the overall crop tolerance. The availability of new tools for genetic improvement (e.g., Marker Assisted Selection) allows breeding for specific traits instead of targeting the overall crop tolerance (Roy et al., 2014) thus increasing the efficiency of the breeding process, since the effectiveness of the changes in the values of different traits varies according to the agro-environmental context.

For the first time, a modelling approach dedicated to ideotyping was developed by explicitly building algorithms around traits for which breeding activities are ongoing. We consider this strategy as the only one able to avoid inconsistencies between model parameters and plant traits, and thus between *in silico* ideotypes and the possibility of realizing them in vivo. Indeed, a high level of detail in the representation of physiological processes cannot be considered as a guarantee of direct relationships between model parameters and plant traits, given the same knowledge can be formalized in a variety of possible modelling structure (Confalonieri et al., 2016). Moreover, during model development, a pronounced process-based perspective was used to properly account for the key physiological processes and feedback mechanisms involved.

Results of model application to design district-specific ideotypes showed that, despite differences between scenarios were mainly limited to the seasonal dynamics of salt concentration in field water, putative traits to increase salt tolerance can rely on completely different physiological mechanisms. Results achieved encourage a paradigm shift towards the development of dedicated trait-based models and their use for supporting breeding programs at district level.

Limits of our study − and thus potential areas for model improvement − deal with the lack of approaches to simulate also the uptake and distribution of K^+, since the toxicity of Na^+ seems to be related also to the Na^+:K^+ ratio and not only to the concentration of Na^+ in itself (e.g., Ren et al., 2005). Also, improved (i.e., more explicit) approaches would be needed for the simulation of the effect of the osmotic component of salt stress on tissue expansion and stomatal reaction, in order better account for osmotic adjustment.

Acknowledgments

We gratefully acknowledge Prof. Gian Attilio Sacchi and his team for the support in the experimental activities and the fruitful discussions that have led to the development of the model for salt stress.

ISIde: A RICE MODELLING PLATFORM FOR *IN SILICO* IDEOTYPING

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Published on
Computers and Electronics in Agriculture (2016), 128, 46-49

6.1. Abstract

Ecophysiological models can be successfully used to analyze genotype by environment interactions, thus supporting breeders in identifying key traits for specific growing conditions. This is especially true for traits involved with resistance/tolerance to biotic and abiotic stressors, which occurrence can vary greatly both in time and space. However, no modelling tools are available to be used directly by breeders, and this is one of the reasons that prevents an effective integration of modelling activities within breeding programs. ISIde is a software platform specifically designed for district-specific rice ideotyping targeting (i) resistance/tolerance traits and (ii) breeders as final users. Platform usability is guaranteed by a highly intuitive user interface and by exposing to users only settings involved with genetic improvement. Other information needed to run simulations (i.e., data on soil, climate, management) is automatically provided by the platform once the study area, the variety to improve and the climate scenario are selected. Ideotypes indeed can be defined and tested under current and climate change scenario, thus supporting the definition of strategies for breeding in the medium-long term. Comparing the performance of current and improved genotype, the platform provides an evaluation of the yield benefits exclusively due to the genetic improvement introduced. An example of the application of the ISIde platform in terms of functionalities and results that can be achieved is reported by means of a case study concerning the improvement of tolerance to heat stress around flowering in the Oristanese rice district (Italy). The platform is currently available for the six Italian rice districts. However, the software architecture allows its extension to other growing areas – or to additional genotypes – via dedicated tools available at the application page.

Keywords: Blast, breeding, climate change, fungal pathogens, spikelet sterility, WARM.

6.2. Introduction

Costs and time resources required to develop new varieties on the one hand, and the availability of new technologies for genetic improvement on the other hand, have given new interest in breeding for specific traits (Peng et al., 2008). In order to identify such traits, genotypes need to be extensively evaluated under the target agroenvironmental and management contexts. This activity is onerous and prevents breeders from testing genotypes under climate change scenarios, despite the time needed to complete breeding programs, as well as their cost, would likely suggest to carefully consider them (Paleari et al., 2015). Despite their level of uncertainty and their exploratory nature, ideotyping studies based on simulation models can be useful to this end (Martre et al., 2015a). Changes in the values of parameters describing morphological and physiological plant traits indeed, allow the definition of *in silico* ideotypes that can be screened under a wide range of conditions. However, possible discrepancies between the *in silico* improved varieties and their *in vivo* realization could limit the effectiveness of model-based ideotyping studies (Hammer et al., 2002). An interdisciplinary approach is thus crucial to develop modelling tools (i) able to provide reliable estimates of differences among genotypes for specific traits and (ii) easily manageable directly by target users (i.e., breeders) without requiring specific crop modelling or IT skills. This would avoid testing hypothesis unfeasible in practical terms.

Usability – defined by ISO as “the capability of a software product to be understood, learned, used and attractive to the user, when used under specified conditions” (ISO, 2001) – thus become a key factor for extending the use of simulation tools to users other than modellers and computational experts (Carpenter et al., 2013).

The objective of this work is to present the first integrated modelling platform specifically developed for the definition and evaluation of rice ideotypes improved for resistance and tolerance traits, targeting breeders as reference users. Ideotypes can be tested over different production districts and under current and climate change scenarios, thus supporting the identification of key traits for specific contexts in both the short and the medium-long term.

6.3. The ISIde platform

ISIde (*In Silico* Ideotyping platform) is a rice-specific modelling platform for ideotyping studies. It is currently available for the six Italian districts and for resistance/tolerance traits dealing with blast disease and with spikelet sterility induced by cold shocks during microsporogenesis and by heat/cold shocks around flowering. However, the component-based architecture allows easily extending the platform to other contexts (i.e., other regions) or processes (i.e., other biotic or abiotic stressors). Simulations are performed with a 5 km × 5 km spatial resolution to capture the heterogeneity of the environmental conditions explored by the crop throughout the districts. ISIde is made available as a .NET application (C#); the setup is freely available at info@cassandralab.com.

6.3.1. The modelling solution

A schematic representation of the modelling solution implemented in ISIde is given in Fig. 1. A full description of the modelling solution is available in Paleari et al. (2015), whereas details on algorithms implemented are available in the seminal literature and in the model web pages (http://www.cassandralab.com/applications/2).

The simulation engine is the hourly-time step version of the rice model WARM (Confalonieri et al., 2009b), which reproduces key processes involved with the rice growth and development, as well as the impact of biotic and abiotic stressors on yield. A micrometeorological model is used to reproduce the floodwater effect on the vertical thermal profile. Biomass accumulation is estimated using a net-photosynthesis approach based on radiation use efficiency (RUE), with the latter modulated by senescence, temperature and saturation of enzymatic chains. Spikelet sterility due to pre-flowering cold shocks and to cold and heat shocks around flowering is simulated by considering the different plant susceptibility during the periods of interest via bell-shaped functions. Given the strong relationship between damage magnitude and specific phenological stages, these functions allow accounting for the between- and within-plant heterogeneity in phenological development (Yoshida, 1981). The simulation of the interaction between the plant and fungal pathogens is performed using the model proposed by Bregaglio et al. (2016) parameterized according to the specific thermal and moisture requirements of the airborne fungal pathogen causing blast disease (*Magnaporthe oryzae* B. Couch).

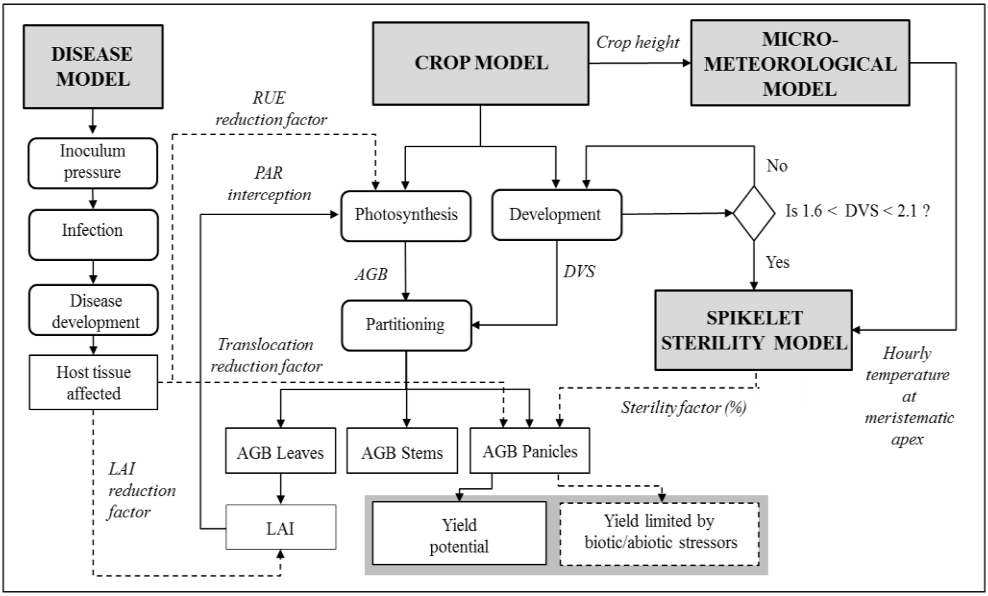


Figure 1. The WARM modelling solution implemented in the ISIde platform. Coupling points between the crop model and models for the impact of biotic/abiotic stressors are represented via dotted arrows. AGB: aboveground biomass; DVS: development stage code (1.6 = panicle initiation, 2.1 = end of flowering); LAI: leaf area index; PAR: photosynthetically active solar radiation, RUE: radiation use efficiency.

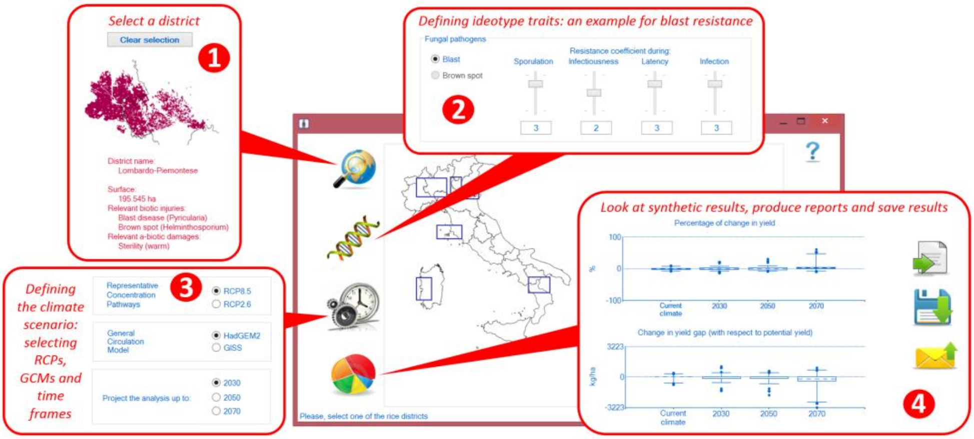
6.3.2. A high-resolution, geo-referenced database for model inputs and parameters

To avoid setup problems due to specific system configurations, the ISIde database is structured in a series of binary files. This solution avoids dealing, e.g., with different ODBC driver versions when installing client applications targeting databases management systems (DBMS), such as Oracle and PostgreSQL. This solution is thus coherent with the plausible background of the target user, i.e., a breeder, who is not required to have system administrator skills in his/her background. The ISIde geo-referenced database includes data on weather (current climate and future projections), soil, crop distribution and management practices (e.g., sowing date) at 5 km × 5 km spatial resolution. District-specific information on the most representative varieties, related parameterizations and on which are the biotic/abiotic constraints affecting rice productions is also included. Daily weather data from the MARS database (http://mars.jrc.ec.europa.eu/mars/) were used for the current climate (i.e., the 1986-2005 baseline), whereas the uncertainty related with future projections was handled by using two contrasting IPCC AR5 representative concentration pathways (RCP2.6 and RCP8.5; IPCC, 2013) and two general circulation models (GCMs): HadGEM2-ES (Hadley Centre, UK) and GISS-E2-R (NASA). 20-year weather series centered on 2030, 2050 and 2070 were then generated for each combination RCP × GCM, using the CLIMAK weather generator (Confalonieri, 2012). Information on rice distribution and sowing dates was derived from European Corine Land Cover data (http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-2) and remote sensing products (10-year median from time series of MODIS 8-Day composite images at 500 m spatial resolution; Boschetti et al., 2009). For each district, the most representative rice varieties were selected according to data provided by the Italian National Rice Authority (ENR; www.enterisi.it). Overall, the platform provides the complete characterization of 34 varieties. The resistance levels to blast diseases were derived by analyzing the results of multi-site and multi-year variety trials carried out by ENR and by the Regional Agency for Agricultural and Forest Services (ERSAF; www.ersaf.lombardia.it). The temperature thresholds triggering cold- and heat-induced spikelet sterility were retrieved from literature for both *Japonica*- and *Indica*-type varieties (Andaya et al., 2003; Shah et al., 2011). The complete description of parameters involved with crop growth and development and of parameters representing the resistance/tolerance level to biotic and abiotic stressors is provided in Paleari et al. (2015).

6.3.3. User interaction

The platform user interface (UI) is highly intuitive, with a set of icons representing the steps needed for the definition and evaluation of the ideotype (Fig. 2). A quick guide – available via the question mark icon – provides users with instructions/suggestions for each specific region of the UI. Moreover, information on which is the next step to perform is always displayed at the bottom left of the working area. To guarantee usability, after a specific district is selected (Fig. 2, step 1) only parameters related to breeding activities focusing on biotic/abiotic stressors are exposed to the user for defining the ideotype (Fig. 2, step 2).

Therefore, only information related with genetic improvements needs to be provided, since all other information is automatically selected by the platform once the district, the variety to improve and the climate scenario (Fig. 2, step 3) are chosen. When all simulations are run, synthetic results are displayed (Fig. 2, step 4), whereas detailed outputs are stored in the database and can be exported in tabular format as text (.csv) files. A MS Word (.docx) report on the ideotyping exercise is also produced (summarizing results as well as the information on settings) to guarantee work reproducibility (Carpenter et al., 2013). A complete documentation and a video tutorial illustrating how to use the platform are available at the application page (www.cassandralab.com/applications/4) and at www.cassandralab.com/components.



Select a district

District name: Lombardo-Piemontese

Surface: 195.545 ha

Relevant biotic injuries: Blast disease (Pyricularia), Brown spot (Helminthosporium)

Relevant a-biotic damages: Sterility (warm)

Defining ideotype traits: an example for blast resistance

Fungal pathogens: ● Blast ○ Brown spot

Resistance coefficient during: Sporulation 3, Infectiousness 2, Latency 3, Infection 3

Defining the climate scenario: selecting RCPs, GCMs and time frames

Representative Concentration Pathways: ● RCP8.5 ○ RCP2.6

General Circulation Model: ● HadGEM2 ○ GISS

Project the analysis up to: ● 2030 ○ 2050 ○ 2070

Please, select one of the rice districts

Look at synthetic results, produce reports and save results

Figure. 2. The ISIde user interface. Windows highlight the consecutive steps needed to define and test ideotypes at district level.

6.4. A case study: defining and testing an ideotype improved for heat tolerance around flowering in the Italian rice district "Oristanese"

The study area has a typical Mediterranean climate, with maximum temperature during summer months frequently close to the upper limit for rice (higher than 35 °C). Damages due to high temperature around flowering could thus potentially threaten rice productions as the climate gets warmer. We hypothesized to improve the *Japonica*-type cultivar Volano – extensively grown in Italy for the preparation of "risotto" – for tolerance to heat-induced spikelet sterility. The ideotype was defined by increasing the threshold temperature for sterility (35 °C; Paleari et al., 2015) up to the value characterizing potential donor varieties available among Italian rice germplasm, as the cultivar Thaibonnet (threshold temperature = 36 °C). The latter is an *Indica*-type cultivar, considered a "founder" for its crucial contribution to the current Italian rice germplasm. To explore a wide range of future climatic projections, we selected two scenarios (HadGEM2-ES GCM − RCP8.5 and GISS-E2-R GCM − RCP2.6, respectively leading to the highest and the lowest expected temperature increase) and three 20-year timeframes centred on 2030, 2050 and 2070. In contrast with a sizable benefit (in terms of yield increase) simulated for the same cultivar and district in case of an improved resistance to blast disease (Paleari et al., 2015), results showed that increasing tolerance to heat-induced spikelet sterility may provide limited advantages under current climate (+2.3%). Benefits increased up to 5% while moving forward the time frame considered with the warmest scenario (HadGEM2-ES GCM − RCP8.5), whereas they remained stable under 3% with the more optimistic scenario (GISS-E2-R GCM − RCP2.6) (Fig. 3). This highlighted the importance of providing the user with more RCP scenarios and GCMs, to test the ideotype under different climate projections.

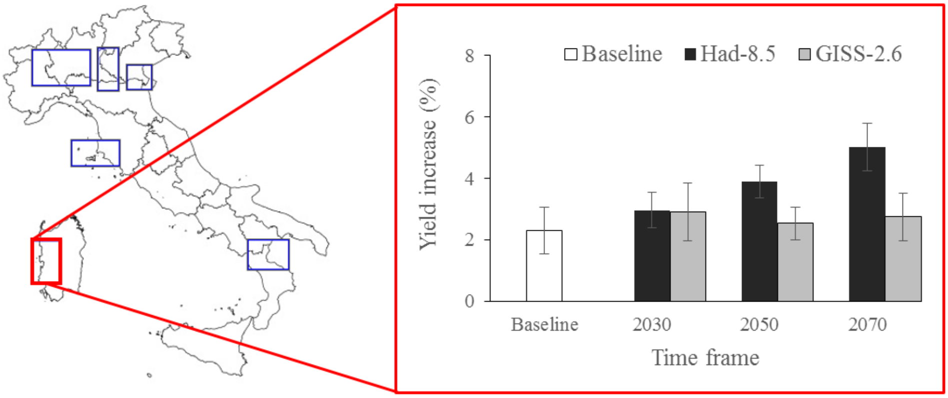


Figure 3. Performance of an ideotype derived by improving the cultivar Volano for tolerance to heat-induced spikelet sterility in the Oristanese rice district. The baseline (1986-2005) and three 20-year scenarios centered on 2030, 2050 and 2070 are reported. Had-8.5: HadGEM2-ES GCM − RCP8.5; GISS-2.6: GISS-E2-R GCM − RCP2.6. The six main Italian rice districts are highlighted with rectangles.

6.5. Conclusion and perspectives

To achieve an effective integration of crop modelling and breeding activities, breeders themselves should be enabled to perform model-based ideotyping studies. This means, in turn, that modelling tools must be developed targeting users who does not have necessarily in-depth skills in agronomy, climatology, agrometeorology, crop modelling, IT. ISIde, deriving from a close collaboration between target users, biophysical modelers and software developers, represents the first prototype of a platform specifically developed for being used by breeders to identify putative traits improving varieties at district level. Taking advantage of software engineering expertise and explicitly targeting breeders as users, it allows testing hypothesis of genetic improvement via an advanced, detailed modelling solution, thus representing a step forward towards an actual role of crop modelling in breeding programs. The availability of supporting software tools (for the creation of new databases and the parameterization of the modelling solution for new varieties) allows extending the platform to new study regions. Indeed, specific applications are available at info@cassandralab.com to generate new weather scenarios and to create the binary files constituting the ISIde database, whereas the application WARM 2 (www.cassandralab.com) can be used to parameterize the simulation engine for other genotypes.

GENERAL CONCLUSIONS

The development of new varieties better adapted to specific growing conditions is one of the most effective strategies to tackle food security issues, alleviate the impact of climate change on agriculture and increase the sustainability of farming systems (Lobell et al., 2011; Ramirez-Villegas et al., 2015). This study addressed the use of crop models to support breeders in designing putative ideotypes for improving quantitative and qualitative aspects of crop productions in specific agro-climatic contexts.

The use of crop models for ideotyping purposes was analyzed from different perspectives, and new solutions were proposed to problems dealing with uncertainty, model suitability, and availability of IT tools. The need of varieties adapted to specific conditions was fully considered by performing analyses at district level and including climate change scenarios.

Part of the researches carried out during the PhD project originated from the consideration that current models are – to a different extent – not completely suitable for ideotyping studies. Indeed, they lack clear relationships between parameters and plant traits. To overcome this limitation, a shift of paradigm was proposed towards the redesign of crop models by building them around traits breeders are working on (trait-based development). According to this new paradigm, a new model for salt stress was developed, by explicitly considering the five traits involved. Compared to existing approaches, the new model demonstrated its full suitability and usefulness in case studies where ideotypes improved for salt tolerance were designed and evaluated in two different contexts.

However, redesigning from the scratch complete crop models will take years. For this reason, another part of this research was focused on decreasing the uncertainty deriving from the use of partially suitable models to maximize their usefulness. This was achieved through the development of a new mathematical procedure for identifying traits for which information on distributions is more important for ideotypes definition, and through the design of ideotyping experiments explicitly accounting for the variability in time and space of the conditions explored by current and improved varieties. Moreover, for the first time, ideotyping studies dealing with plant-pathogen interactions were carried out, demonstrating how yield benefits deriving from increased resistance can be markedly affected by spatial and temporal heterogeneity. Similar considerations were derived when the potential benefits of breeding for tolerance to abiotic stressors were simulated.

A third part of the research focused on the development of the first software platform for *in silico* ideotyping designed to target breeders as final users. The aim of the platform was bridging the gap between the modelling and breeding communities. Although it is currently available for the six Italian rice districts and limited to resistance/tolerance traits, the platform could be easily extended to other growing areas, additional genotypes and phenotypic traits.

Future developments could be driven by the limits behind this research: (i) absence of explicit interactions between traits, since epistasis and pleiotropy could potentially bind the combination of traits to be explored (e.g., Chenu et al., 2009), (ii) no adaptation strategies considered (e.g., alternative sowing date; Zheng et al., 2012), and (iii) lack of evolutionary potential of the pathogen in response to long-term climate variations and increased host resistance (Chakraborty, 2013). Finally, the definition of ideotypes could be extended by including the genotypic variation in yield response to elevated CO_2 (Ziska et al., 2012).

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SUMMARY

The development of new cultivars better adapted to specific growing conditions is a key strategy to meet an ever-increasing growing global food demand and search for more sustainable cropping systems. This is even more crucial in the context of a changing climate.

Ecophysiological models and advanced computational techniques (e.g., sensitivity analysis, SA) represent powerful tools to analyze genotype (G) by environment (E) interactions, thus supporting breeders in identifying key traits for specific agro-environmental contexts. However, limits for the effective use of mathematical models within breeding programs are represented by the uncertainty in the distribution of plant trait values, the lack of processes dealing with resistance/tolerance traits in most ideotyping studies, the partial suitability of current crop models for ideotyping purposes, and the absence of modelling tools directly usable by breeders.

The aim of this research was to address these issues improving methodologies already in use, proposing new paradigms for the development of crop models explicitly targeting ideotyping applications and developing tools that would encourage a deep interaction of the modelling and breeding communities. The focus was on rice, for its role as staple food for more than a half of world’s population, and on resistance/tolerance traits to biotic/abiotic stressors, for their central role in increasing crop adaptation. Moreover, current conditions and climate change projections were considered, to support the definition of strategies for breeding in the medium-long term.

A standard procedure to quantify − and manage − the impact of the uncertainty in the distribution of plant trait values was developed, using the WARM rice model and the Sobol’ method as case study. The approach is based on a SA (generating sample of parameter distributions) of a SA (generating samples of parameters for each generated distribution) using

distributions of jackknife statistics calculated on literature values to reproduce the uncertainty in defining parameters distributions. As a practical implication, the procedure developed allows identifying plant traits whose uncertainty in distribution can alter ideotyping results, i.e., traits whose distributions could need to be refined.

Global SA was then used to identify rice traits putatively producing the largest yield benefits in five contrasting districts in the Philippines, India, China, Japan and Italy. The analysis involved phenotypic traits dealing with light interception, photosynthetic efficiency, tolerance to abiotic stressors, resistance to fungal pathogens and grain quality. Results suggested that breeding for traits involved with disease resistance and tolerance to cold- and heat-induced spikelet sterility could provide benefits similar to those obtained from improving traits affecting potential yield. Instead, advantages resulting from varying traits involved with grain quality were markedly frustrated by inter-annual weather variability. Since results highlighted strong G×E interactions, a new index to derive district-specific ideotypes was developed.

Given the key role of biotic/abiotic stressors in determining actual yield and the deep impact of related G×E interactions, a study was carried out by explicitly focusing on the definition of rice ideotypes improved for their resistance to fungal pathogens and tolerance to abiotic constraints (temperature shocks inducing sterility). The analysis was carried out at district level with a high spatial resolution (5 km × 5 km elementary simulation unit), targeting the improvement of the most representative 34 varieties in six Italian rice districts. Genetic improvement was simulated via the introgression of traits from donor varieties. Results clearly showed that breeders should focus on increasing resistance to blast disease, as this appears as a factor markedly limiting rice yields in Italy, regardless of the districts and climate scenarios, whereas benefits deriving from improving tolerance to cold-induced sterility could be markedly affected by G×E interactions.

To reduce the risk of discrepancies between *in silico* ideotypes and their *in vivo* realizations, both studies involved only model parameters with a close relationship with phenotypic traits breeders are working on. However, a long-term strategy to overcome limitations related with the partial suitability of available models would be building new ideotyping-specific models explicitly around traits involved in breeding programs. This proposal for a paradigm shift in model development was illustrated taking salt stress tolerance and rice as a case study. Dedicated growth chamber experiments were conducted to develop a new model explicitly accounting for tolerance traits modulating Na^+ uptake and distribution in plant tissues, as well as the impact of the accumulated Na^+ on photosynthesis, senescence and spikelet sterility. An ideotyping study was conducted at two sites (in Greece and California) characterized by different seasonal dynamics of salinity in field water. Results showed how, under different scenarios, traits assuring the largest contribution to the overall tolerance could refer to completely different physiological mechanisms: tissue tolerance in one case, sodium exclusion in the other. This encourages the development of explicit trait-based approaches to increase the integration of crop models within breeding programs.

A parallel path to achieve this goal is the development of modelling platforms targeting breeders as final users, who does not have necessarily in-depth skills in crop modelling and IT. The platform ISIde, derived from a close collaboration between target users, biophysical modelers and IT specialists, represents the first prototype of a platform specifically developed for being used directly by breeders to evaluate *in silico* improved varieties at district level.

This thesis demonstrated the usefulness of simulation models for the definition of ideotypes for specific agro-environmental conditions. Targeting ideotyping applications, new methodologies, paradigms for model development and modelling tools were developed, thus contributing to improve the potential of crop modelling to support breeding programs. Future developments will target researches aimed at overcoming the limits

behind this study, i.e., (i) absence of explicit interactions between traits, (ii) no adaptation strategies considered, and (iii) lack of approaches for the simulation of the evolutionary potential of pathogens in response to long-term climate variations and increased host resistance.

Keywords

Ideotyping, global sensitivity analysis, Morris method, parameter distribution, Sobol' method, WARM rice model, WOFOST-GT2, modelling, blast, *Magnaporthe oryzae* B. Couch, fungal pathogens, canopy structure, chalkiness, head rice, photosynthetic efficiency, genotype × environment interactions, spikelet sterility, cold tolerance, heat stress, *Oryza sativa* L., rice, salinity, salt stress, breeding, climate change, crop adaptation, resistance, tolerance, modelling platform.

CURRICULUM VITAE

The numbers between curly brackets refer to the publications listed at the end of this document.

Livia Paleari was born on 22 September 1984 in Trescore Balneario (BG). In 2002/2003 she completed the high school (Second Level College of Science) with a grade of 100/100. After the high school, she directed an agricultural farm for five years. In 2008 she started a 5-year University course (including B.Ag. and M.Sc. degrees) at the University of Milan (Faculty of Agriculture). B.Ag. and M.Sc. were completed, respectively in 2010/2011 (110/110 cum laude; dissertation on a research about relationships between urea content in milk and nitrogen balance in goats) and in 2012/2013 (110/110 cum laude; dissertation on a research on a prototype of an ideotyping platform based on simulation models). In 2013 she started the PhD project on ideotyping and ecophysiological models at the University of Milan.

Livia Paleari is co-author of the first simulation platform for the *in silico* evaluation of improved rice varieties at district level and under climate change scenarios {9}. The platform was used within the Ager-RISINNOVA project for the definition and evaluation of rice ideotypes improved for blast resistance and for tolerance to cold-induced spikelet sterility {7}. She is the main author of the first model for the simulation of salt-stress on rice explicitly developed around traits for which breeding programs are ongoing. She developed the first method for the quantification of the distributional assumptions on the results of global sensitivity analysis of mathematical models {10} and co-authored the first statistical procedures for the quantification of the amount of uncertainty in model predictions due to user subjectivity {8}. She also co-authored a protocol for the evaluation of in vivo field methods based on an adaptation of the ISO 5725 norm {2} and two smart app for non-destructive estimates of leaf area index {1} and nitrogen concentration in plant tissues {5}, later adapted to different operational contexts {4}.

Involved in the European projects EU-FP7 MODEXTREME (KBBE), grant no. 613817, and JPI-FACCE BarPlus. Co-coordinator of a project funded by PhosAgro Trading SA on heavy metals in rice crops.

Awarded with the Highly Cited Research Certificate form the ISI journal Computers and Electronics in Agriculture for the paper "Development of an app for estimating leaf area index using a smartphone. Trueness and precision determination and comparison with other indirect methods". The paper was among the five most cited in the Journal since 2014.

Author of 10 publications on ISI Journals with Impact Factor (3 as first author). *h*-index = 3 (ISI Web of Science Core Collection), = 4 (Scopus), = 4 (ResearchGate). RG Score = 17.59.

Inventor of a technology registered by the University of Milan on the use of digital imagery for nitrogen determination in plant tissues.

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