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DATA AND MODEL-BASED RESOURCES TO SUPPORT ITALIAN RICE BREEDING

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ABSTRACT

The central challenge that humanity is facing is the need to meet the nutritional needs of a growing population. After the tremendous progress achieved during the green revolution, the yields of the primary cereal crops are now stagnating and the undergoing climatic changes represent a further threat. Among the technologies available to allow a further increase in yield, genetic improvement is the most promising. Plant breeding, though, is an expensive, time consuming and labour-intensive activity which relies on a thorough knowledge of the available germplasm for its efficient exploitation requiring the integration of the phenotypic expression with molecular data.

The analysis of the interactions between genetic makeup, pedo-climatic conditions and management practices is thus essential to guide breeding programs aimed at improving the agronomic traits of the main herbaceous crops. Crop simulation modelling can be used to support such activities, via a cost- and time-efficient analysis of the performances of a wide range of phenotypes in different weather, soil and management conditions. The requirement is the minimum deviation between the phenotypic expression and its model representation, which should consider the known physiological limits and compensatory effects among traits.

The lack of an extensive characterisation of available germplasm often impedes the availability of exhaustive data to support breeding programs via crop modelling. This applies to Italian rice agriculture, being characterized by a long history of cultivation with a vast varietal landscape. Crop model-based studies and services have already been developed in the area to support rice growers and local stakeholders, thus outlining a proficient case study for their implementation in breeding programs.

This doctoral project aimed at analysing the morpho-physiological characteristics of the Italian rice germplasm mostly contributing to the yield increase in the 20th century, highlighting the evolutionary trends, and the associations with published molecular data. The released information enlarges previous findings and can be used to guide genetic improvement programs aimed at further improve current rice varieties. The field experimental activity produced ready-to-use quantitative data to further refine crop modelling capabilities in the area. Their integration in a crop model study allowed correlating yield component traits and model parameters, fostering the design of synthetic cultivars to facilitate and prioritize new breeding efforts.

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Chapter 1 INTRODUCTION

1.1 CHARACTERISATION OF EUROPEAN RICE CULTIVATION

Rice is the 6th most cultivated cereal crop in Europe (EU), with a total annual production of 1.83 million milled-equivalent tons in 2017 (European Commission, 2017). Although not being a staple food crop in EU, rice plays a central sociocultural (Picazo-Tadeo, Reig-Martinez, & Estruch, 2009) and ecological (Fasola & Ruiz, 1996; Longoni, 2010) role in several Mediterranean countries, where the consumption is steadily increasing (Ferrero, 2007). Cultivation is mainly located in Italy, Spain, Greece, Portugal and it is typically conducted in paddies under continuous flooding during most part of the crop cycle, with water drainages to allow rooting, top-dressing fertilization, herbicide spraying and ripening (Ferrero, 2005). European rice varietal landscape is mostly constituted by cultivars belonging to the *temperate japonica* ecotype and representing 65-70% of the total EU production (Courtois et al., 2012). Rice is a key component of the Mediterranean diet especially in Italy and Spain where traditional foods like paella and risotto are prepared.

Rice-growing area in Italy was 234 132 ha in 2016 (Ente Nazionale Risi, 2016) contributing to nearly 50% of the total EU rice production (Agriculture and rural development - European Commission, 2016). Modern Italian rice varieties also belong to the *temperate japonica* ecotype, with main differences in the duration of the growing cycle and in morpho-physiological traits such as height, grain weight and shape, blast resistance and milling yield (Faivre-Rampant et al., 2010), as these features were targeted by rice breeders throughout the whole 20th century.

One of the main environmental constraint to rice production in Mediterranean countries is cold stress (Jena & Hardy, 2012), as rice originated from sub-tropical and tropical areas. Low temperatures can damage rice plant at any growth stage, being particularly severe at early stages. Before emergence low temperature can affect germination and result in poor crop establishment. At seedling stage, cold temperatures can delay heading and determine pollen sterility (Julia & Dingkuhn, 2013)1. Exposition to high temperature can also increase the risk of pollen sterility, with variable intensity according to the growth stage (Fu et al., 2016). The climatic projections in the near future indicate a increase in the frequency of heat stress on temperate rice cropping systems and recent studies indicate that heat sterility will likely occur even in Mediterranean countries, especially in warm and humid years (Julia & Dingkuhn, 2013).

1.2 RICE BREEDING IN ITALY

The introduction of rice cultivation in Italy dates back to the second half of the fifteenth century, when the Sforza family spread it in less-favoured, marginal marshy areas where other agricultural activities were hindered (Courtois et al., 2012). The first recognized Italian rice variety was "Nostrale", which most probably differentiated into different ecotypes depending on the area of cultivation, with common features as considerable height, tendency to lodging, and high susceptibility to rice blast. In 1903, 44 Italian rice varieties were documented (Gobbetti, 1903); among these, the first varieties obtained from mass selection were present, like “Novara” and “Ostiglia”. Dramatic outbreaks of rice blast occurred in the first half of the 19th century pushing the import of new genetic material from eastern countries, mainly China and Japan, in search of less susceptible varieties (Cai et al., 2013). The first artificial crossing between two distinct varieties was accomplished in 1925: with this technique and the novel genetic material imported from the United States (e.g., the cultivar “Lady Wright”), the Rice Experimental Institute in Vercelli constituted many key varieties of the Italian varietal landscape like “Vialone Nano” and “Carnaroli”. The selection of grain varieties with high amylose content started in the same period; these were also characterized by a stiffer and flimsier stem with respect to other cultivars of that time (Tinarelli & Mezza, 1981).

The need of a grain classification based on its shape characteristics started to arise: Italian rice varieties were then divided according to grain biometrics in four categories that were subsequently adopted by the European Union with slight modifications (Tamborini & Lupotto, 2006): these categories are Round (caryopsis shorter than 5.2 mm), Medium (caryopsis length comprised between 5.2 and 6 mm), Long A (caryopsis longer than 6mm, with a length/width ratio under 3) and Long B (caryopsis longer than 6mm, with a length/width ratio equal to or greater than 3).

Until 1980, Long A grain varieties were preferred. Starting from the 80s and through the 90s, Long B cultivars started to arouse interest and to spread in Italian rice cultivation areas. The causes were two-fold: the need for competing with non-EU countries in the export market, especially in Northern Europe, and the introduction of long-grain cultivars suitable for cultivation in Italian climate, like the variety “Thaibonnet”, which was obtained from mass selection from the American “L202”. The next years evidenced this expanding interest towards long B and long A cultivars, the latter for transformation with parboiling technique. In 1998 the long B grain cultivar “Gladio” was released: its popularity quickly arouse and it became the most cultivated Italian variety from 2001 to 2010. In 2003, Louisiana State University firstly introduced *imidazolinone*-tolerant genotypes, (Croughan, 1999) that were commercialized in the next years under the name of Clearfield® rice. These varieties are not currently considered GMOs since they were obtained using chemical mutagenesis (Croughan, 2003). Italy was the first EU country to express interest in and promote the cultivation of Clearfield (R) rice varieties. In 2005, the first Italian Clearfield® cultivar “Libero” was listed in the National Catalogue (Tamborini, 2016b). After that, many other Clearfield varieties were released. The area cultivated with Clearfield® varieties in Italy reached its peak in 2015, with 37.4% of the total rice-growing area (Tamborini, 2016a).

A distinctive feature of the history of the rice breeding in Italy, not easily found in other cereals, lies in the important and significant activities pursued by private breeders in the development process of new varieties. The manifold independent initiatives carried out by different researchers and private breeders driven by market demands and pest outbreaks make the current varietal landscape markedly heterogeneous (Tamborini & Lupotto, 2006). Italian rice germplasm is an invaluable source of biodiversity. The importance of having a broad genetic diversity to improve important agronomic traits such as resistance to stresses, early maturity, grain quality and tolerance to lodging is undeniable (Nwachukwu et al., 2016).

1.3 MODEL-ASSISTED BREEDING

Crop simulation modelling is rapidly gaining scientific momentum because of its proved value in agricultural research, being able to formally describe the behaviour of a specific cropping system via mathematical relationships. Applications encompass the study of large-scale ecological interactions (Young, 1998), yield forecast and gap analysis (Van Wart, Kersebaum, Peng, Milner, & Cassman, 2013), the assessment of climate change impacts (Rötter, Carter, Olesen, & Porter, 2011), and *in silico* ideotyping (Martre et al., 2015; Paleari, 2016). The worldwide relevance of rice as a staple food crop fostered the development of various crop simulation models describing its peculiar soil-water-plant-atmosphere inter-relationships, largely different from the other cereal crops. The phenological, physiological, and morphological features are described by models of plant processes, which can be defined as interlinked sets of equations with morpho-physiological features embedded into parameters (Bregaglio, Titone, et al., 2017b). The gene-environment interactions are mediated by these parameters which can thus be considered the "genetic coefficients" of the crop (Boote, Kropff, & Bindraban, 2001). Currently, the use of rice models is mainly oriented towards the assessments of climate change impacts on agricultural productivity and the development of novel breeding strategies: in these contexts, the use of model ensembles has proven to be an effective tool to increase the robustness of predictions (Diffenbaugh & Giorgi, 2012; Makowski et al., 2015; Martre et al., 2014; Tao et al., 2017).

The vast diversity of the Italian rice germplasm was accounted for by developing specific parameter sets specific to groups of similar phenotypes or maturity class (Bocchi, Boschetti, Stroppiana, & Pietro Alessandro Brivio, 2006; Confalonieri & Bocchi, 2005; Confalonieri, Rosenmund, & Baruth, 2009), using field or greenhouse experiments referred to few representative varieties (Bregaglio, Titone, et al., 2017b; Paleari et al., 2015). This approach does not fully account for the large phenotypic variability of Italian rice (Faivre-Rampant et al., 2010; Mantegazza et al., 2008; Spada, Mantegazza, Biloni, Caporali, & Sala, 2004), its yield potential (Ben Hassen et al., 2017) and economic value (Ferrero, 2007), as even small differences in model parameter values could significantly impact the outcomes of modelling studies performed at regional or larger resolution (Angulo et al., 2013; Teixeira et al., 2017). Advances in crop modelling capabilities are hindered by the absence of accurate field data collected in multiple environments, required for model calibration and validation (Mavromatis et al., 2001), and the high turn-over rate of cultivated varieties quickly vanishes the efforts made for their characterisation (Tamborini, 2016b).

A fruitful integration of genetic knowledge and model parameters is the missing piece in the effective exploitation of crop modelling to support advanced breeding strategies, as recently demonstrated in many different studies (Collins, Tardieu, & Tuberosa, 2008; Hammer et al., 2006; Reymond, Muller, Leonardi, Charcosset, & Tardieu, 2003; Tardieu & Tuberosa, 2010; YIN, Stam, Kropff, & Schapendonk, 2003; YIN, Struik, van Eeuwijk, Stam, & Tang, 2005).

Particularly, modelling simulation studies aimed at defining and testing new breeding strategies (Khush, 1995) should account for the known physiological limits and the correlations and compensatory effects that exists among traits (Picheny et al., 2017). Because of the costs related to the characterisation of germplasm, there is a scarcity of such data that is invaluable to crop scientist, to support breeding for superior yields (Nwachukwu et al., 2016).

1.4 OBJECTIVES AND ORGANISATION OF RESEARCH

The project aims at releasing data and model-based tools to assist novel breeding programs targeting the Italian rice area.

The specific objectives of this doctorate are:

- Release of resources to extend the state of the art of rice cultivation in Italy, with emphasis on the aspects related to plant breeding and the biodiversity of the Italian rice varietal landscape, its evolutionary trends and the associations with published molecular data.
- Improving crop modelling capabilities in the area to support new integrated model-assisted breeding programs by providing ready-to-use data and context towards a detailed representation of cultivars in crop models.

The project articulates in three main work packages:

- *Phenotypic characterisation of Italian rice germplasm.*
  A database containing quantitative information on seven yield-related traits (days to heading, days to maturity, stem and panicle length, caryopsis length and width, thousand seeds weight) for 351 cultivars representing the rice varieties released in Italy since 1900 will be created with previous morphological data collected from the Vercelli Office of CREA-DC (Council for Agricultural Research and Economics, Research Centre for Plant Protection and Seed Certification). An exploratory uni- and multivariate analysis will be performed to quantify their phenotypic variability, to analyse the evolutionary trends, and to identify grouping patterns that could be used to drive future breeding programs (Chapter 2).
- *Analysis of yield-related traits in Italian rice germplasm.*
  The developed database will be screened to derive a sample of 40 cultivars which will be further characterised in open field with a 2-year dedicated experiment (2016-2017). The selection method that will be used aims at maximising the sample variance associated to the known traits. Collected data will be subjected to multivariate analysis to highlight correlations and evaluate traits variability (Chapter 3).
- *Development of a method to derive cultivar-specific parameters sets from common agronomic data.*

Experimental data collected in the Value and Cultivation of Use (VCU) trials on Italian rice varieties in 2004-2015 will be used to perform a cultivar-specific calibration of the crop model WOFOST_GT. A minimum data set including phenological observations of heading and maturity, and canopy height and grain yield measurements were used as reference variables for model calibration. The study will rely on the availability of a dedicated sensitivity analysis performed on the same crop and in similar agro-environmental conditions. The methodology will be used to obtain parameters sets representing the most cultivated Italian rice varieties of the last twenty years (Chapter 4). At last, the method will be put at test with a hazelnut crop model to perform a multi-environment calibration and model sensitivity analysis (Chapter 5).

CHAPTER 2 EVOLUTIONARY TRENDS AND PHYLOGENETIC ASSOCIATION OF KEY MORPHOLOGICAL TRAITS IN THE ITALIAN RICE VARIETAL LANDSCAPE

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2.1 ABSTRACT

Efficient germplasm exploitation in crop breeding requires comprehensive knowledge of the available genetic diversity. Linking molecular data to phenotypic expression is fundamental for the profitable utilisation of genetic resources. Italian rice germplasm is an invaluable source of genes, being characterised by marked heterogeneity. A phenotypic characterisation is presented in this paper, with a focus on the evolutionary trends, and on the comparison with available molecular studies. A panel of 351 Italian rice varieties was analysed using seven key morphological traits, employing univariate and multivariate analyses. Considerable variability was found, with clear morphological trends towards reduced plant height, earliness, and spindle-shaped caryopses. Previous findings indicating that genetic diversity was maintained throughout time could not be confirmed, as small phenotypic variability was found in the most recent rice varieties. Consistency with phylogenetic data from previous studies was partial: one phylogenetic subgroup was phenotypically well distinct, while the others had overlapping characteristics and encompassed a wide range of phenotypic variation. Our study provides a quantitative ready-to-use set of information to support new breeding programs, as well as the basis to develop variety-specific calibrations of eco-physiological models, to identify promising traits in light of climate change conditions and alternative management scenarios.

2.2 INTRODUCTION

Germplasm is the foundation of agricultural production as it constitutes the living genetic resource making plant breeding viable (Nachimuthu et al., 2015). The primary objective of germplasm collection is to preserve the genetic diversity of a given species, envisaging its utilisation in breeding programs to improve crop varieties in the evolving agricultural landscape (Peeters & Galwey, 1988). The conservation of germplasm collections is of paramount importance nowadays, given the need of feeding a steadily increasing population, while seeking for adaptation strategies to mitigate the impacts of climate change on agriculture (Food and Agriculture Organization, 2007). Efficient use of germplasm collections in plant breeding relies on the understanding of the existing genetic diversity, including its characterisation, evaluation, and classification (Beuselinck & Steiner, 1992). The variability of key agronomic traits in available germplasm is indeed crucial for their fruitful utilisation via recombination, breeding and selection. There is broad agreement in recognising the scarcity of these data to support breeders in screening the available genetic material, before applying molecular breeding techniques (Food and Agriculture Organization of the United Nations, 2010). While molecular breeding proved essential in identifying the relationships among groups of accessions and in estimating the genetic diversity within a population, the analysis of morpho-physiological traits will keep playing a significant role in assessing the degree of similarity among genotypes. This method was indeed successfully applied to evaluate diversity on landraces and ancestral lines of rice varieties in the Philippines (Caldo, Sebastian, & Hernandez, 1996), China (Yawen et al., 2003), and Nepal (Bajracharya, Steele, Jarvis, Sthapit, & Witcombe, 2006). Furthermore, the adoption of morpho-physiological characterisation is needed under the International Union for the Protection of New Varieties of Plants and therefore for the registration of new cultivars, their certification, and seed production (Button & Jördens, 2011).

With incrementing germplasm collections by the number of accessions, and the massive amount of available data on molecular, biochemical, morphological and agronomic traits, the adoption of multivariate statistical analysis (MVA) methods is unavoidable (Maji A T & T, 2012). Most MVA methods are used for exploratory purposes, such as the extraction of principal components in large data sets, the detection of underlying data structures, and the representation of complex biological patterns. MVA methods - particularly factor and cluster analyses - have also been applied in the evaluation of germplasm collections, studying various traits on a large number of accessions (Chakravarthi & Naravaneni, 2006; Lasalita-Zapico, Namocatcat, & Cariño-Turner, 2010; Nachimuthu et al., 2014), thanks to their capability to deal with multicollinearity.

Modern plant breeders generally prefer focusing on elite germplasm, that is constituted either by recently released cultivars or by cultivars that are no longer grown (Nwachukwu et al., 2016). This germplasm indeed presents a highly productive potential and it is relatively easy to access, other than offering a gene combination adapted to specific agro-environmental conditions (Acquaah, 2012).

All these considerations apply to the history of rice breeding in Italy, whose cultivation extends on 234,132 hectares in 2016 (Ente Nazionale Risi, 2016), contributing to 50% of the total EU rice production (Agriculture and rural development - European Commission, 2016). Italian rice varieties mostly belong to the *temperate japonica* ecotype, which represents the vast majority of the European rice varietal landscape (65-70% of the total EU production (Courtois et al., 2012)); these type of varieties are grown for traditional foods like paella and risotto, with primary differences in the duration of growing cycle and morpho-physiological traits such as plant height, grain weight and shape, milling yield and resistance to blast disease (*Magnaporthe grisea*, T.T. Hebert, M.E. Barr) (Faivre-Rampant et al., 2010). Multiple breeding programs in Italy focused on these aspects throughout the 20th century.

Documented Italian rice cultivation dates back to the second half of the fifteenth century when the noble Sforza family promoted it in the wetlands of the Po Valley, where other cereals were difficult to grow (Motta, 1913). The pioneer Italian rice variety was *Nostrale*, which most probably encompassed different ecotypes sharing a considerable height, with a tendency to lodging, and high susceptibility to blast disease (Piacco, 1954). In 1903, the first Italian varieties obtained from mass selection were introduced. Outbreaks of rice blast occurred in the first half of the 19th century pushing for the import of less susceptible varieties from China and Japan (Cai et al., 2013). In 1925, the first artificial crossing using novel genetic material imported from the United States was performed. The selection of Long A grain varieties with high amylose content started immediately after (Tinarelli & Mezza, 1981): these represent an Italian agricultural excellence.

Between 1980 and 1990, 'long B' grain varieties (long spindle-shaped grain) started to spread in Italy, to meet the international market demand. This trend continued in the next years when the cultivation of 'long B' and 'long A' (for parboiling technique) varieties significantly rose. In 2003, Louisiana State University introduced *imidazolinone*-resistant rice cultivars (Croughan, 2003), which started to be commercialised under the name of Clearfield® rice. Italy was the first country in Europe to show interest in these varieties and, in 2005, the first Clearfield® rice variety, *Libero*, was listed in the National Catalogue (European Commission, 2015), followed by others in the next years. The area cultivated with Clearfield® varieties reached its peak in 2015, with 37.4% of the national total rice-growing area (corresponding to about 85021 hectares) (Tamborini, 2016b).

Despite a large number of rice cultivars listed in the Italian National Catalogue (203), about 78% of the rice area in 2016 was cropped with 20 cultivars (Ente Nazionale Risi, 2016). Italian rice germplasm is an invaluable source of useful genes, as it presents a broad genetic diversity which can be used to improve agronomic performance of new varieties (Mantegazza et al., 2008). A distinctive feature of the history of Italian rice is the crucial role played by farmers in enriching the genetic diversity while maintaining hundreds of varieties for many years (Tamborini & Lupotto, 2006). Furthermore, the lack of coordination between public institutions and private companies in planning medium-long term genetic improvement contributed to generating a heterogeneous rice varietal landscape. Differently from what has been done for other cereals, an analysis of the morphological diversity of Italian rice varieties is still lacking. Studies focusing on the genetic characterisations of Italian rice germplasm via molecular markers have been performed in the past (Courtois et al., 2012; Faivre-Rampant et al., 2010; Mantegazza et al., 2008; Spada et al., 2004). Therefore a phenotypic analysis on the expression of agronomic traits would integrate available knowledge to identify genetic material to guide new breeding efforts.

This study presents a phenotypic characterisation of the Italian rice germplasm, focusing on the evolutionary trends of rice breeding of the 20th century and the beginning of 21st century. We aimed at highlighting patterns of similarity between phenotypic and molecular characterisation, which could support the selection of germplasm for novel breeding programs.

2.3 RESULTS

2.3.1 EXPLORING EVOLUTIONARY TRENDS IN ITALIAN RICE VARIETAL LANDSCAPE.

Seven morphological traits (stem length, panicle length, days to heading, days to maturity, thousand seeds weight, caryopsis width, caryopsis length) were analysed on a dataset of 351 Italian rice varieties through univariate and multivariate analyses. These traits are used in the official tests for variety registration as the driving characteristics to group rice varieties, other than being of paramount agronomic interest and hence the target of many breeding efforts in Italy. We also grouped rice cultivars by four categorical variables, i.e. *grain shape*, *time of release*, *phylogenetic subgroup*, and *flag leaf attitude*. These variables were used to further support our findings, by tracing back the evolutionary trends in Italian rice breeding as well as to compare specific results to previous studies. The selection of the categorical variables was driven by the aims of the study, i.e., assessing the evolutionary trends (*time of release*) and the phylogenetic associations (*phylogenetic subgroup*) in Italian rice varietal landscape, also considering market classification (*grain shape*) and a phenotypic feature directly impacting on plant productivity (*flag leaf attitude*). Specifically, *time of release* has been derived from a previous study in which the loss of genetic variability was assessed throughout the Italian rice breeding history; each group represented a crucial step (mass selection, hybridisation, grain quality, dwarf genotypes, and imidazolinone-tolerant varieties) in the evolution of the Italian rice varietal landscape. Additional details regarding these traits and the categorical variables were provided in the 'Methods' section. The distribution of the categorical variables *grain shape*, *phylogenetic subgroup*, and *flag leaf attitude* divided by *time of release* among rice varieties is shown in Supplementary Figure 2.1. A complete list of genotypes comprised within each *time of release* group is reported in Table 2.1; the number of genotypes within each group reflected the rate of release of Italian varieties, indicating an increasing trend over time.

Table 2.1 – List of varieties included in this study, grouped by time of release. Group size is indicated in parenthesis.

Period	Group	Rice accessions
1850 - 1927	G1 (32)	Agostano, Airone, Allorio, Americano 1600, Ardizzone, Bertone, Feronio, Fulgente, Greggio, Greppi, Ice, IR64, Italico, Italico Livorno, Lady Wright, Lencino, Lucero, Maratelli, Originario, Orion, Orione (historic), Ostiglia, Pierrot, Raffaello, Ranghino, Romanico, Roncarolo, S.Rocco, Sancio P6, Sirion, Teqing, Vialone 190, Vialone Nero.
1928 - 1962	G2 (28)	Adelaide Chiappelli, Arborio, Balilla, Balilla Gg, Balocco, Balzaretti, Baraggia, Bellardone, Benito, Carnaroli, Corbetta, Ferraris, Fortuna, Gigante Vercelli, La Ferla, Lomello, Mantova, Novara, Olcenengo, Oldenico, Precoce Corbetta, Precoce Monticelli, Razza77, Ribe, Rinaldo Bersani, Rizzotto, Senatore Novelli, Trionfo Fassone, Vialone Nano.
1963 - 1990	G3 (74)	Akitakomachi, Anseatico, Arborio Precoce, Argo, Ariete, Artiglio, Auro, Baldo, Bali, Bomba, Bonni, Castello, Cervo, Cripto, Dedalo, Drago, Elio, Europa, Faro, Giovanni Marchetti, Graldo, Gritna, Icaro, Idra, Italico Roncarolo, Italpatna, Koral, Lemont, Lido, Lieto, Lomellino, Loto, Medusa, Mida, Molinella, Molo, Monticelli, Navile, Neretto, Nero, Nova, Onda, Padano, Panda, Pecos, Pegaso, Piemonte, Prometeo, Radon, Redi, Rialto, Ribello, Ringo, Riva, Rocca, Rodio, Roma, Romeo, Roncolo, Rosa Marchetti, Rubino, S. Petronio, S.Andrea, Selenio, Sesila, Smeraldo, Sorriso, Strella, Tarriso, Titanio, Torio, Veneria, Vitro, Volano.
1991 - 2004	G4 (102)	A201, Adelio, Aiace, Albatros, Alice, Alpe, Ambra, Andolla, Apollo, Arco, Ares, Armonia, Arona, Artico, Asia, Asso, Astro, Augusto, Bastia, Bianca, Bravo, Cadet, Castelmochi, Centauro, Cesare, Chimera, Cistella, Cobra, Creso, CRT2, Delfino, Dixiebelle, Dorella, Ebro, Elba, Elvo, Eolo, Eurosis, Fenis, Flipper, Fragrance, Galileo, Gange, Garda, Gemini, Genio, Ghibli, Giada, Giano, Gigante, Giove, Gladio, Ibis, Italmochi, Jacinto, Jefferson, Karnak, Koala, Lamone, Lampo, Marte, Mercurio, Minerva, Nebbione, Nembo, Nuovo Maratelli, Orta, Otello, Perla, Perseo, Pony, Poseidone, Prezioso, Primo, Puntal, Rodeo, Romolo, Rova, S.Pietro, Santerno, Sara, Saturno, Savio, Scirocco, Sereno, Sesiamochi, Silla, Sillaro, Sirmione, SISR215, Spina, Sprint, Stresa, Tanaro, Tea, Tejo, Thaibonnet, Top, Vega, Venere, Zena, Zeus.
2005 - 2015	G5 (112)	Agata, Allegro, Antares, Arpa, Arsenal, Artemide, Atlantis, Bacco, Barone CL, Brezza, Brio, BS1, Calipso, Cammeo, Carmen, Carnaval, Carnise, Carnise Precoce, Casanova, Castore, Centro, Cerere, CL 12, CL 46, CL 80, CL111, CL15, CL26, CL31, CL71, Corimbo, CRLB1, Crono, CRW3, Dante, Dardo, Deneb, Ducato, Ecco 51 CL, Ecco 61, Ecco 63, Elettra, Ellebi, Ercole, Eridano, Ermes, Falco, Fast, Febo, Fedra, Fenice, Festa, Furia CL, Galassia, Generale, Ghiaccio, Giglio, Gloria, Iarim, King, Lagostino, Leonida CL, Libero, Libra, Lince, LT 155, Luna Cl, Luxor, Mare Cl, Meco, Medea, Megumi, Mirko, Musa, Nerone, Neve, Ninfa, Oceano, Onice, Opale, Orione, Pato, Presto, Proteo, Puma, Reperso, RG200, Ribaldo, Risrus, Rombo, Ronaldo, Sagittario, Salvo, Samba, Scudo, Sfera, Sirio Cl, Sole Cl, Sp55, Telemaco, Terra CL, Teseo, Teti, Tosca, Ulisse, Unico, Urano, Vasco, Virgo, Vulcano, Wang, Yume.

Considerable variability was found in the seven morphological traits among the Italian genotypes (Table 2.2).

Table 2.2 – Summary statistics of the considered morphological traits characterising the Italian rice varieties. The table reports minimum value, first quartile (1Q), median, third quartile (3Q), maximum value, trimmed standard deviation (Trim. SD), range, and coefficient of variation (CV) for each morphological trait.

Trait	Min.	1Q	Median	3Q	Max.	Trim.SD	Range	CV
Days to heading (days)	71	85	90	97	118	8.28	47	9.07%
Days to maturity (days)	115	136	142	150	175	9.83	60	6.9%
Culm length (cm)	46	63	73	85	119	16.38	73	22.27%
Panicle length (cm)	11	16	18	20	25	3.39	14	18.97%
Caryopsis length (mm)	4.64	5.95	6.7	7.14	8.35	0.87	3.71	13.16%
Caryopsis width (mm)	1.93	2.54	2.88	3.11	3.63	0.42	1.7	14.64%
Thousand seeds weight (g)	19.4	27.05	29.85	33.95	51.79	5.35	32.39	17.69%

The trait ‘stem length' had the highest coefficient of variation (CV = 22.27%), varying from 46 (recent and short varieties like *Pony* or *Artico*) to 119 cm (old ‘long A (IC)' grain varieties like Carnaroli or Vialone Nero), while trait ‘panicle length' had the second largest variability (CV = 19.03%; ranging from 11 cm to 25 cm). The trait ‘thousand seeds weight' also largely varied in the dataset due to the different grain shapes (CV = 17.69%, with a range of 32.39 g), even if ‘caryopsis length' and ‘caryopsis width' had a lower relative variability. The dependency of grain weight on length and width, with their multiple combinations - from long spindle-shaped to round-shaped grains - led to a largely variable seeds weight. The two traits associated with phenological development (‘days to heading’ and ‘days to maturity’) showed a lower degree of variability.

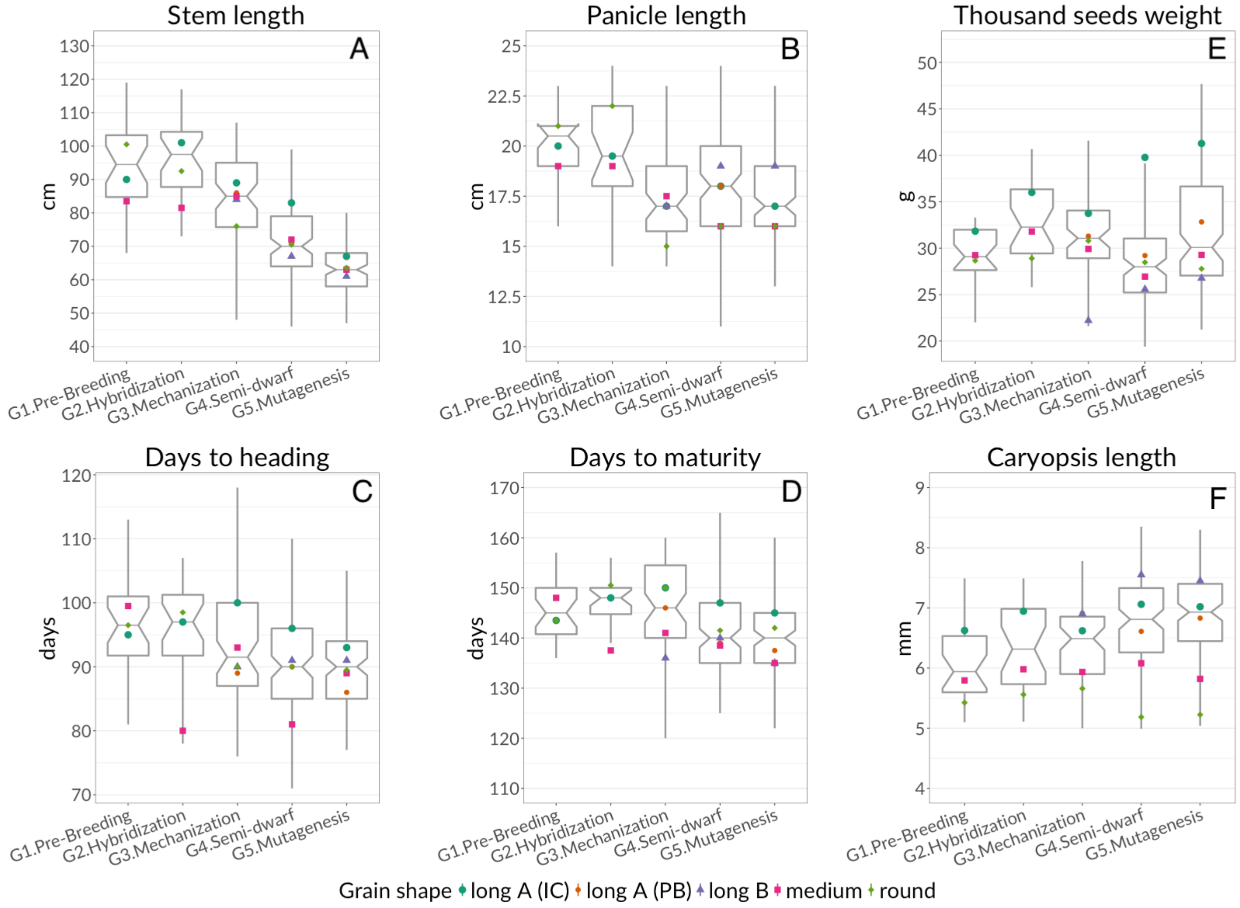


Figure 2.1 – Improved boxplots for traits 'stem length' (A), 'panicle length' (B), 'days to heading' (C), 'days to maturity' (D), thousand seeds weight (E), and caryopsis length (F) divided per groups based on time of release. The boxplot notches show the 95% confidence intervals around the median. Dots indicate median values for grain shape-*based groups.*

Traits evolution in the Italian rice varieties is presented in Figure 2.1. Significant differences (95% confidence) were found among the groups defined by *time of release* (groups from G1 to G5, see Methods section) regarding traits 'stem length', 'panicle length', 'days to heading', and 'days to maturity'. 'Stem length' (Figure 2.1A) remained quite stable in the first two groups G1 (1850 - 1927) and G2 (1928 - 1962), with median values of 94.5 cm and 97.5 cm, respectively, then progressively decreased to 63 cm in G5 (2005 - 2016). There was a significant decrease in 'stem length' (95% confidence) among subsequent *time of release* groups, except between G1 and G2; the decadal reduction rate since 1980 was 6-8 cm (Supplementary Figure 2.2). The group with the highest median 'stem length' was G2, likely due to the massive introduction of taller genotypes from Asia and the United States. The marked reduction in 'stem length' in G4 (1991 - 2004) was likely associated with the introduction of *semi-dwarf* genotypes. The G5 (2005 - 2016) group presented the smallest variability, indicating the focus of modern Italian breeders on short-stem varieties. G1 (1850 - 1927) and G2 (1928 - 1962) presented a significantly (95% confidence) higher median ‘panicle length’ (20.5 cm and 19.5 cm, respectively) than the other groups (Figure 2.1B). This trait also presented a significant reduction (95% confidence) in G3 (1963 - 1990, median 17 cm) and remained quite stable to date with a slight increase in G4 (1991 - 2004), due to the release of ‘long B’ grain varieties with longer panicles. The evolutionary trend in the duration of vegetative and reproductive development showed a gradual shift towards early varieties in the last 25 years. The median of ‘days to heading’ significantly reduced (95% confidence) from G1 (96.5 days) to G3 (91.5 days) and then stabilised at 90 days in G4 and in G5 (2005 - 2016, Figure 2.1C). The same trend was detected for the trait ‘days to maturity’, with a significant reduction (95% confidence) by eight days in G4 and G5 median values, compared to G2 (Figure 2.1D). The G3 group showed the highest variability for this trait (interquartile range – IQR – 15 days, range 55 days), determined by the co-existence of late and early varieties. The number of late varieties (‘days to maturity’ > 145 days) had markedly reduced in the most recent period (G5), and the IQR decreased from 15 to 10 days. The analysis showed a decreasing trend in trait ‘thousand seeds weight’ from G2 to G4 (Figure 2.1E) likely due to the gradual shift in market preference towards long spindle-shaped grains (Figure 2.1F). A renewed interest in ‘long A (IC)’ varieties in recent years could partly explain their increase from G4 to G5, caused by a drop in sale prices of the other grain types.

Differences among *grain shape*-based groups were also evaluated (Supplementary Figure 2.3, Supplementary Figure 2.4, and Supplementary Figure 2.5) to consider the phenotypic differences among these groups. ‘Long A (IC)’ (median 85 cm) and ‘long B’ (median 64.5 cm) varieties were at the extremes of ‘stem length’, the latter presenting small variability (IQR 11 cm) and the highest median value of ‘panicle length’ (19 cm). ‘Long A (IC)’ varieties also had long panicles (median 18 cm), with a long growing cycle (median ‘days to heading’ and ‘days to maturity’ equal to 95 and 146 days, respectively). ‘Round’ genotypes had a median ‘days to maturity’ of 144 days (ranked second highest among groups) while having a median ‘days to heading’ of 90 days (ranked second lowest). We observed a clear increasing trend in ‘thousand seeds weight’ in the varieties belonging to the ‘long A (IC)’ and ‘long B’ groups. ‘Long A (IC)’ grain varieties passed from 31.83 g median value in G1 to 41.27 g in G5, with a strongly skewed distribution towards high values and a maximum of 51.79 g (Supplementary Figure 2.4). The median of ‘thousand seeds weight’ in ‘long B’ grain varieties increased over time from 22.2 g in G3 to 26.8 g in G5. The median ‘thousand seeds weight’ for ‘long A (PB)’ varieties increased from 29.2 in G4 to 32.83 in G5, with a smaller IQR (Supplementary Figure 2.4). The G3 group had a higher median of ‘thousand seeds weight’ compared to G4, even if with a lower number of varieties. There were no detectable trends for ‘medium’ and ‘round’ grain shapes. The evolution observed in the trait ‘thousand seeds weight’ was congruent with ‘caryopsis width’ and ‘caryopsis length’ (Supplementary Figure 2.5 and

Supplementary Figure 2.6). The distribution of traits among the phylogenetic subgroups was compared with Faivre-Rampant *et al.* (2010), from which we mutated the nomenclature used in Table 3 of the seminal paper (Supplementary Figure 2.7). The groups considered were: ‘IIa’, consisting of the majority of Northern American (US) varieties, the Spanish *Puntal* and a set of Italian varieties derived from US varieties (mostly registered in G4, 1991 - 2004) such as *Thaibonnet*, *Gange*, and *Gladio*; ‘IIe’, for the most part, comprised accessions with ‘long A (IC)’ or ‘medium’ grain shape, and a lower number of ‘round’ grain varieties; it also included many of the ancient Italian rice varieties; ‘IIf’, included East-Asian accessions (the Japanese *Akitakomachi*), as well as Italian, US, Egyptian, Spanish and French varieties that were derived by Asian accessions as, e.g., the foundation variety *Balilla* derived from *Originario Chinese* (i.e. “originated from China”). We labelled as “Not available” all the genotypes for which phylogenetic data was unknown, plus the varieties belonging to groups ‘I’, ‘IIb’, ‘IIc’, and ‘IId’, as they comprised a limited number of accessions. A large variability was detected both within and between the phylogenetic subgroups. *Phylogenetic subgroup* ‘IIa’ showed the lowest internal variability, with a significantly different (95% confidence) data distribution compared to the other subgroups. This is due to the vast majority of ‘long B’ grain varieties, having specific phenotypic characteristics markedly different from the other *grain shape*-based groups. The *phylogenetic subgroups* ‘IIe’ and ‘IIf’ had similar data distributions, both encompassing the entire range of phenotypic variation for each trait, differing only in grain-related traits (‘thousand seeds weight’, ‘caryopsis length’), due to the aforementioned differences in *grain shape*. Correlations coefficients among traits were calculated and evaluated before executing Principal Component Analysis (PCA, Supplementary Figure 2.8). Several strong positive correlations were found between ’caryopsis width’ and ‘thousand seeds weight’ (r = 0.67), ‘days to heading’ and ‘days to maturity’ (r = 0.63), ‘stem length’ and ‘panicle length’ (r = 0.35). Moreover, ‘caryopsis length’ was inversely related with ‘caryopsis width’ (r = -0.48) that, in turn, was positively related with stem length (r = 0.39). Further analysis of the relationships among traits was included in the characterisation of Principal Components (PCs), where the linear relationships between variables were investigated by detecting the principal dimensions of variability(Husson, Lê, & Pagès, 2010a).

2.3.2 PRINCIPAL COMPONENTS ANALYSIS

PCA was performed to summarise the data with a multivariate approach and to provide a visual representation of the reciprocal phenotypic distance. The first three components, explaining 74.22% of the total variance, were selected for data interpretation. Characterisation of Principal Components (PCs) was performed by calculating the correlation coefficients with the original traits and the associated significance level (Table 2.3). Moreover, one-way ANOVA models were constructed including the Principal Components as response variables and *grain shape*, *time of release*, *phylogenetic group*, and *flag leaf attitude* as explanatory variables (see Methods section); coefficients of determination (R^2) and related p-values were reported in Supplementary Table 2.1; the categories coefficient estimates, tested for significant differences from zero ($\alpha = 0.05$), were listed in Supplementary Table 2.2 to Supplementary Table 2.4.

*Table 2.3 – Correlation coefficients between each analysed variable and the first three Principal Components (PC) with an indication about the significance of differences from 0, and the amount of variance explained by each PC. Significance codes: '***' $p < 0.00$*

Trait	PC1	Sig.	PC2	Sig.	PC3	Sig.
Caryopsis length	-0.33	* * *	0.5	* * *	0.72	* * *
Caryopsis width	0.74	* * *	-0.61	* * *	0.15	* * *
Days to heading	0.61	* * *	0.53	* * *	-0.18	* * *
Days to maturity	0.72	* * *	0.27	* * *	-0.23	* * *
Panicle length	0.2	* * *	0.65	* * *	0.24	* * *
Stem length	0.72	* * *	0.2	* * *	-0.05	
Thousand seeds weight	0.51	* * *	-0.33	* * *	0.78	* * *
Time of release	0.32	* * *	0.05	* * *	0.06	* * *
Flag leaf attitude	0.05	* * *	0.03	*	0.06	* * *
Phylogenetic subgroup	0.27	* * *	0.06	* * *	0.12	* * *
Grain shape	0.51	* * *	0.41	* * *	0.44	* * *
Explained variance	33.9%		21.9%		18.5%	

The first component (PC1) accounted for 33.9% of total variance and had a strong positive correlation with traits 'stem length', 'days to heading', and 'days to maturity'. PC1 was also positively correlated with trait 'caryopsis width', confirming the results of univariate analysis. Overall, PC1 opposed long-stem, late and wide grain varieties, such as *La Ferla* or *Vialone Nero*, to small, early, and narrow-grain varieties such as *CRLB1* and *Tea*.

The second principal component (PC2) explained 21.9% of the total variance (Table 2.3) and was positively correlated with 'panicle length' and negatively with 'caryopsis width'. This suggested an inverse correlation between these traits indicating that narrow-grain varieties generally presented longer panicles (e.g. accessions *Ecco 63* and *Libero*, that had extreme positive values on this axis) than wide-grain varieties (e.g. 'round' grain varieties like *Megumi* or *Ducato*, that had the lowest negative coordinates on this axis).

The third component (PC3), explaining 18.5% of the total variability, was mostly positively correlated with grain traits, i.e. ‘thousand seeds weight’ and ‘caryopsis length’ (Table 2.3). In fact, ‘long A (IC)’ grain varieties (e.g. accession *Neve*) had the highest value on PC3 while ‘round’ grain varieties (e.g. accession *Top*) the lowest (Supplementary Table 2.4). All the categorical variables *grain shape*, *time of release*, *phylogenetic group*, and *flag leaf attitude* explained a significant amount of variance on the first three PCs (Supplementary Table 2.1).

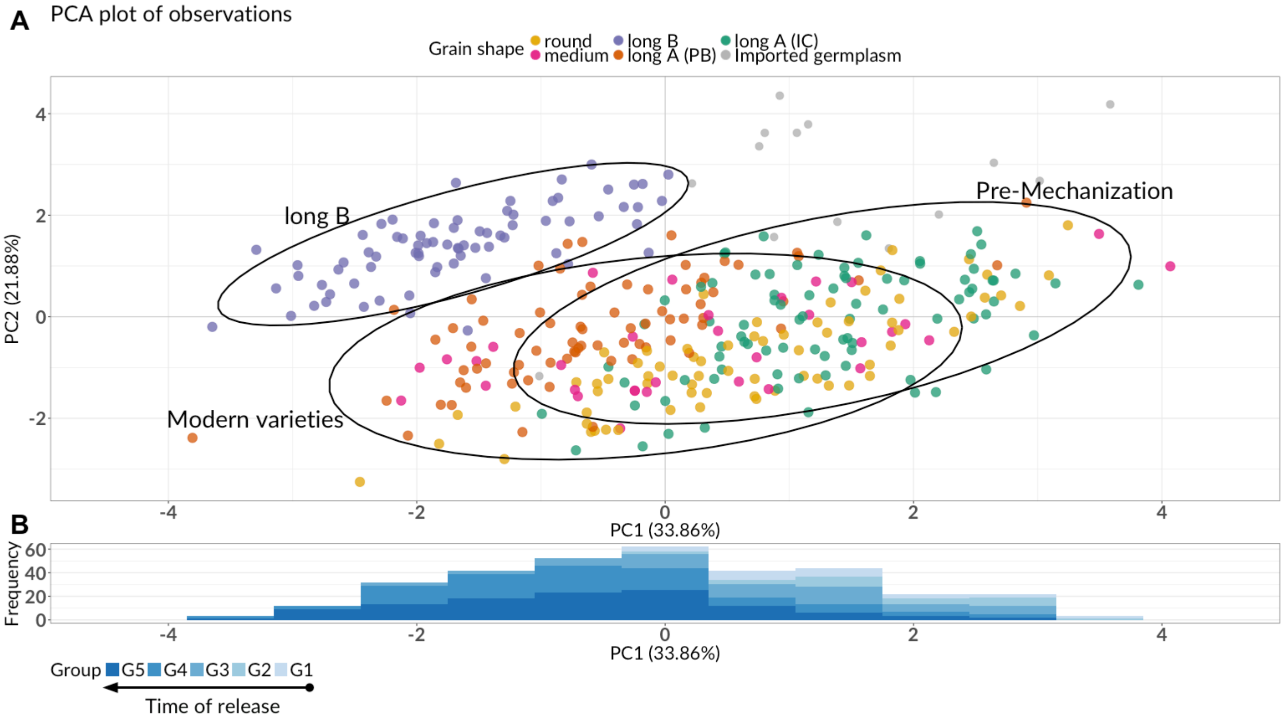


Figure 2.2 – A) Biplot of the genotypes in the Principal Component space, colour-coded by the categorical variable grain shape. *Grey dots represent the 13 supplementary individuals, i.e. foreign varieties introduced in Italy as a source of new genetic material. Confidence ellipses for ‘Long B’, modern (bred after 1962), and pre-mechanization varieties are showed. B) Frequency histogram of the supplementary categorical variable* time of release *on PC1.*

We classified as modern the varieties bred from G3 (1963 - 1990) onwards since during this period the primary driver of genetic improvement was the mechanisation of Italian rice agriculture. Modern and old varieties substantially overlapped (Figure 2.2A), the latter obtaining positive values on PC1, with few exceptions. The frequency histogram in Figure 2.2B showed rice varieties roughly following an inverse chronological order on PC1, from left to right.

‘Long B’ grain varieties were grouped and well isolated from the other grain shapes on the first two dimensions. Genotypes belonging to this group had negative values on PC1 and positive values on PC2 since these varieties typically had a short stem, narrow-grain, and long panicle. The majority of ‘long A (IC)’ grain varieties had positive values on PC1: only eight cultivars from this group had negative values, both on PC1 and PC2: these were *Falco*, *Fedra*, *Fulgente*, *Galileo*, *Neve*, *Pato*, *Proteo* and *Tosca*. With the exception of *Fulgente,* these are all modern varieties, bred after the year 2000, having a short stem and panicle and an earlier growing cycle than the other varieties of ‘long A (IC)’ group. Similarly, ‘round’ grain varieties were mostly on the negative side of PC2, except for 13 old varieties which were bred in the G1 or G2 period (1850 - 1962). These were *Agostano*, *Ambra*, *Americano 1600*, *Balocco*, *Benito*, *Feronio*, *Ferraris*, *Lencino*, *Neretto*, *Originario*, *Precoce Monticelli*, *Roncarolo*, *Sesiamochi*, and *Sorriso*. ‘Long A (PB)’ varieties were split between both axes at negative and positive values, covering an ample spectrum of variation: *Rodio* and *Tea* were at the extremes of the ‘long A (PB)’ group, with *Rodio* and *Tea* presenting the highest and lowest positive coordinates on both axes, respectively. ‘Medium’ grain varieties were evenly distributed on PC1 and had negative values on PC2 except for few accessions, which were bred in early periods (G1 to G3). The imported foreign varieties, considered as supplementary individuals (not taking part in the computation of the PCs, see Methods section) had positive coordinates on both PC1 and PC2 and were separated from the Italian varieties, with the unique exception of *Akitakomachi*.

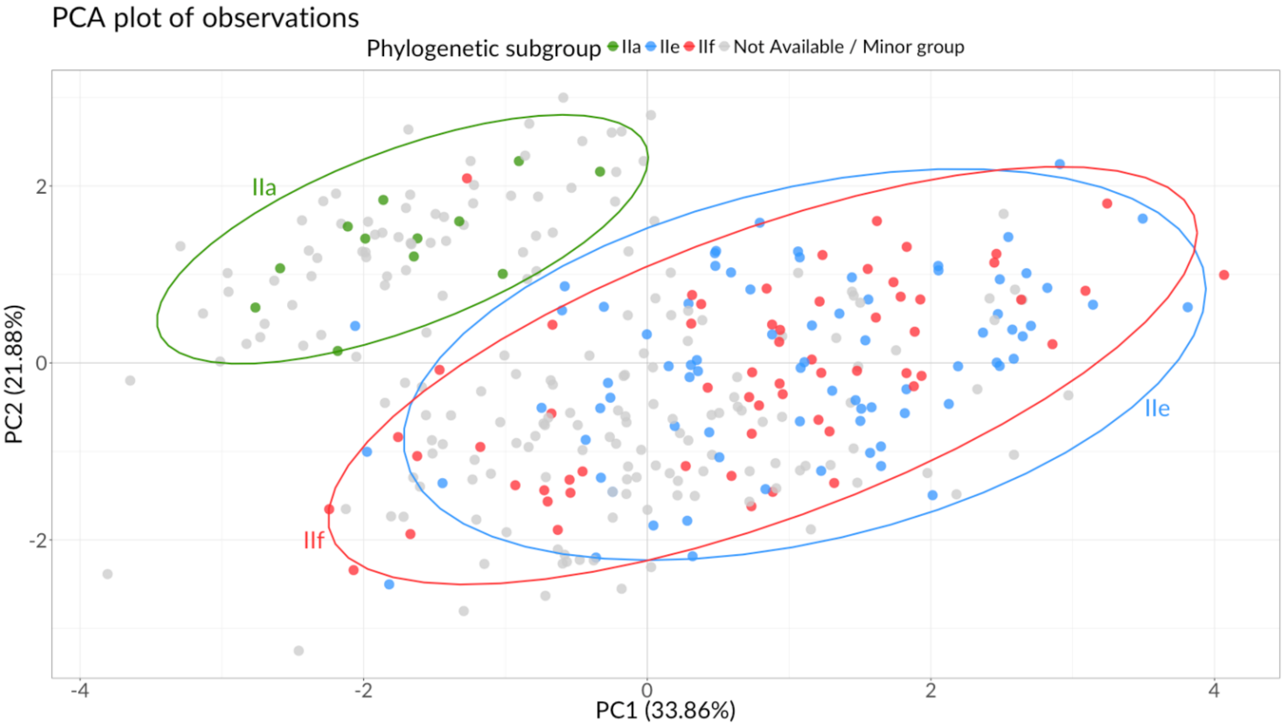


Figure 2.3 – PCA biplot comparing phenotypic and phylogenetic data coming from previous studies. Genotypes are colour-coded by the categorical variable phylogenetic subgroup*, derived from a previous study involving molecular characterisation (see Methods section); confidence ellipses (95% confidence) are drawn for the three major phylogenetic subgroups IIa, IIe, and IIf.*

The comparison with the available phylogenetic data was performed by drawing confidence ellipses (95% confidence) on the biplot for the three major phylogenetic subgroups (Fig. 3). As expected, varieties from *phylogenetic subgroup* 'IIa' (mostly composed by 'long B' grain varieties) clustered together in PCA biplot and were separated from the others; on the contrary, confidence ellipses of groups 'IIe' and 'IIf' highly overlapped, thus indicating a wide range of phenotypic variation.

2.3.3 CLUSTER ANALYSIS

A hierarchical clustering algorithm was applied to the extracted PCs (HCPC) to detect inner structures in the data and to provide a phenotypic classification of the Italian rice varietal landscape. Only the first three PCs were used in the analysis to reduce noise. Three clusters were selected to maximise the relative loss of inertia(Husson, Lê, & Pagès, 2010a). We provide the complete list of varieties in each *cluster* in Supplementary Table 2.5; data plotted in PCs space colour-coded by *cluster* is presented in Figure 2.4. The obtained partition was compared to the original qualitative and quantitative variables, using an alpha level $\alpha = 0.05$ for all statistical tests. The proportion of between-clusters variance over the total variance explained by each trait was evaluated (Supplementary Table 2.6). Trait 'caryopsis width' explained the most variance among clusters (η^2 = 0.657, p < .001), followed by 'stem length' (η^2 = 0.448, p < .001), and 'days to heading' (η^2 = 0.367, p < .001). A v-test (see Methods section) was calculated on the means of quantitative variables: the null hypothesis (H_0) was that the cluster average did not differ from general average, with the sign of the test statistic indicating a lower (-) or greater (+) cluster mean than the overall mean. Tests results were listed in Supplementary Table 2.7 to Supplementary Table 2.9; a visual representation of traits distributions within clusters was provided in Supplementary Figure 2.9.

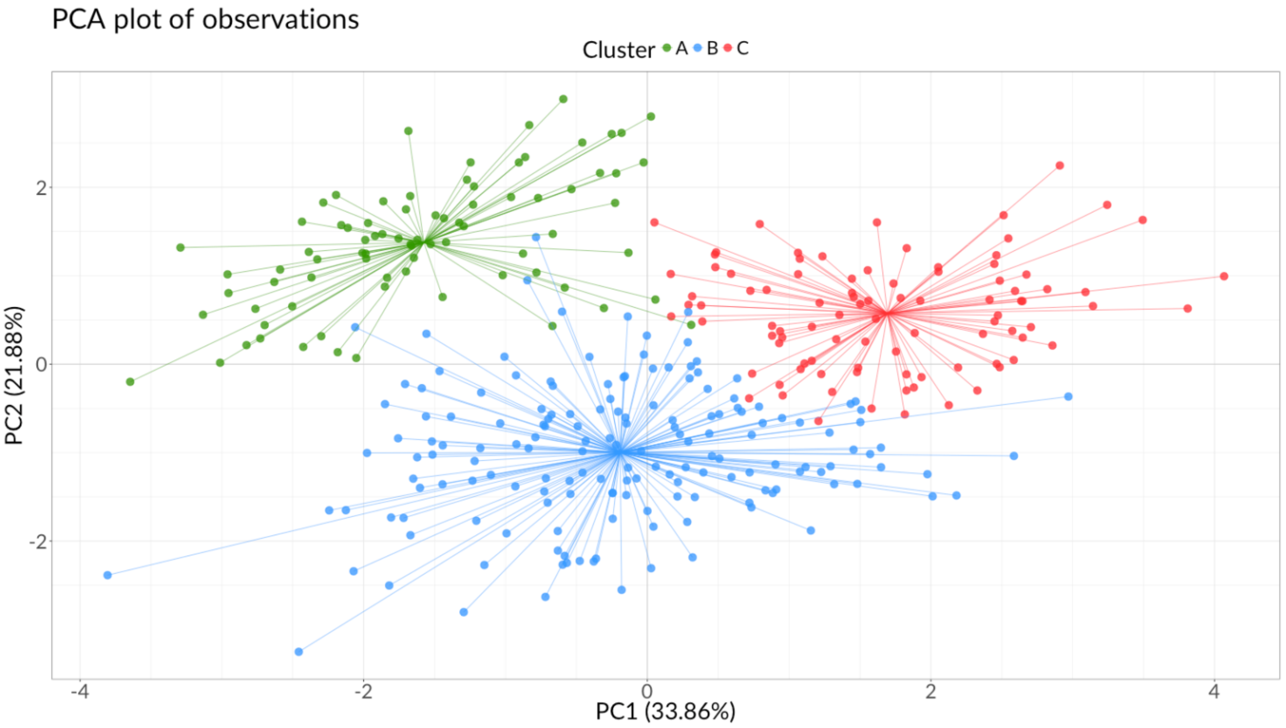


Figure 2.4 – PCA biplot comparing phenotypic and phylogenetic data coming from previous studies. Genotypes are colour-coded by the categorical variable phylogenetic subgroup*, derived from a previous study involving molecular characterisation (see Methods section); confidence ellipses (95% confidence) are drawn for the three major phylogenetic subgroups IIa, IIe, and IIf.*

Category frequencies distributions within clusters for all the four categorical variables were significantly different ($p < .001$) from the overall frequency distribution according to χ^2 test (Supplementary Table 2.10). The p-values were then calculated for each category with a hypergeometric test (Supplementary Table 2.11 to Supplementary Table 2.13): in this case, a positive or negative sign of the v-statistic indicates an over- or under-representation of a category in the cluster, respectively.

The 90% of varieties in cluster were 'long B' grain varieties ($v = 16.54$, $p < .001$), including 97.3% of the total 'long B' group; all the other grain shapes were under-represented or absent. Almost all the varieties included in this group had been released in the most recent periods (92.5%), specifically in G4 (1991 - 2004, $v = 4.04$ $p < .001$) and G5 (2005 - 2016, $v = 1.98$, $p = 0.0479$). All the varieties in the *phylogenetic subgroup* 'IIa' were included in this cluster ($v = 5.65$, $p < .001$), while varieties in group 'IIe' ($v = -5.67$, $p < .001$) and 'IIf' were nearly absent ($v = -4.2$, $p < .001$); the remaining 75% of the varieties included in this cluster had no phylogenetic data, either because they were not included or were released after Faivre-Rampant *et al.* (2010). Cluster A included varieties with short stems ($\overline{x} = 65$ cm, $v = -6.49$, $p < .001$) and long panicles ($\overline{x} = 19$ cm, $v = 4.05$, $p < .001$). Average grain biometrics in this cluster reflected the extensive presence of 'long B' grain varieties, with long and narrow caryopses, and low 'thousand seeds weight'. The 'erect' category in *flag leaf attitude* was significantly represented in cluster A ($v = 2.86$, $p = 0.0043$); on the contrary, the 'horizontal' level was under-represented ($v = -2.85$, $p = 0.0043$). Variety *Mare CL*, a recent 'Long B' grain Clearfield® variety, was close to the barycentre of Cluster A, while variety *CRLB1*, an early, short height, 'Long B' variety, was well distinct from the other clusters.

Cluster B was characterised by 'long A (PB)' ($v = 4.98$, $p < .001$) and 'round' grain ($v = 3.07$, $p = 0.0021$) genotypes; 'long B' *grain shape* was strongly under-represented ($v = -9.77$, $p < .001$). The 65.2% of the varieties bred in G5 (2005 - 2016) were included in this cluster ($v = 4.27$, $p < .001$), which encompassed the 44.5% of the total number of varieties; only 13 genotypes were released in G1 ($v = -3.03$, $p = 0.0024$) and G2 ($v = -2.61$, $p = 0.009$). No *phylogenetic subgroup* was significantly present in Cluster A, probably due to data unavailability in 63.4% of the genotypes. Genotypes in Cluster B had short stems ($\bar{x} = 70$ cm, $v = -5.74$, $p < .001$) and panicles ($\bar{x} = 17$ cm, $v = -5.74$, $p < .001$), and the lowest mean of trait 'days to heading' (88 days, $v = -9.53$, $p < .001$). This cluster was characterised by an above-average 'thousand seeds weight' ($\bar{x} = 32.62$ g, $v = 5.05$, $p < .001$), slightly higher than Cluster C, presenting greater variability, and very similar mean values of 'caryopsis length' and 'caryopsis width' to Cluster C. Varieties in Cluster B mainly presented a 'semi-erect' *flag leaf attitude* (55.4%, $v = 2.06$, $p = 0.0394$). *Opale*, a 'Long A (PB)' medium-cycle modern variety was one of the closest to the barycentre of this cluster, while *Tea*, a very short-height and early maturing genotype, was one of the farthest.

Half of the genotypes in Cluster C had 'long A (IC)' grain shape ($v = 5.8$, $p < .001$), while 'long B' genotypes were absent. The 75% of genotypes bred in G1 ($v = 5.4$, $p < .001$) and G2 ($v = 5.4$, $p < .001$), and 48.4% of the varieties in G3 ($v = 3.95$, $p < .001$) were included in this cluster, implying that roughly 80% of these genotypes were bred before 1990. *Phylogenetic subgroups* 'IIe' ($v = 5.38$, $p < .001$) and 'IIf' ($v = 5.14$, $p < .001$) strongly characterised Cluster C, which comprised 52.56% and 55.74%, of the varieties in these two subgroups, respectively. Since Cluster C included many genotypes from pre-mechanisation periods, it had the highest mean for traits 'stem length' ($\bar{x} = 93$ cm; $v = 12.60$, $p < .001$), 'days to heading' ($\bar{x} = 99$ days; $v = 10.28$, $p < .001$), 'days to maturity' ($\bar{x} = 149$ days; $v = 8.16$, $p < .001$), and 'panicle length' ($\bar{x} = 19$ cm, $v = 6.55$, $p < .001$). As previously mentioned, the average grain biometrics in Cluster C were very similar to Cluster B, with lower variability. The 'horizontal' category of *flag leaf attitude* was significantly present in this cluster ($v = 4.04$, $p < .001$), describing over 60% of the genotypes; the 'semi-erect' category only occurred in 35% of the cases ($v = -3.59$, $p = .0039$). Many historic accessions were included in this cluster, i.e., *Originario*, *Americano 1600*, *Sancio P6*, *Lencino*, *Agostano*, *Rizzotto*, *Vialone Nero*.

2.4 DISCUSSION

A panel of 351 rice varieties bred or imported as a source of new genetic material in Italy was investigated using seven morphological markers, to determine its main phenotypic variability, and to allow comparing with molecular data in order to identify evolutionary trends of genetic improvement; evolutionary trends were evaluated by grouping genotypes on the basis of their release date, as reported in Table 2.1. The most recent group (G5) corresponded to the higher number of varieties, proving that the Italian rice breeding activity is still active in recent years.

A large phenotypic variability emerged in the considered traits. The total amount of phenotypic variation was relatively high compared to other germplasm collections (Bosetti et al., 2011; Rabara, Ferrer, Diaz, Newingham, & Romero, 2014; Roy & Sharma, 2014), and it decreased over time in disagreement with other molecular studies (Faivre-Rampant et al., 2010; Mantegazza et al., 2008; Spada et al., 2004). The phenotypic variability in G5 (2005 - 2016) was indeed the smallest, with limited range. The analysis of traits evolution revealed clear trends of genetic improvement in Italian rice breeding: stem length has been gradually reduced as well as the duration of the vegetative and reproductive cycles, similarly to what has been observed for other cereal crops during the green revolution (Ormoli, Costa, Negri, Perenzin, & Vaccino, 2015; Worland, 1999). It can be noted that few variations in the considered traits occurred in G2 (1928 - 1962), probably because of the breeders focus on the resistance to blast disease, without primary concern on mechanisation. Earliness was instead a desirable feature, to ease the adoption of weed management strategies, especially against weedy rice (Faivre-Rampant et al., 2010). Furthermore, a trend towards bigger and heavier caryopses was found in 'long A' and 'long B' genotypes: this could contribute to explain the large yield increase throughout the twentieth century since grain biometrics are commonly considered to be yield-related traits (Samonte, PB Samonte, Wilson, & McClung, 1998; Veni, Lakshmi, & Ramana, 2013). A similar trend did not occur on 'round' and 'medium' genotypes that had, on average, lighter grains after G3. Although not supported with quantitative analysis, it can be hypothesised that the large reduction in plant height of 'round' varieties determined a reduction in caryopses weight, since these two traits are known to be highly correlated (Hittalmani et al., 2003; Samonte et al., 1998; Weng et al., 2008). Furthermore, the breeding strategy adopted to increase yield levels focused on the enhancement of tillering capacity rather than on panicle biomass. For 'medium' grain genotypes, the lack of a detectable trend could be due to the low number of accessions and to their diminished agronomic interest, which were gradually replaced with 'long A (PB)' varieties.

The analysis of correlations highlighted distinct multilinear relationships among traits, further confirmed by the relations between the extracted principal components and the original variables. For example, the first component (PC1) positively correlated with growth characteristics as stem length and the duration of vegetative and reproductive cycles, and negatively with ‘caryopsis width’.

To some extent, PC1 represented a proxy of a timeline, with old, tall, and late varieties on the positive side while newer, short, and early varieties on the negative one. In fact, varieties in G1, G2, and G3 generally had positive coordinates on this axis; contrariwise, varieties in G4 and G5 obtained negative coordinates. The distinctive order of varieties on PC1 suggested two considerations: earliness and the reduction of plant height were slowly achieved over time since the 19th century, and the release of varieties with narrower grain as ‘long A (PB)' and ‘long B' increased in the last 20 years. In fact, in early periods of Italian rice cultivation, ‘medium’ and ‘long A (IC)’ varieties were mostly cultivated (Spada et al., 2004); nowadays, Italian rice-growers prefer spindle-shaped grain varieties (like ‘long A (PB)’ and ‘long B’) (Ente Nazionale Risi, 2016). Furthermore, a temporal trend toward ‘erect’ and ‘semi-erect’ *flag leaf attitude* emerged: in fact, groups defined by ‘horizontal’ and ‘recurved’ categories were at the positive side of PC1, the latter with highest positive values; on the contrary, varieties with ‘semi-erect’ and ‘erect’ flag leaf were at negative values, the latter with the lowest negative coordinate. The explanation is twofold: firstly, after 1990 ‘long B’ grain varieties with a more erect attitude of flag leaf were introduced in the Italian varietal landscape; secondly, leaves erectness was directly or indirectly selected to increase rice yield since a small insertion angle of leaves on stem leads to larger light interception (Peng, Khush, Virk, Tang, & Zou, 2008). Italian rice varieties evolved from tall, late, and horizontal-to-recurved leaves varieties to short, early and erect (or semi-erect) leaves. The evolution was even more evident after 1990, following the introduction of the long B varieties carrying *semi-dwarf* genes.

The second principal component (PC2) determined an evident cluster of ‘long B’ grain varieties, because of the relationships with the original traits. PC2 was mostly related with ‘panicle length’ and inversely related to ‘caryopsis width’, so that ‘long B’ grain varieties (having a long spindle-shaped grain and long panicles) positioned at positive coordinates while on the negative side of the first component due to their short height. Other grain shapes also clustered together, with overlaps. The reason is that varieties with the same caryopsis shape generally showed similar phenotypic characteristics, confirming the findings of other molecular studies on Italian rice germplasm (Spada et al., 2004). ‘Long A (IC)’ typically showed a considerable plant size, late growing cycle, and large panicles. Italian 'Long A (PB)' varieties were shorter, had small panicles and narrower grains compared to the varieties for internal consumption (IC). 'Medium' grain varieties had similar features but were taller, while 'round' grain featured, on average, shorter stems and were late-maturing. 'Long B' grain varieties were short and had, on average, long growing cycles, other than presenting long panicles and narrow grains.

Cluster analysis further validated our results, allowing the identification of the variables determining most between-clusters variance, i.e., 'caryopsis width', 'stem length', and 'days to heading'. In fact, each cluster was predominantly constituted by varieties belonging to at least one *grain shape*, and to two or three subsequent *time of release* groups. The time component also emerged from the cluster analysis: modern varieties generally clustered together, because of their shared characteristics of reduced plant height and short duration of vegetative/reproductive cycles.

There is general agreement between the available molecular analysis on Italian rice germplasm (Faivre-Rampant et al., 2010) and our clustering: rice varieties from *phylogenetic subgroup* 'IIa', corresponding mostly to 'long B' grain varieties derived from US accessions, were grouped in Cluster A. On the contrary, varieties from phylogenetic subgroups 'IIe' and 'IIf', comprising all other genotypes, were distributed among the other two clusters. These varieties showed considerable heterogeneity in phenotypic characteristics, congruently with other studies, even though they presented a lower genetic variability due to common ancestors. The most significant distance emerged between Cluster A and C, in agreement with measured genetic distances, their history and pedigree data (Spada et al., 2004).

Genetic improvement of Italian rice during the 20th century was partly driven by the emerging mechanised and chemical agriculture, as the technological advancements were among the primary determinants of yield gains for all crops worldwide. The limited phenotypic variability characterising the modern rice varieties emphasises the importance of safeguarding the genetic resources stored in germplasm banks. As an example, crop modelling studies aimed at forecasting future crop yields under climate change scenarios indicate that the adoption of varieties with longer crop cycle could be a profitable adaptation strategy to counteract the effects of rising temperatures (Bregaglio, Hossard, et al., 2017a). Although our study did not consider the impact of climatic conditions on the evaluated morphological traits, it provides an overall picture of the evolutionary trends in Italian rice cultivars, integrating the information coming from molecular studies. Our characterisation could be used to support classic rice breeding programs, as well as the basis to develop ideotype breeding analyses with eco-physiological models. Furthermore, with the new genome-editing techniques like CRISPR/Cas9, the direct introduction of a natural or novel mutation into elite germplasm will be possible and cost-effective: this type of technology had been successfully used to improve climate-related agronomic traits like pathogen resistance in various crops (Scheben, Yuan, & Edwards, 2016). Efficient use of germplasm in rice genetic improvement could indeed lead to enhance the genetic variability of the crop, potentially allowing to break the actual yield barriers and developing new varieties suited for cultivation in climate change conditions.

2.5 METHODS

2.5.1 PLANT MATERIAL AND EXPERIMENTAL CONDITIONS.

A panel of 351 varieties, bred in Italy or imported as a source of new genetic material, was used to perform our analysis (Table 2.1). Germplasm used in the trials belongs to the reference collection of the Vercelli Laboratory of the Research Centre for Plant Protection and Certification of the Council for Agricultural Research and Economics (CREA-DC): CREA-DC maintains this collection for the execution of the official technical exams needed for variety registration and protection of plant breeders' rights. The sample represents the 94% (187) of the registered and 69% (64) of the deleted Italian rice varieties until 2016 in Italy. Eighty-seven varieties that have never listed in the National Catalogue were also used in the analysis: these were bred before the constitution of the National Catalogue or rejected after official testing. Thirteen foreign varieties imported from China (*Teqing*), Japan (*Akitakomachi*), USA (*Lady Wright*, *Pecos*, *Lemont*, *A201*, *Dixiebelle*, *Jefferson*, *Jacinto* and *Orion*), Spain (*Bomba* and *Puntal*), and Philippines (*IR64*) were included as 'supplementary individuals' i.e. not taking part in statistical analysis but only used for comparison afterwards (Lê, Josse, & Husson, 2008).

The phenotypic characterisation was done in open-field trials in Garbagna Novarese (N45°23'11.558, E8°39'55.976,), in the middle of the rice cultivation area of North-West Italy. Data were collected during eight cropping seasons (2009 to 2016) with a minimum of two consecutive years of cultivation for each variety. Reference varieties were sown each year in the trials, as prescribed by the technical protocols (Community Plant Variety Office, 2012), to spot and eventually to exclude experiments with possible strong environmental effects interfering on the expression of each considered morphological trait. Trials data were then averaged to obtain a dataset with one single observation per variety.

The agricultural management of the field trials reflected the standard practices of rice farmers in the area with flooded conditions, prevention of nitrogen limitations/excesses and losses due to weeds, pests, and diseases. Randomized complete block design with two replications was adopted. Seeds were seed-drilled in plots of 2.5 m^2 (6-rows plots, interspaced by 0.2 m, spaced 1 meter, and 2 meters long), providing at least 500 plants per plot.

2.5.2 PHENOTYPIC CHARACTERISATION

The assessment of quantitative traits was performed according to the methods described by the technical protocol CPVO-TP/16/2 (Community Plant Variety Office, 2012), issued by the Community Plant Variety Office (CPVO). The selected traits are part of rice 'grouping characteristics' (Community Plant Variety Office, 2012; Ministero delle Politiche Agricole Alimentari e Forestali, 2014), i.e. used to arrange varieties in homogenous phenotypic groups with a limited number of traits. They also are of relevant agronomic importance, especially in Italy, having been targeted by multiple breeding programs throughout the 21st century. 'Stem length' (cm) was measured at milk maturity stage, as the mean length of culms from the ground to the panicle node. 'Panicle length' (cm) was measured along with 'Stem length', from the panicle node to the tip of panicle, excluding awns. Trait 'days to heading' was recorded as the number of days after sowing when 50% of culms showed emerging panicles while 'days to maturity' as the number of days from sowing to the moment when spikelets reached 22% average relative humidity (assessed with a thermogravimetric scale model Sartorius MA-150). 'Thousand seeds weight' (g) was expressed as the average weight of 1000 fully developed spikelets at reference moisture content (14% relative humidity). 'Caryopsis length' and 'caryopsis width' (mm) were assessed on 100 fully developed caryopses using an image analysis software (WinSEEDLE Pro v. 2007d). Genotypes were also described by four categorical variables (data presented in Supplementary Table 2.5): *grain shape*, *time of release*, *phylogenetic subgroup,* and *flag leaf attitude*. These were used to support the interpretation of results, to evaluate the evolutionary trends, and to allow comparison of our results with previous studies or available classification. *Grain shape* variable categorises varieties as 'Round', 'Medium', 'Long B', 'Long A (for internal consumption, IC)', and 'Long A (for parboiling transformation process, PB)' based on market classification defined by Regulation (EU) No 1308/2013. Even though this Regulation does not distinguish 'Long A' grain varieties for internal consumption from those used for parboiling, a further distinction was adopted in light of their phenotypic differences and market specialisation. *Time of release* classifies varieties in five groups by their year of release or registration according to historical records or, when available, in the National Catalogue. Group 1 (G1, 1850 - 1927) includes varieties released before 1927 when mass selection was used as the primary breeding technique. Group 2 (G2, 1927-1962) collects varieties bred in the period when hybridisation programs started to increase. Varieties in Group 3 (G3, 1963-1990) were released at a time when the main driver of Italian genetic improvement was grain quality. The varieties released in the period covered by Group 4 (G4, 1991-2005) were characterised by the introduction of *semi-dwarf* genes carried by novel 'Long B' grain varieties (e.g., Thaibonnet). Lastly, Group 5 (G5, 2005 - 2016) covers the time during which Clearfield®

varieties were introduced in Italy. These groups were extracted from a previous study (Mantegazza et al., 2008), except for G5, and were chosen to allow both the evaluation of evolutionary trends and the comparison with the study. The categorical variable *phylogenetic subgroup* was mutated from a past molecular characterisation (Faivre-Rampant et al., 2010) and was used to explore similarities and links between genotypic and phenotypic data, as both studies shared 181 out of 351 varieties. Groups of this categorical variable are ‘I’, ‘IIa’, ‘IIb’, ‘IIc’, ‘IId’, ‘IIf’, and ‘IIg’: when no information was available, we classified the variety as ‘Not Available’ (NA). Since groups I, IIb, IIc, and IId only contained a limited number of genotypes, they were included in the ‘Not Available’ group in the plots. *Flag leaf attitude* was assessed during anthesis and classified as ‘erect’, ‘semi-erect’, ‘horizontal’, or ‘recurved’, according to CPVO-TP/16/2 (Community Plant Variety Office, 2012). This categorical variable was included as the insertion angle of the flag leaf markedly influences the patterns of light interception and saturation of upper leaves, therefore impacting plant productivity (YoshidaS, 1981).

2.5.3 DATA ANALYSIS

Descriptive statistics of morphological traits are showed using improved box-plots (McGill, Tukey, & Larsen, 1978) allowing to represent the significance of differences between groups, other than intra-group variability. The notches in improved boxplots indicate the 95% confidence interval around the median calculated as ($95\%CI_{median} = Median \pm 1.57 \cdot \frac{IQR}{\sqrt{n}}$), where IQR is the interquartile range and *n* the sampling number. The medians of two groups are (roughly) significantly different at a 95% confidence level when their notches do not overlap. Boxplot figures were created using *ggplot2* R Package (Wickham, 2009).

Principal Components Analysis (PCA) was used to summarise the information related to the morphological traits of the Italian rice varieties, also providing a visual representation of their phenotypic distance. We obtained Principal Components (PCs) on centred and scaled active quantitative traits, through diagonalisation of the correlation matrix and extraction of the associated eigenvectors and eigenvalues. Traits ‘stem length’, ‘panicle length’, ‘days to heading’, ‘days to maturity’, ‘thousand seeds weight’, ‘caryopsis length’, and ‘caryopsis width’ were set as active quantitative variables, i.e. used to compute PCs; *grain shape*, *time of release*, *phylogenetic subgroup*, and *flag leaf attitude* were used as supplementary categorical variables (see section “Phenotypic characterization). The adjective "supplementary" indicates that these variables did not take part in the computation of PCs and their coordinates were calculated after the analysis as the barycentre of the corresponding individuals in the Principal Component space. The *FactoMineR* R package (Lê et al., 2008) was adopted to perform the analysis. The biplot was drawn using *ggplot2* R Package (Wickham, 2009). A one-way ANOVA model was constructed using the supplementary categorical variables as predictors and the PCs as response variables. The significance of the relationships between the PCs and the categorical variables was evaluated via *F-test*. Several *t-tests* were then conducted for each level of the categorical variables to determine if the coordinates of the individuals of the sub-population defined by one category are significantly different from 0. These tests were performed with the function *dimdesc()* of the *FactoMineR* package (Lê et al., 2008).

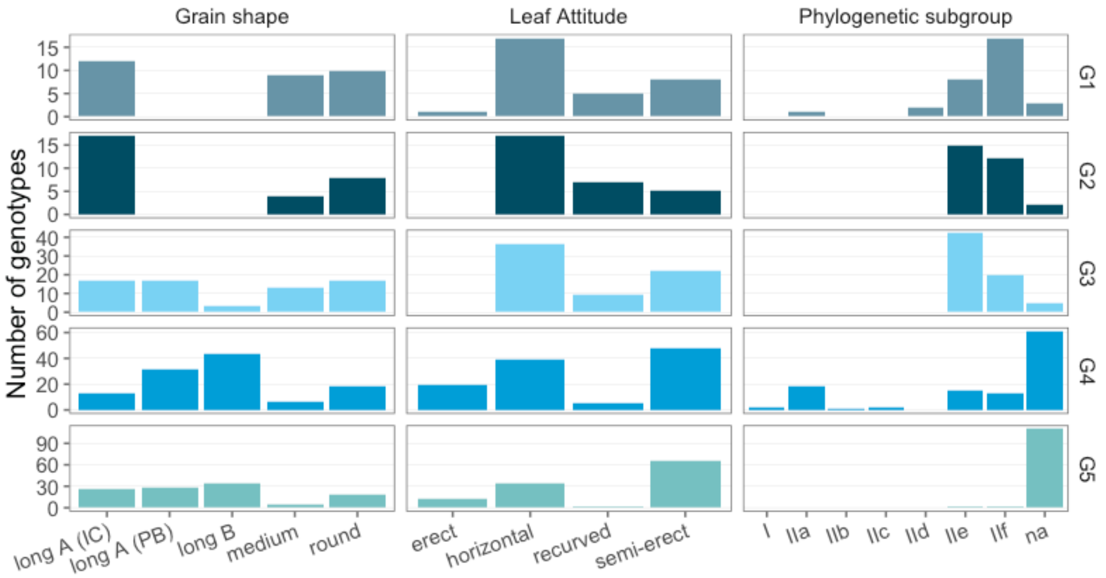
A Hierarchical Clustering on Principal Components (HCPC) (Lê et al., 2008) was then performed to detect any new or to confirm data structures previously detected (Luxburg, Williamson, & Guyon, 2012) and to provide a phenotype-based classification of the Italian rice genotypes. The analysis was performed using function *HCPC()* of the *FactoMineR* package (Lê et al., 2008). η^2 was calculated for quantitative variables (i.e. the traits) to measure the proportion of total variance associated with the extracted clusters and explained by each variable. Clusters were then characterised using both the quantitative and qualitative variables with a *v-test*[30]. In the first case, cluster mean ($\overline{x}_q$) was compared to the overall mean ($\overline{x}$), to see if there was a significant difference within the cluster. The following quantity was calculated:

$$u = \frac{\overline{x}_q - \overline{x}}{\sqrt{\frac{s^2}{n_q}\left(\frac{N - n_q}{N - 1}\right)}}$$

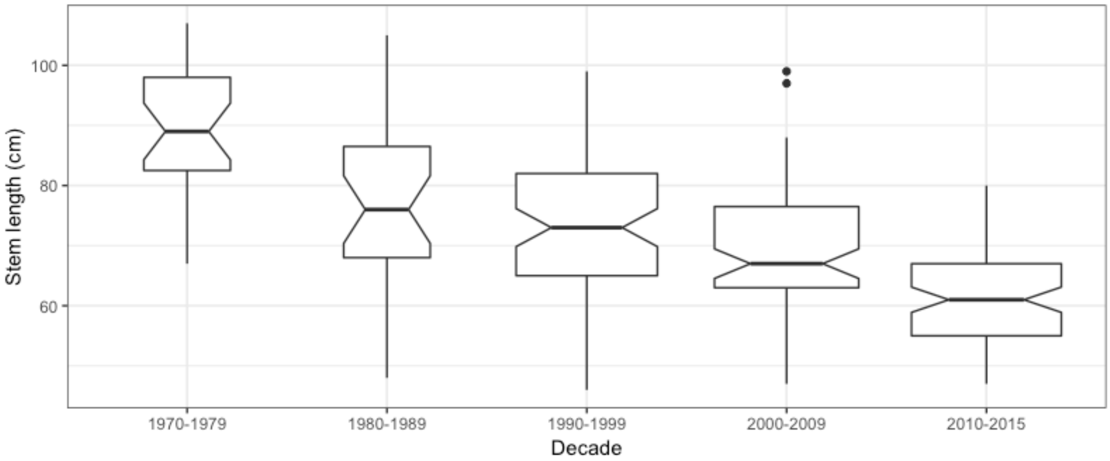
where n_q is the number of genotypes in cluster q, N the total number of genotypes, s the global standard deviation. The value of u is then confronted to the corresponding quantile of the normal distribution; therefore, an absolute value higher than 1.96 indicate $p < 0.05$ and then a discriminating variable to describe the cluster; the sign indicates the direction of the difference from the global mean (Lê et al., 2008). For qualitative variables the objective was to identify the sub-populations (defined by the category levels) being over- or under-represented within the clusters. A χ^2 test was at first performed between each categorical variable and the cluster variable. For the significant ones, the frequency N_{qj} (number of individuals of the group q in the category level j) was distributed as an hypergeometric distribution with the parameters $N, n_j, \frac{n_q}{N}$ (where n_j is the number of individuals that have taken the category j) and a p-value was calculated. The p-value was then transformed to the correspondent value in quantile of the Gaussian distribution. Positive and negative signs indicate an over- or under-representation respectively of the category to which it referred within the examined cluster. These analyses were performed using *catdes()* function of *FactoMineR* R package[30]. All the data analyses were performed under R 3.2.3 environment (R Core Team, 2017).

2.6 SUPPLEMENTARY MATERIAL

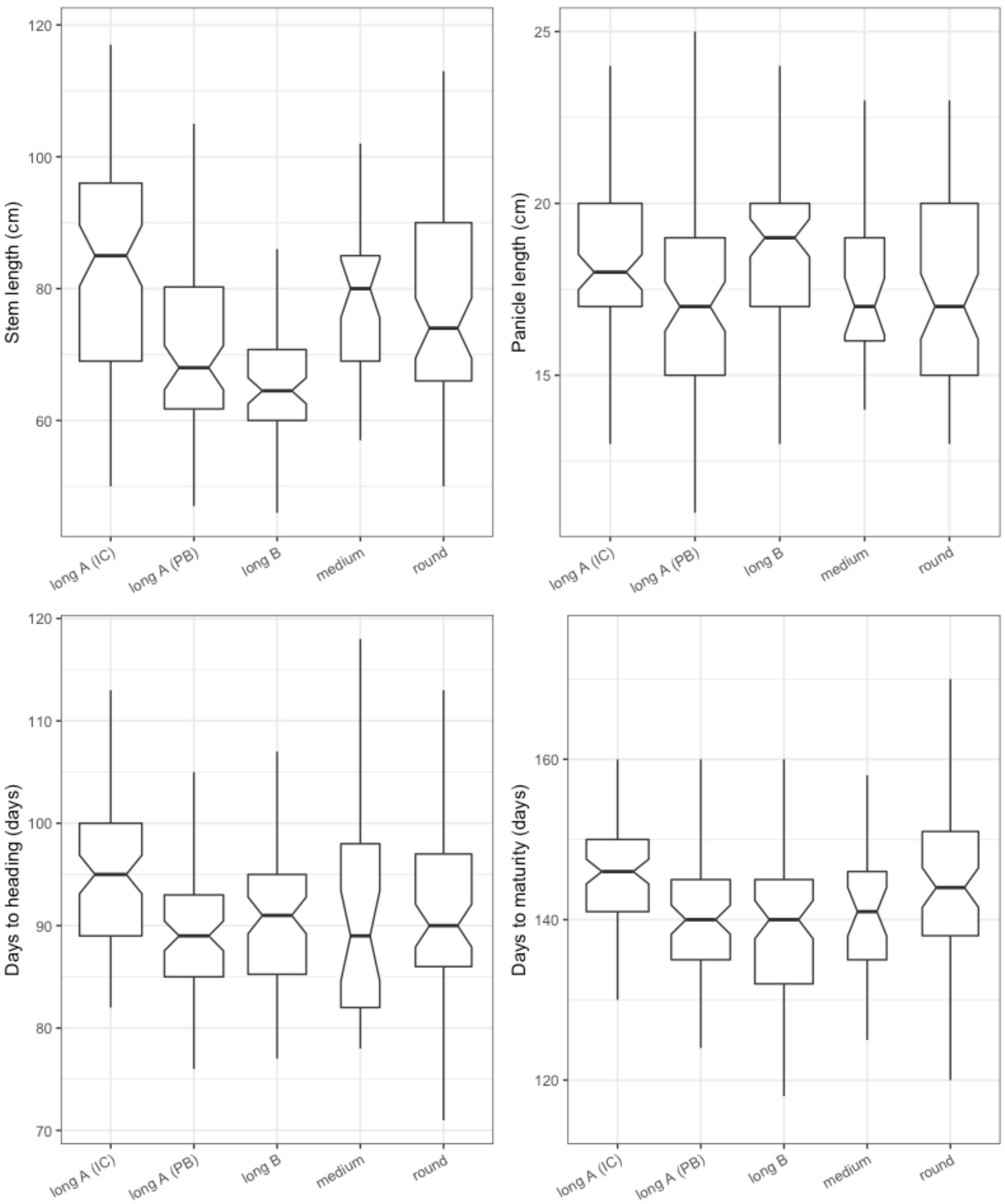
2.6.1 UNIVARIATE ANALYSIS



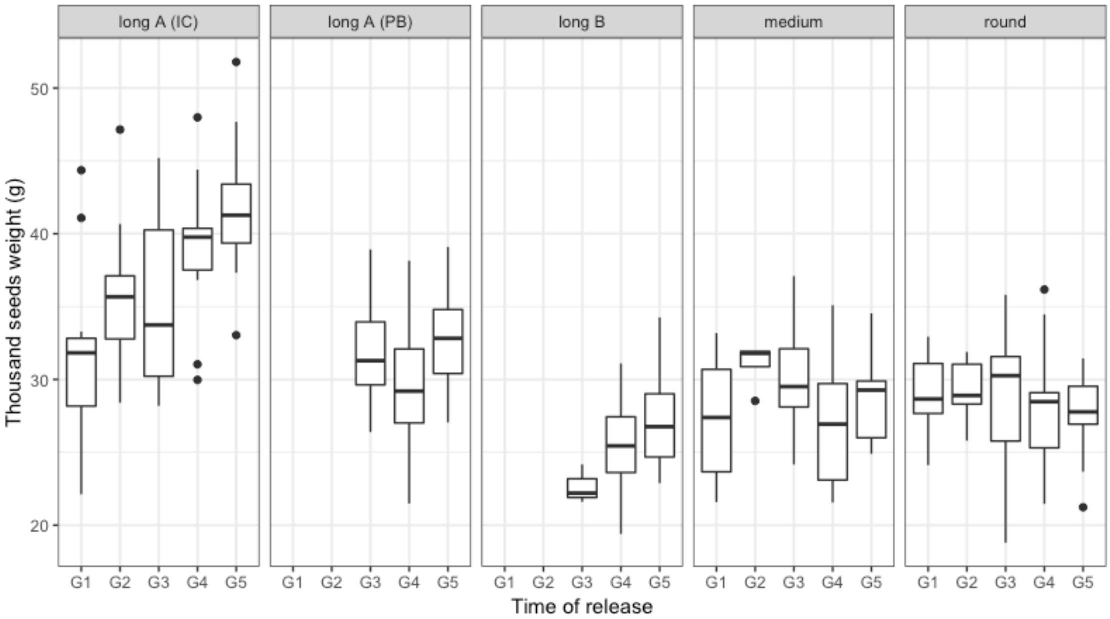
Supplementary Figure 2.1 – Frequencies of the supplementary categorical variables: grain shape, phylogenetic subgroup, and flag leaf attitude calculated for the whole dataset and grouped by time of release groups G1 (1850 - 1927), G2 (1928 - 1962), G3 (1963 - 1990), G4 (1991 - 2004), and G5 (2005 - 2016).



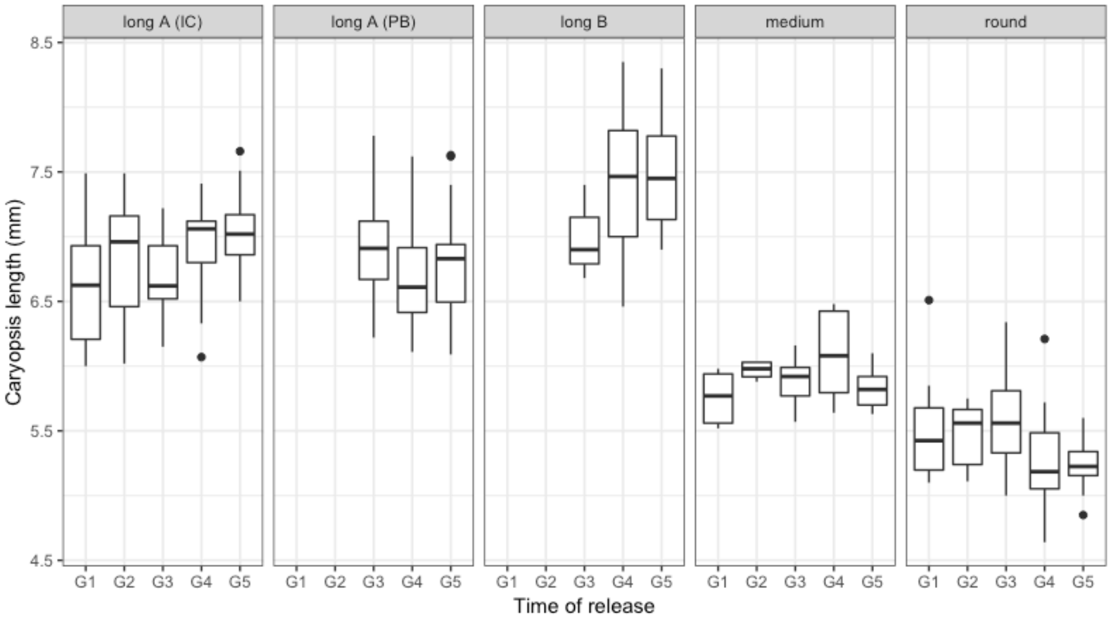
Supplementary Figure 2.2 – Stem length data distribution by decades, starting after the institution of National Register. The width of the boxes provides a visual cue for group size; the notches indicate the 95% confidence interval around the median



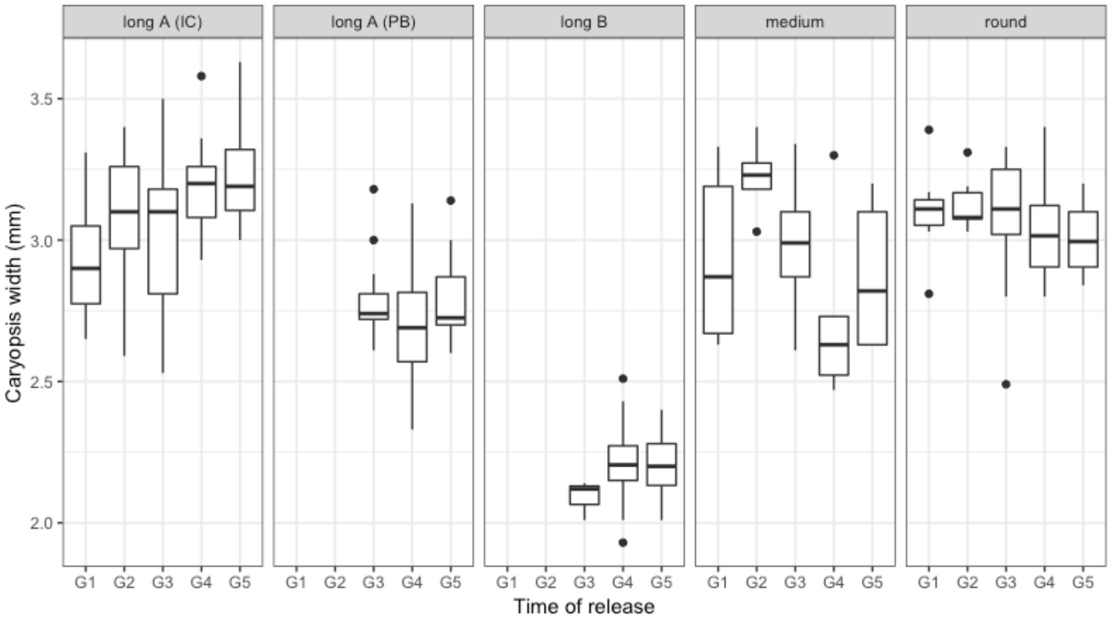
Supplementary Figure 2.3 – Box plots for traits 'stem length', 'panicle length', 'days to heading', and 'days to maturity', divided by grain shape-based groups of varieties. The notches indicate the 95% confidence interval around the median



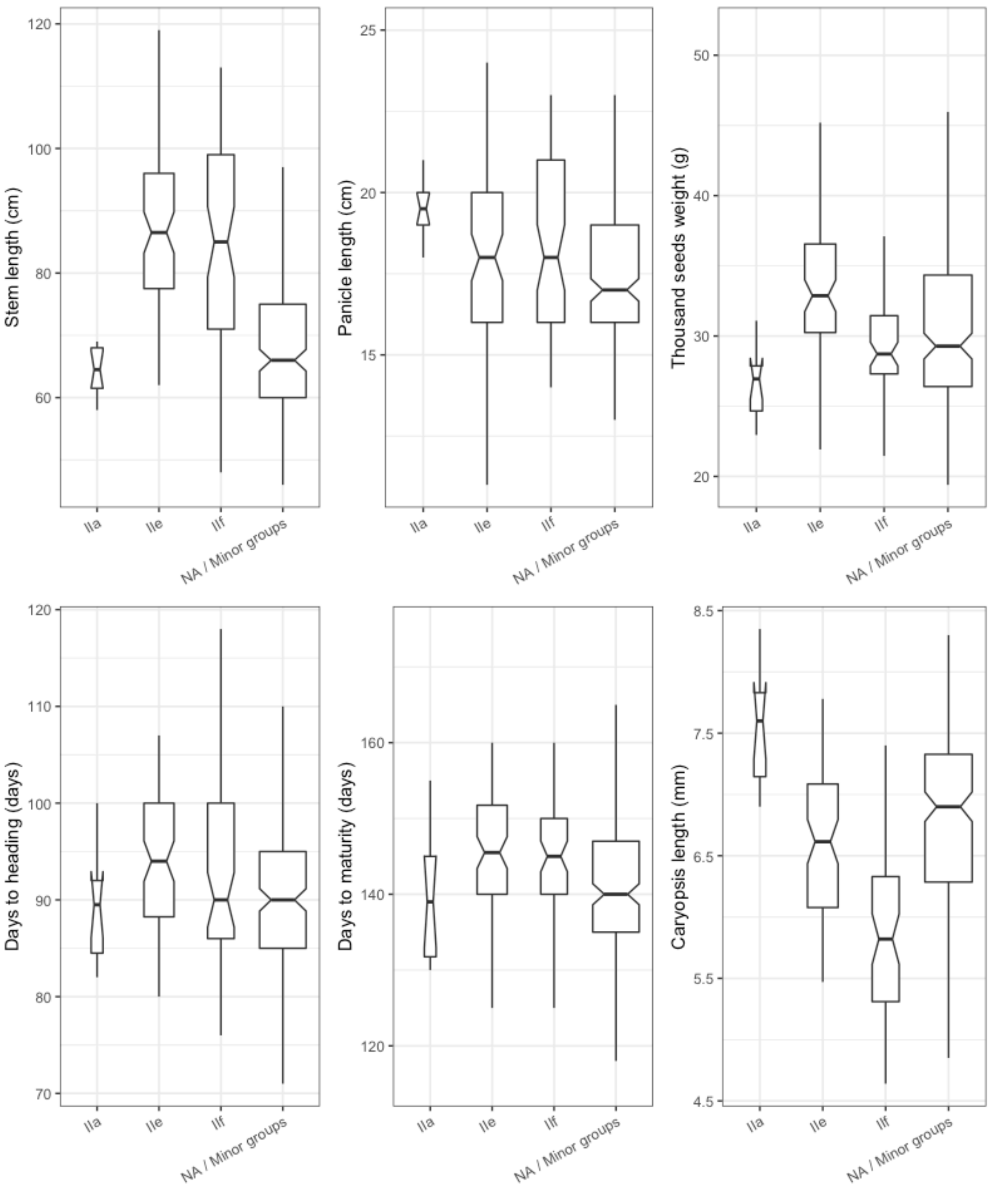
Supplementary Figure 2.4 – Box plots for trait 'thousand seeds weight', divided by time of release; each panel correspond to one category of grain shape.



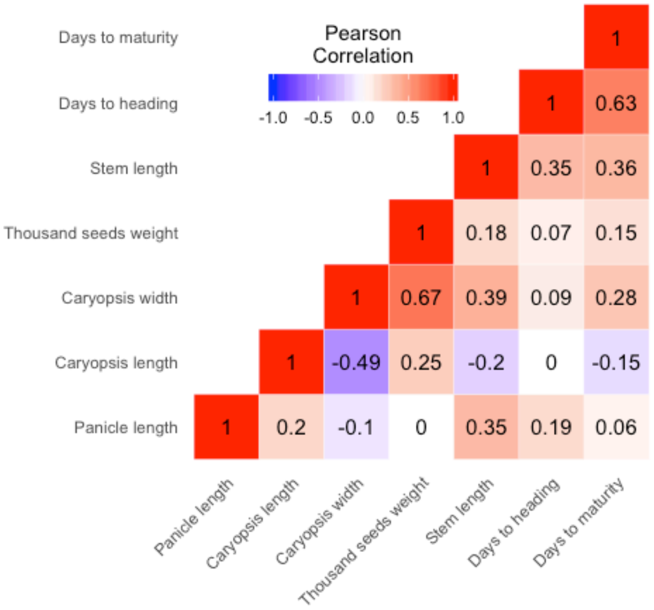
Supplementary Figure 2.5 – Box plots for trait 'caryopsis length', divided by time of release; each panel correspond to one category of grain shape



Supplementary Figure 2.6 – Box plots for trait 'caryopsis width', divided by time of release; each panel correspond to one category of grain shape



Supplementary Figure 2.7 – Box plots for traits 'stem length', 'panicle length', 'days to heading', 'days to maturity', 'thousand seeds weight', and 'caryopsis length' grouped by categorical variable phylogenetic subgroup, derived from Faivre-Rampant et al. (2011, see Methods section). The notch indicates the 95% confidence interval around the median. Varieties from minor groups 'I', 'IIb', 'IIc', and 'IId' were merged in category 'Not Available' (NA) due to their limited size.



Supplementary Figure 2.8 – Correlation matrix showing Pearson's correlation coefficients calculated between the seven considered traits. Cells are color coded to indicate the direction and the stregth of correlations.

2.6.2 PRINCIPAL COMPONENTS ANALYSIS

*Supplementary Table 2.1 – Coefficients of determination (R^2) and associated p-values resulting from the one-way ANOVA models constructed with Principal Components as the response variables and the four categorical variables (grain shape, time of release, phylogenetic subgroup, and flag leaf attitude) as explanatory variables ($\alpha = 0.05$). Significance codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

Variable	PC1	Sig.	PC2	Sig.	PC3	Sig.
Grain shape	0.509	* * *	0.411	* * *	0.437	* * *
Time of release	0.325	* * *	0.047	* *	0.061	* * *
Phylogenetic subgroup	0.266	* * *	0.061	* *	0.117	* * *
Flag leaf attitude	0.055	* * *	0.027	*	0.059	* * *

*Supplementary Table 2.2 – Ouput of the function dimdesc(). Coefficients estimates and associated p-values resulting from the one-way ANOVA models constructed with Principal Component 1 as the response variable and each of the four categorical variables (grain shape, time of release, phylogenetic subgroup, and flag leaf attitude) as explanatory variable ($\alpha = 0.05$). Coefficients are ordered by p-value, only significant were reported. Significance codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

Category	Estimate	Sig.
Grain shape=long A (IC)	1.277	* * *
Phylogenetic subgroup=IIe	1.438	* * *
Time of release=G2	1.182	* * *
Time of release=G1	0.844	* * *
Time of release=G3	0.271	* * *
Grain shape=round	0.663	* * *
Phylogenetic subgroup=IIf	1.114	* * *
Attitude of flag leaf=horizontal	0.364	* *
Attitude of flag leaf=erect	-0.784	* *
Grain shape=long A (PB)	-0.545	* * *
Phylogenetic subgroup=IIa	-1.299	* * *
Time of release=G4	-1.121	* * *
Time of release=G5	-1.176	* * *
Phylogenetic subgroup=na	-0.158	* * *
Grain shape=long B	-1.692	* * *

*Supplementary Table 2.3 – Ouput of the function dimdesc(). Coefficients estimates and associated p-values resulting from the one-way ANOVA models constructed with Principal Component 2 as the response variable and each of the four categorical variables (grain shape, time of release, phylogenetic subgroup, and flag leaf attitude) as explanatory variable ($\alpha = 0.05$). Coefficients are ordered by p-value, only significant were reported. Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.*

Category	Estimate	Sig.
Grain shape=long B	1.482	* * *
Phylogenetic subgroup=IIa	0.606	* * *
Time of release=G4	0.13	*
Time of release=G1	0.367	*
Attitude of flag leaf=recurved	0.305	*
Grain shape=medium	-0.498	* *
Time of release=G5	-0.38	* *
Grain shape=round	-0.77	* * *

*Supplementary Table 2.4 – Ouput of the function dimdesc(). Coefficients estimates and associated p-values resulting from the one-way ANOVA models constructed with Principal Component 2 as the response variable and each of the four categorical variables (grain shape, time of release, phylogenetic subgroup, and flag leaf attitude) as explanatory variable ($\alpha = 0.05$). Coefficients are ordered by p-value, only significant were reported. Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.*

Category	Estimate	Sig.
Grain shape=long A (IC)	1.058	* * *
Time of release=G5	0.431	* * *
Attitude of flag leaf=horizontal	0.249	* * *
Phylogenetic subgroup=na	0.688	* *
Phylogenetic subgroup=IIe	0.748	*
Time of release=G1	-0.411	*
Grain shape=medium	-0.535	* * *
Attitude of flag leaf=semi-erect	-0.284	* * *
Phylogenetic subgroup=IIf	-0.233	* * *
Grain shape=round	-1.069	* * *

2.6.3 CLUSTERING

Supplementary Table 2.5 – List of varieties included in this study, with indication of grain shape, time of release, phylogenetic subgroup according to Faivre-Rampant et al. (2011), flag leaf attitude, and cluster membership resulted from the Hierarchical Clustering on Principal Components (HCPC).

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Greppi	G1	medium	semi-erect	IId	1
Graldo	G3	long B	horizontal	na	1
Lido	G3	medium	semi-erect	IIe	1
Onda	G3	long A (PB)	horizontal	IIe	1
Panda	G3	long B	recurved	IIf	1
Tarriso	G3	long B	horizontal	na	1
Adelio	G4	long B	horizontal	na	1
Aiace	G4	long A (PB)	horizontal	IIa	1
Ambra	G4	round	semi-erect	IIf	1
Andolla	G4	long B	horizontal	na	1
Apollo	G4	long B	semi-erect	IIa	1
Armonia	G4	long B	erect	na	1
Artico	G4	long B	erect	na	1
Artiglio	G4	long B	semi-erect	na	1
Asia	G4	long B	semi-erect	IIa	1

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Cadet	G4	long B	horizontal	na	1
Condor	G4	long B	semi-erect	na	1
Creso	G4	long A (PB)	semi-erect	IIf	1
Dedalo	G4	long B	horizontal	na	1
Eolo	G4	long B	semi-erect	IIa	1
Fenis	G4	long B	horizontal	na	1
Fragrance	G4	long B	semi-erect	IIa	1
Gange	G4	long B	erect	IIa	1
Gemini	G4	long B	recurved	na	1
Ghibli	G4	long B	recurved	na	1
Giada	G4	long B	erect	na	1
Giano	G4	long B	horizontal	IIa	1
Giove	G4	long B	semi-erect	na	1
Gladio	G4	long B	semi-erect	IIa	1
Icaro	G4	long B	semi-erect	na	1
Idra	G4	long B	erect	na	1
Lamone	G4	long B	semi-erect	IIa	1
Lampo	G4	long A (PB)	erect	na	1
Mercurio	G4	long B	horizontal	na	1
Mida	G4	long B	semi-erect	na	1
Orta	G4	long B	horizontal	na	1
Perseo	G4	long B	recurved	na	1
Prezioso	G4	long B	erect	na	1
Santerno	G4	long B	erect	IIb	1
Saturno	G4	long B	erect	IIa	1
Sillaro	G4	long B	horizontal	na	1
SISR215	G4	long A (PB)	erect	IIa	1

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Sprint	G4	long B	semi-erect	na	1
Tanaro	G4	long B	semi-erect	na	1
Thaibonnet	G4	long B	semi-erect	IIa	1
Zena	G4	long B	semi-erect	IIc	1
Arsenal	G5	long B	horizontal	na	1
Artemide	G5	long B	semi-erect	na	1
Atlantis	G5	long B	semi-erect	na	1
Brezza	G5	long B	semi-erect	na	1
Centro	G5	long B	semi-erect	na	1
CL 46	G5	long B	erect	na	1
CL 80	G5	long B	semi-erect	na	1
CL111	G5	long B	semi-erect	na	1
CL26	G5	long B	horizontal	na	1
CL71	G5	long B	recurved	na	1
Corimbo	G5	long B	semi-erect	na	1
CRLB1	G5	long B	horizontal	na	1
Ecco 51 CL	G5	long B	erect	na	1
Ecco 61	G5	long B	erect	na	1
Ecco 63	G5	long B	semi-erect	na	1
Elettra	G5	long B	semi-erect	na	1
Ellebi	G5	long B	horizontal	na	1
Ermes	G5	long B	horizontal	na	1
Fast	G5	long B	semi-erect	na	1
Febo	G5	long B	semi-erect	na	1
Giglio	G5	long B	semi-erect	na	1
Iarim	G5	long B	erect	na	1
King	G5	long B	horizontal	na	1

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Libero	G5	long B	semi-erect	na	1
Mare Cl	G5	long B	semi-erect	na	1
Mirko	G5	long B	semi-erect	na	1
Ninfa	G5	long B	horizontal	na	1
Oceano	G5	long B	horizontal	na	1
Sagittario	G5	long B	semi-erect	na	1
Salvo	G5	long B	semi-erect	na	1
Scudo	G5	long B	semi-erect	na	1
Sirio Cl	G5	long B	horizontal	na	1
Teseo	G5	long B	semi-erect	na	1
Urano	G5	long B	horizontal	na	1
Bertone	G1	long A (IC)	recurved	IIe	2
Fulgente	G1	long A (IC)	horizontal	na	2
Maratelli	G1	medium	semi-erect	IIf	2
S.Rocco	G1	long A (IC)	horizontal	IIe	2
Sirion	G1	long A (IC)	recurved	na	2
Vialone 190	G1	round	recurved	IIe	2
Adelaide Chiappelli	G2	long A (IC)	recurved	IIe	2
Balilla	G2	round	semi-erect	IIf	2
Baraggia	G2	round	horizontal	IIe	2
Corbetta	G2	medium	horizontal	IIe	2
Novara	G2	medium	recurved	IIf	2
Precoce Corbetta	G2	medium	horizontal	na	2
Razza77	G2	long A (IC)	horizontal	IIf	2
Arborio Precoce	G3	long A (IC)	horizontal	IIe	2
Ariete	G3	long A (PB)	semi-erect	IIf	2
Auro	G3	round	horizontal	IIe	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Baldo	G3	long A (IC)	horizontal	IIe	2
Bali	G3	round	semi-erect	na	2
Castello	G3	round	semi-erect	IIf	2
Cervo	G3	long A (PB)	semi-erect	IIe	2
Cripto	G3	medium	semi-erect	IIf	2
Drago	G3	long A (PB)	horizontal	IIe	2
Elio	G3	round	semi-erect	IIf	2
Giovanni Marchetti	G3	medium	horizontal	IIe	2
Lomellino	G3	round	horizontal	IIe	2
Loto	G3	long A (PB)	horizontal	IIf	2
Medusa	G3	medium	horizontal	na	2
Molo	G3	long A (PB)	recurved	IIf	2
Nova	G3	medium	recurved	IIf	2
Piemonte	G3	round	semi-erect	IIe	2
Ringo	G3	long A (PB)	horizontal	IIe	2
Riva	G3	long A (PB)	semi-erect	IIe	2
Roma	G3	long A (IC)	recurved	IIe	2
Romeo	G3	round	semi-erect	IIe	2
Roncolo	G3	round	semi-erect	IIe	2
Rosa Marchetti	G3	medium	horizontal	IIe	2
Selenio	G3	round	semi-erect	IIf	2
Sesila	G3	medium	horizontal	IIf	2
Smeraldo	G3	long A (PB)	horizontal	IIe	2
Titanio	G3	round	horizontal	IIe	2
Volano	G3	long A (IC)	horizontal	IIe	2
Albatros	G4	long B	erect	IIe	2
Alice	G4	long A (PB)	horizontal	IIe	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Alpe	G4	long A (PB)	semi-erect	IIe	2
Ares	G4	long A (PB)	horizontal	na	2
Arona	G4	long A (PB)	horizontal	na	2
Astro	G4	long A (PB)	horizontal	na	2
Augusto	G4	long A (PB)	semi-erect	IIe	2
Bastia	G4	round	semi-erect	na	2
Bianca	G4	long A (IC)	erect	IIe	2
Bravo	G4	long A (PB)	semi-erect	na	2
Castelmochi	G4	round	horizontal	IIf	2
Centauro	G4	round	horizontal	IIf	2
Chimera	G4	round	semi-erect	na	2
Cobra	G4	long A (PB)	semi-erect	na	2
CRT2	G4	round	semi-erect	na	2
Delfino	G4	long A (PB)	horizontal	IIf	2
Dorella	G4	long A (PB)	horizontal	na	2
Ebro	G4	long A (PB)	horizontal	na	2
Elvo	G4	round	semi-erect	na	2
Eurosis	G4	long A (PB)	semi-erect	IIc	2
Flipper	G4	long A (PB)	semi-erect	IIf	2
Galileo	G4	long A (IC)	semi-erect	IIe	2
Garda	G4	long A (IC)	horizontal	na	2
Genio	G4	long A (IC)	horizontal	na	2
Gigante	G4	long A (IC)	horizontal	na	2
Italmochi	G4	medium	horizontal	na	2
Koala	G4	long A (PB)	erect	na	2
Marte	G4	round	semi-erect	IIf	2
Minerva	G4	medium	semi-erect	IIf	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Nuovo Maratelli	G4	medium	horizontal	IIe	2
Pegaso	G4	long B	horizontal	na	2
Perla	G4	round	semi-erect	IIf	2
Pierrot	G4	round	semi-erect	IIf	2
Pony	G4	long A (PB)	semi-erect	na	2
Poseidone	G4	long A (IC)	horizontal	na	2
Primo	G4	long A (PB)	erect	na	2
Rodeo	G4	long A (PB)	horizontal	IIf	2
Romolo	G4	long A (IC)	horizontal	na	2
Sara	G4	medium	semi-erect	IIe	2
Savio	G4	medium	semi-erect	IIe	2
Scirocco	G4	long A (PB)	semi-erect	IIf	2
Sereno	G4	round	horizontal	na	2
Silla	G4	long A (PB)	recurved	IIe	2
Sirmione	G4	long A (PB)	horizontal	na	2
Spina	G4	round	semi-erect	na	2
Stresa	G4	round	erect	na	2
Tea	G4	long A (PB)	horizontal	na	2
Top	G4	round	erect	na	2
Vega	G4	round	semi-erect	na	2
Venere	G4	medium	horizontal	IIe	2
Agata	G5	round	erect	na	2
Allegro	G5	long A (IC)	horizontal	na	2
Antares	G5	long A (PB)	horizontal	na	2
Arpa	G5	round	semi-erect	IIf	2
Bacco	G5	long A (IC)	horizontal	na	2
Barone CL	G5	long A (IC)	semi-erect	na	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Brio	G5	round	semi-erect	na	2
BS1	G5	long A (IC)	horizontal	na	2
Calipso	G5	long A (PB)	semi-erect	na	2
Carmen	G5	long A (PB)	horizontal	na	2
Carnise Precoce	G5	long A (IC)	horizontal	na	2
Casanova	G5	long A (IC)	semi-erect	na	2
Castore	G5	round	semi-erect	na	2
Cerere	G5	round	semi-erect	na	2
CL 12	G5	round	horizontal	na	2
CL15	G5	round	semi-erect	na	2
CL31	G5	long A (PB)	semi-erect	na	2
Crono	G5	medium	semi-erect	na	2
CRW3	G5	round	semi-erect	na	2
Dante	G5	long A (PB)	semi-erect	na	2
Dardo	G5	long A (PB)	semi-erect	na	2
Deneb	G5	long A (PB)	semi-erect	na	2
Ducato	G5	round	semi-erect	na	2
Eridano	G5	round	semi-erect	na	2
Falco	G5	long A (IC)	horizontal	na	2
Fedra	G5	long A (IC)	horizontal	na	2
Fenice	G5	long A (PB)	semi-erect	na	2
Festa	G5	long A (IC)	semi-erect	na	2
Furia CL	G5	medium	horizontal	na	2
Galassia	G5	long A (IC)	horizontal	na	2
Generale	G5	long A (IC)	semi-erect	na	2
Ghiaccio	G5	long A (PB)	semi-erect	na	2
Gloria	G5	long A (IC)	horizontal	na	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Lagostino	G5	round	semi-erect	na	2
Libra	G5	long A (PB)	horizontal	na	2
Lince	G5	long A (PB)	horizontal	na	2
LT 155	G5	long A (IC)	semi-erect	na	2
Luna Cl	G5	long A (PB)	semi-erect	na	2
Luxor	G5	long A (IC)	semi-erect	na	2
Meco	G5	long A (PB)	semi-erect	na	2
Medea	G5	medium	horizontal	na	2
Megumi	G5	round	semi-erect	na	2
Musa	G5	long A (PB)	horizontal	na	2
Nerone	G5	long A (PB)	horizontal	na	2
Neve	G5	long A (IC)	horizontal	na	2
Onice	G5	long A (PB)	semi-erect	na	2
Opale	G5	long A (PB)	semi-erect	na	2
Orione	G5	medium	semi-erect	IIe	2
Pato	G5	long A (IC)	semi-erect	na	2
Presto	G5	long A (PB)	semi-erect	na	2
Proteo	G5	long A (IC)	horizontal	na	2
Puma	G5	long A (PB)	horizontal	na	2
Reperso	G5	long A (IC)	semi-erect	na	2
RG200	G5	long A (PB)	erect	na	2
Ribaldo	G5	long A (PB)	horizontal	na	2
Risrus	G5	long A (PB)	semi-erect	na	2
Rombo	G5	long A (PB)	erect	na	2
Ronaldo	G5	long A (PB)	semi-erect	na	2
Samba	G5	long A (IC)	semi-erect	na	2
Sfera	G5	round	erect	na	2

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Sole Cl	G5	round	semi-erect	na	2
Sp55	G5	round	semi-erect	na	2
Telemaco	G5	long A (IC)	semi-erect	na	2
Terra CL	G5	round	semi-erect	na	2
Teti	G5	long A (PB)	semi-erect	na	2
Tosca	G5	long A (IC)	horizontal	na	2
Ulisse	G5	long A (IC)	semi-erect	na	2
Unico	G5	long A (PB)	erect	na	2
Vasco	G5	long A (PB)	erect	na	2
Virgo	G5	round	erect	na	2
Vulcano	G5	long A (IC)	semi-erect	na	2
Wang	G5	medium	erect	na	2
Yume	G5	round	semi-erect	na	2
Agostano	G1	round	horizontal	IIf	3
Airone	G1	long A (IC)	horizontal	IIf	3
Allorio	G1	long A (IC)	semi-erect	IIf	3
Americano 1600	G1	round	horizontal	IIf	3
Ardizzone	G1	long A (IC)	horizontal	IIf	3
Feronio	G1	round	recurved	IIf	3
Greggio	G1	long A (IC)	horizontal	IIf	3
Ice	G1	long A (IC)	horizontal	IIe	3
Italico	G1	medium	horizontal	IIf	3
Italico Livorno	G1	long A (IC)	horizontal	IIe	3
Lencino	G1	round	semi-erect	IIf	3
Lucero	G1	round	semi-erect	IIf	3
Originario	G1	round	horizontal	IIf	3
Orione (historic)	G1	medium	semi-erect	na	3

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Ostiglia	G1	round	semi-erect	IIf	3
Raffaello	G1	medium	horizontal	IIf	3
Ranghino	G1	round	horizontal	IIf	3
Romanico	G1	long A (IC)	horizontal	IIf	3
Roncarolo	G1	round	horizontal	IIe	3
Sancio P6	G1	long A (IC)	horizontal	IIf	3
Vialone Nero	G1	medium	horizontal	IIe	3
Arborio	G2	long A (IC)	semi-erect	IIe	3
Balilla Gg	G2	round	semi-erect	IIf	3
Balocco	G2	round	semi-erect	IIf	3
Balzaretti	G2	long A (IC)	horizontal	IIf	3
Bellardone	G2	round	horizontal	IIf	3
Benito	G2	round	horizontal	IIf	3
Carnaroli	G2	long A (IC)	horizontal	IIe	3
Ferraris	G2	round	horizontal	na	3
Gigante Vercelli	G2	long A (IC)	horizontal	IIe	3
La Ferla	G2	long A (IC)	horizontal	IIe	3
Lomello	G2	long A (IC)	horizontal	IIf	3
Mantova	G2	long A (IC)	horizontal	IIe	3
Olcenengo	G2	long A (IC)	recurved	IIe	3
Oldenico	G2	long A (IC)	recurved	IIe	3
Precoce Monticelli	G2	round	recurved	IIf	3
Ribe	G2	long A (IC)	horizontal	IIe	3
Rinaldo Bersani	G2	long A (IC)	horizontal	IIe	3
Rizzotto	G2	long A (IC)	horizontal	IIf	3
Senatore Novelli	G2	long A (IC)	horizontal	IIe	3
Trionfo Fassone	G2	long A (IC)	semi-erect	IIf	3

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Vialone Nano	G2	medium	recurved	IIe	3
Anseatico	G3	long A (IC)	recurved	IIe	3
Argo	G3	medium	semi-erect	IIe	3
Bonni	G3	long A (PB)	recurved	IIe	3
Europa	G3	long A (PB)	semi-erect	IIe	3
Faro	G3	long A (IC)	horizontal	IIe	3
Gritna	G3	long A (PB)	recurved	IIe	3
Italico Roncarolo	G3	round	horizontal	IIe	3
Italpatna	G3	long A (PB)	horizontal	IIf	3
Koral	G3	long A (PB)	horizontal	IIe	3
Lieto	G3	long A (IC)	horizontal	IIe	3
Molinella	G3	long A (IC)	horizontal	IIe	3
Monticelli	G3	round	horizontal	IIf	3
Navile	G3	medium	horizontal	IIf	3
Neretto	G3	round	horizontal	IIe	3
Nero	G3	long A (IC)	horizontal	IIe	3
Padano	G3	medium	horizontal	IIf	3
Prometeo	G3	medium	recurved	IIf	3
Radon	G3	long A (IC)	semi-erect	IIe	3
Redi	G3	long A (IC)	horizontal	IIe	3
Rialto	G3	long A (PB)	horizontal	IIe	3
Ribello	G3	long A (IC)	horizontal	IIe	3
Rocca	G3	long A (IC)	horizontal	IIe	3
Rodio	G3	long A (PB)	horizontal	IIe	3
Rubino	G3	medium	semi-erect	IIe	3
S.Andrea	G3	long A (IC)	recurved	IIf	3
San Petronio	G3	round	semi-erect	IIf	3

Variety	Time of release	Grain shape	Attitude of Flag leaf	Phylogenetic subgroup	Cluster
Sorriso	G3	round	horizontal	IIe	3
Strella	G3	long A (PB)	horizontal	IIe	3
Torio	G3	long A (IC)	semi-erect	na	3
Veneria	G3	long A (IC)	horizontal	IIe	3
Vitro	G3	long A (IC)	semi-erect	IIf	3
Arco	G4	long A (PB)	recurved	IIe	3
Asso	G4	long A (PB)	horizontal	na	3
Cesare	G4	long A (PB)	horizontal	na	3
Cistella	G4	long A (IC)	horizontal	na	3
Elba	G4	long A (IC)	horizontal	na	3
Ibis	G4	round	semi-erect	na	3
Karnak	G4	long A (IC)	semi-erect	IIe	3
Nebbione	G4	long A (IC)	horizontal	na	3
Nembo	G4	long A (PB)	semi-erect	IIf	3
Otello	G4	long A (IC)	erect	na	3
Rova	G4	long A (IC)	horizontal	IIe	3
S.Pietro	G4	round	semi-erect	IIe	3
Sesiamochi	G4	round	semi-erect	na	3
Tejo	G4	long A (PB)	erect	I	3
Zeus	G4	long A (PB)	horizontal	na	3
Cammeo	G5	long A (IC)	horizontal	na	3
Carnaval	G5	long A (IC)	semi-erect	na	3
Carnise	G5	long A (IC)	semi-erect	na	3
Ercole	G5	long A (PB)	horizontal	na	3
Leonida CL	G5	long A (IC)	semi-erect	na	3

DESCRIPTION OF CLUSTERS BY QUANTITATIVE VARIABLES

Supplementary Table 2.6 – η^2 *values calculated for each trait, indicating the amount of explained variance between clusters.*

	η^2	*p*-value
Caryopsis width	0.6572	2.265e-78
Stem length	0.4882	2.652e-49
Days to heading	0.3669	6.944e-34
Caryopsis length	0.2903	1.338e-25
Panicle length	0.2621	9.03e-23
Thousand seeds weight (g)	0.2236	4.39e-19
Days to maturity	0.1997	7.021e-17

Supplementary Table 2.7 – v-test results for Cluster A; only significant results are showed. A positive or negative test statistic indicates a Cluster mean significantly higher or lower, respectively, than the overall mean. Cluster and Overall mean and standard deviation are also reported. Variables are ordered by value of v-test.

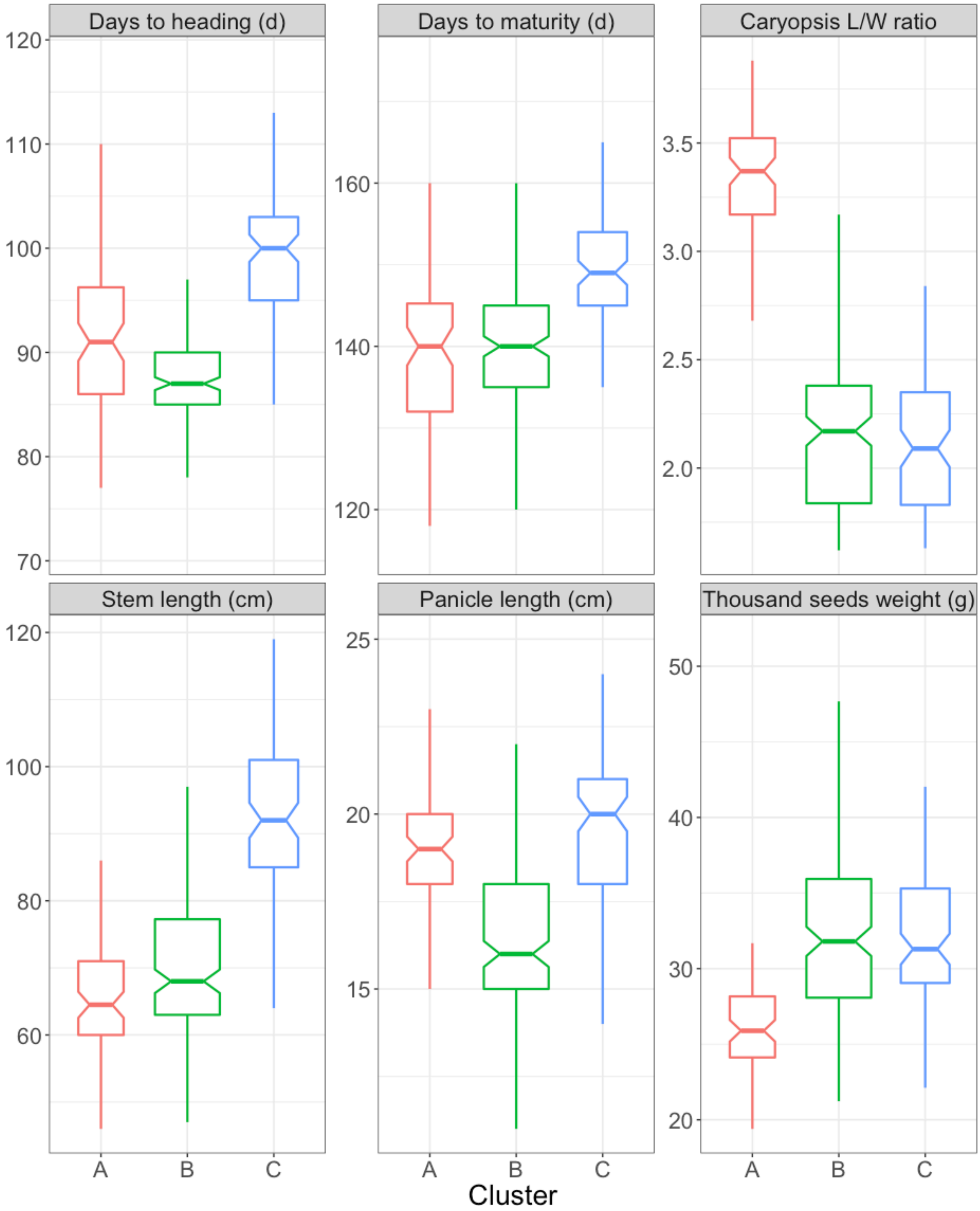
Variable	v-test	*p*	Cluster mean	Global mean	Cluster SD	Global SD
Caryopsis length (mm)	9.877	5.253e-23	7.369	6.581	0.5561	0.8156
Panicle (cm)	4.051	5.094e-05	18.94	17.89	2.094	2.636
Days to maturity (days)	-3.488	0.000487	139.6	142.7	8.926	9.079
Stem length (cm)	-6.49	8.591e-11	65.4	75.27	9.02	15.55
Thousand seeds weight (g)	-8.658	4.785e-18	26.06	30.98	2.839	5.818
Caryopsis width (mm)	-14.83	1.009e-49	2.24	2.81	0.166	0.3932

Supplementary Table 2.8 – v-test results for Cluster B; only significant results are showed. A positive or negative test statistic indicates a Cluster mean significantly higher or lower, respectively, than the overall mean. Cluster and Overall mean and standard deviation are also reported. Variables are ordered by value of v-test.

Variable	v-test	p	Cluster mean	Global mean	Cluster SD	Global SD
Caryopsis width (mm)	7.201	5.987e-13	2.969	2.81	0.2633	0.3932
Thousand seeds weight (g)	5.048	4.46e-07	32.63	30.98	6.256	5.818
Days to maturity (days)	-4.328	1.508e-05	140.5	142.7	8.1	9.079
Caryopsis length (mm)	-5.335	9.534e-08	6.337	6.581	0.7578	0.8156
Stem length (cm)	-5.745	9.213e-09	70.26	75.27	11.47	15.55
Panicle (cm)	-9.308	1.307e-20	16.52	17.89	2.32	2.636
Days to heading (days)	-9.53	1.569e-21	87.71	91.85	5.468	7.761

Supplementary Table 2.9 - v-test results for Cluster C; only significant results are showed. A positive or negative test statistic indicates a Cluster mean significantly higher or lower, respectively, than the overall mean. Cluster and Overall mean and standard deviation are also reported. Variables are ordered by value of v-test.

Variable	v-test	p	Cluster mean	Global mean	Cluster SD	Global SD
Stem length (cm)	12.6	2.086e-36	92.58	75.27	12.1	15.55
Days to heading (days)	10.28	8.478e-25	98.9	91.85	6.484	7.761
Days to maturity (days)	8.159	3.383e-16	149.2	142.7	7.403	9.079
Panicle (cm)	6.551	5.703e-11	19.42	17.89	2.306	2.636
Caryopsis width (mm)	6.06	1.363e-09	3.021	2.81	0.2148	0.3932
Thousand seeds weight (g)	2.597	0.009408	32.32	30.98	4.39	5.818
Caryopsis length (mm)	-3.435	0.0005917	6.334	6.581	0.657	0.8156



Supplementary Figure 2.9 – Boxplot showing the distributions for the 7 quantitative variables within each of the extracted clusters. Caryopsis length and width were summarized using their ratio.

DESCRIPTION OF CLUSTERS BY CATEGORICAL VARIABLES

Supplementary Table 2.10 – Results of the χ^2 tests performed between the supplementary categorical variables and the extracted clusters. Variables are ordered by their p-value.

	χ^2	Df	*p*-value
Grain shape	307.2	8	1.192e-61
Time of release	127.7	8	8.461e-24
Phylogenetic subgroup	140.4	14	5.977e-23
Flag leaf attitude	33.18	6	9.701e-06

Supplementary Table 2.11 – Output of the catdes() function, used to characterize the extracted clusters. 'Cla/Mod' is the percentage of all individuals belonging to the category indicated by row name included in Cluster A; 'Mod/Cla' is the percentage of all the individuals in Cluster A that express the category indicate by row name; 'Global' is the percentage of all individuals in the dataset that belong to the category indicated by row name; 'v.test' indicates the quantile of the normal distribution and its associated 'p-value'. Categories are ordered by v.test value and only significant categories are showed.

	Cla/Mod	Mod/Cla	Global	p.value	v.test
Grain.shape=long B	97.3	90	21.96	1.662e-61	16.55
Phylogenetic.subgroup=IIa	100	15	3.56	1.64e-08	5.65
Phylogenetic.subgroup=na	33.15	75	53.71	9.729e-06	4.42
Time.of.release=G4	38.1	50	31.16	5.382e-05	4.04
Attitude.of.flag.leaf=erect	46.67	17.5	8.9	0.004259	2.86
Time.of.release=G5	30.36	42.5	33.23	0.04792	1.98
Grain.shape=medium	6.06	2.5	9.79	0.007022	-2.7
Attitude.of.flag.leaf=horizontal	16.08	28.75	42.43	0.004373	-2.85
Time.of.release=G1	3.57	1.25	8.31	0.004095	-2.87
Time.of.release=G3	7.81	6.25	18.99	0.00038	-3.55
Time.of.release=G2	0	0	8.31	0.0003488	-3.58
Phylogenetic.subgroup=IIf	4.92	3.75	18.1	2.708e-05	-4.2
Grain.shape=long A (PB)	6.58	6.25	22.55	1.625e-05	-4.31
Grain.shape=round	1.45	1.25	20.47	1.788e-08	-5.63
Phylogenetic.subgroup=IIe	2.56	2.5	23.15	1.453e-08	-5.67
Grain.shape=long A (IC)	0	0	25.22	1.613e-12	-7.06

Supplementary Table 2.12 – Output of the catdes() function, used to characterize the extracted clusters. 'Cla/Mod' is the percentage of all individuals belonging to the category indicated by row name included in Cluster B; 'Mod/Cla' is the percentage of all the individuals in Cluster B that express the category indicate by row name; 'Global' is the percentage of all individuals in the dataset that belong to the category indicated by row name; 'v.test' indicates the quantile of the normal distribution and its associated 'p-value'. Categories are ordered by v.test value and only significant categories are showed.

	Cla/Mod	Mod/Cla	Global	p.value	v.test
Grain.shape=long A (PB)	73.68	34.15	22.55	6.203e-07	4.98
Time.of.release=G5	65.18	44.51	33.23	1.928e-05	4.27
Phylogenetic.subgroup=na	57.46	63.41	53.71	0.0005222	3.47
Grain.shape=round	65.22	27.44	20.47	0.00213	3.07
Attitude.of.flag.leaf=semi-erect	55.4	46.95	41.25	0.03939	2.06
Time.of.release=G2	25	4.27	8.31	0.008969	-2.61
Time.of.release=G1	21.43	3.66	8.31	0.002455	-3.03
Phylogenetic.subgroup=IIa	0	0	3.56	0.0002764	-3.64
Grain.shape=long B	2.7	1.22	21.96	1.539e-22	-9.77

Supplementary Table 2.13 – Output of the catdes() function, used to characterize the extracted clusters. 'Cla/Mod' is the percentage of all individuals belonging to the category indicated by row name included in Cluster C; 'Mod/Cla' is the percentage of all the individuals in Cluster C that express the category indicate by row name; 'Global' is the percentage of all individuals in the dataset that belong to the category indicated by row name; 'v.test' indicates the quantile of the normal distribution and its associated 'p-value'. Categories are ordered by v.test value and only significant categories are showed.

	Cla/Mod	Mod/Cla	Global	p.value	v.test
Grain.shape=long A (IC)	52.94	48.39	25.22	6.686e-09	5.8
Time.of.release=G2	75	22.58	8.31	6.81e-08	5.4
Time.of.release=G1	75	22.58	8.31	6.81e-08	5.4
Phylogenetic.subgroup=IIe	52.56	44.09	23.15	7.47e-08	5.38
Phylogenetic.subgroup=IIf	55.74	36.56	18.1	2.747e-07	5.14
Attitude.of.flag.leaf=horizontal	39.16	60.22	42.43	5.424e-05	4.04
Time.of.release=G3	48.44	33.33	18.99	7.695e-05	3.95
Phylogenetic.subgroup=IIa	0	0	3.56	0.01922	-2.34
Attitude.of.flag.leaf=erect	6.67	2.15	8.9	0.003952	-2.88
Attitude.of.flag.leaf=semi-erect	17.27	25.81	41.25	0.0003309	-3.59
Time.of.release=G4	14.29	16.13	31.16	0.0001556	-3.78
Grain.shape=long B	0	0	21.96	1.02e-12	-7.13
Time.of.release=G5	4.46	5.38	33.23	2.599e-13	-7.31
Phylogenetic.subgroup=na	9.39	18.28	53.71	2.647e-16	-8.19

CHAPTER 3 PHENOTYPIC CHARACTERISATION OF THE MAIN SOURCES OF VARIATION IN ITALIAN RICE CULTIVARS.

GABRIELE MONGIANO, PATRIZIA TITONE, SIMONE PAGNONCELLI, DAVIDE SACCO, LUIGI TAMBORINI, ROBERTO PILU, SIMONE BREGAGLIO.

3.1 ABSTRACT

3.1.1 BACKGROUND

Plant breeding is amongst the most promising technologies to further enhance crop yields. Efficient breeding strategies involve the phenotypic characterisation of the available germplasm, which is often impeded by the lack of extensive field experimental datasets.

3.1.2 FINDINGS

We characterised the phenotypic expression of fourteen yield components traits in a panel of 40 cultivars selected to maximize the phenotypic diversity in the Italian rice germplasm via a two-season field experiment. The measured range of phenotypic variation was high and multiple strong associations emerged in all the yield component traits analysed.

3.1.3 CONCLUSIONS

Our findings confirmed that a proper balance between sink and source organs favours grain production, as well as the extended duration of vegetative and reproductive phases. Aboveground biomass accumulation before flowering also positively correlated with yield. The released dataset represents the phenotypic variability in Italian rice cultivars and could be used to implement crop modelling applications in the area.

3.2 BACKGROUND

After the tremendous increases reached during the green revolution, the yields of the primary cereal crops - rice, wheat and maize - are now stagnating (Brisson et al., 2010; Lobell, Schlenker, & Costa-Roberts, 2011; Van Wart et al., 2013). New strategies are needed to breeding programs towards enhanced crop productivity, especially in light of climate change projections that will threaten food security while hampering the environmental sustainability of cropping systems (Bocchiola, 2015; Diffenbaugh & Giorgi, 2012). The effects of climate change on agricultural production are still uncertain, despite there is wide agreement on the need of designing mitigation and adaptation strategies to tackle the future challenges of global agriculture (Asseng et al., 2013; Bregaglio, Hossard, et al., 2017a; Confalonieri, Bregaglio, & Acutis, 2016). Among the available technologies, plant breeding is considered as the most promising for the purpose (Slafer, Araus, Royo, & Garcìa Del Moral, 2005). Plant breeding is indeed an expensive, time consuming and labour-intensive activity (Acquaah, 2012), and its effectiveness is limited by different causes like, e.g. narrowed genetic diversity or poor adaptability of genotypes across environments. Scientists are thus developing new techniques to speed up and support standard breeding programs like genetic editing (Song et al., 2016), crop simulation modelling (Tardieu, 2003), and ideotype breeding (Martre et al., 2015). All these techniques rely on a thorough knowledge of the available genetic variability and of the relationships between the crop traits of interest.

Particularly, crop simulation modelling aimed at defining and testing new breeding strategies (Khush, 1995) should account for the known physiological limits and for the correlations and compensatory effects among traits (Picheny et al., 2017). The high cost connected to the characterisation of available germplasm often impedes the availability of exhaustive data to support breeding programs via crop modelling (Nwachukwu et al., 2016). These considerations apply to rice agriculture in Italy where the genetic diversity is significantly narrowing in the last 20 years, despite the long history of cultivation and the vast biodiversity of rice genotypes (Mongiano, Titone, Tamborini, Pilu, & Bregaglio, 2018). Rice sale price has fallen considerably since the 90s due to the competition of developing countries (Ferrero, 2007). Moreover, the EU Common Agricultural Policy endorsed a gradual reduction of the subsidies granted to rice growers aiming at equable income support to farmers, and decoupled from the cultivated species (European Parliament, 2010). Rice cultivation is also in the spotlight for ecological issues like groundwater pollution, high greenhouse gases (mostly methane) emissions, and land degradation (Blengini & Busto, 2009), although providing socio-economic benefits like water catchment (e.g. used as floodplains areas) and creation of favourable habitats for water birds in lowland areas like the Italian Po valley (Fasola, Canova, & Saino, 1996; Longoni, 2010). Apart from the ecological benefits (achievable with different strategies), the environmental costs of rice cultivation and the occupation of primary agricultural land must be at least justified by the economic return of the crop, while actively researching for reducing its impacts (Dingkuhn et al., 2015).

We analysed here the most important agronomic traits of 40 rice cultivars, selected to maximize the variance found in the Italian varietal landscape. The aims of this study were a) to broaden the knowledge of yield components in Italian rice cultivars b) to investigate the between- traits relationships, and c) to highlight pattern of similarities.

3.3 FINDINGS

The 40 rice cultivars were chosen from a collection of 351 Italian cultivars (Mongiano et al., 2018), which were characterised for seven key morphological traits – i.e., vegetative and reproductive stages duration, culm and panicle length, caryopses length, width, and weight - via Kennard-Stone algorithm (Kennard & Stone, 1969) to obtain a sub-sample of cultivars which maximises traits variance, including individuals at the tails of the distributions. We assumed that the measured variance in the considered seven crop traits reflected in the variability of the other traits, because of their internal multiple correlations. The resulting dataset encompassed cultivars released along the 20th century, with few cultivars established in early 1900 (Americano 1600, Originario, Fortuna, Senatore Novelli) and many modern varieties (Dante, Reperso, Megumi, Brezza, Carnise precoce, Meco), also including Clearfield® (*imidazolinone*-tolerant) genotypes (Terra CL, Leonidas CL).

A two-year (2016 and 2017) field trial was carried out at Cascina Boraso (45°19'16.8"N 8°21'35.6"E, Vercelli, Italy), adopting a typical agricultural management plan of the area (i.e., direct seeding and permanent flooded conditions with some draining for chemical weeding and mineral fertilisation) with practices aimed at avoiding any biotic and abiotic stress (e.g. fungicide applications). The management practices implemented in the trial is reported in Supplementary Table 3.1, and the thermal conditions in the two seasons are plotted in Supplementary Figure 3.1. We used a completely randomised design with the experimental unit consisting of a 10 m^2 plot (six rows, interspaced 0.2 m and 8 m long) and two replications. The trial was seed drilled on April 27th, 2016 and April 24th, 2017, with seed rates adjusted to reach 1500 viable seeds for each plot, according to germinability and seed weight of the different cultivars. Fourteen yield-related traits (Table 3.1) were selected from literature (Katsura, Maeda, Horie, & Shiraiwa, 2007) and measured in the field to characterise the main growth and development processes represented in crop models (Confalonieri et al., 2009; Jamieson, Semenov, Brooking, & Francis, 1998; Li et al., 2017; Stöckle, Donatelli, & Nelson, 2003; L. Tang et al., 2009; Van Diepen et al., 1989).

Table 3.1 – List of the considered traits with notation of abbreviations used, measure unit, and sample size.

Code	**Trait**	**Unit**	**Sample size**	**Notes**
Phyll	Average phyllochron	°C day^{-1}	5 plants	Average value, phyllochron is not constant through time
Heading	Days from emergence to heading	days	Plot	Date determined when 50% of shoots have reached heading
GDDflo	Degree days from emergence to flowering	°C day^{-1}	Plot	Calculated from the date of heading
Maturity	Days from heading to maturity	days	Plot	Date dermined at complete final toning of the hulls
GDDmat	Degree days from heading to maturity	°C day^{-1}	Plot	Calculated from the date of maturity
CulmLen	Culm length	cm	20 shoots	Measured from crown root to the panicle node
ShootDM	Biomass of a single complete shoot at full flowering	g	5 plants	Average biomass of a single shoot (i.e. culm, leaves, sheats, and complete unfilled panicle)
Density	Final culm number	shoots m^{-2}	0.5 m^2	Measured on a randomly chosen area before harvest
FinLeafNum	Final leaf number	unitless	5 plants	Counted on the main stem
LAI	Leaf area index at full flowering	unitless	10 measures	Estimated using PockeLAI app (Confalonieri, Francone, & Foi, 2014)
PaniLen	Panicle length (main axis)	cm	20 plants	Measured from panicle node to the tip
PaniDM	Panicle biomass	g	10 panicles	-
RachisNum	Number of rachis branches per panicle	unitless	10 panicles	-
SpikesWeight	Weight of 1000 fully developed spikelets (thousand seeds weight)	g	800 seeds	-
GrainNum	Number of spikelets per panicle	unitless	5 panicles	-

Code	Trait	Unit	Sample size	Notes
Sterility	unitless	unitless	5 panicles	-
Yield	t ha^{-1}	t ha^{-1}	Plot	-

We followed the standard protocols reported either in the IRRI Standard Evaluation System (IRRI, 2002) or in the CPVO's technical protocol for rice (Community Plant Variety Office, 2012) to measure the fourteen crop traits, when available. The phyllochron (Phyl) was calculated as the average thermal time for complete leaf emission, since it varies through time. We monitored leaf emission on five plants on the main stem by weekly visual assessment, tagging the leaves and considering a leaf formed when the collar was visible according to Counce *et al.* (2000). Days to heading (Heading) was measured as the number of days from emergence to heading (i.e., when 50% of the shoots showed emerging panicle). Days to maturity (Maturity) was recorded as the number of days from heading to maturity, which was visually estimated using the toning of the hulls as a morphological marker, i.e. when all the spikelets turned brown at the end of the dry-down processes (around 22% of relative humidity) (Counce et al., 2000). Phyl and the growing degree days to reach flowering (GDDflo) and maturity (GDDmat) were then estimated using the average number of days for leaf emission, Heading, and Maturity. Growing degree days (*GDD*) were calculated from the daily average air temperature (*T*), according to Yan and Hunt (1999):

$$GDD = r\ (T_{opt} - T_{min})$$

with:

$$r = \left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)\left(\frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max} - T_{opt}}}$$

where T_{min}, T_{opt}, and T_{max} are the minimum, optimum, and maximum cardinal temperatures. We used the cardinal temperatures for rice (sub-specie *japonica*) reported by Sanchéz et al (2014), i.e. 10.5 °C, 29.7 °C, 42.5 °C for Phyl; 13.5 °C, 28 °C, 36 °C for GDDflo; 20.7 °C, 24.2 °C, 31.3 °C for GDDmat. The culm length (CulmLen, cm) was measured from soil surface to panicle base, at the end of milk stage. The biomass of a single complete shoot at flowering (ShootDM, grams) was calculated as the average dry weight of a complete (culm, panicle, leaves) shoot without roots at full flowering, by destructive sampling of five complete plants (main stem plus tillers) weighted after oven drying until constant weight (~72 hours). Final culm number (Density, shoots m^{-2}) was assessed one week before harvest by counting the total number of tillers at maturity on two samples of 0.5 m^2 area per plot. Final leaf number (FinLeafNum, unitless) was the final number of leaves counted on the main stem. Leaf area index at full flowering (LAI, unitless) was estimated with the PocketLAI app (Confalonieri et al., 2014), taking ten pictures at the base of the canopy randomly in the plot, four days after the date of heading. Panicle length (PaniLen, cm) was measured on ten plants per plot from the panicle node (base) to its tip. Panicle biomass (PaniDM, grams) was determined as the average weight of five panicles, harvested at maturity by cutting at the panicle node, and oven dried until constant weight. The number of secondary rachis-branches per panicle (RachisNum, unitless) was counted on the same five-panicles sample. The weight of 1000 fully developed spikelets, or thousand seeds weight (SpikesWeight, g), was determined on four samples of 200 fully developed spikelets, measured at ~14% of relative humidity, and the values were adjusted to dry weight. The total number of spikelets per panicle (GrainNum, unitless) was assessed on five randomly chosen panicles by counting the total number of spikelets (both filled and unfilled). Panicle sterility (Sterility, %) was the average ratio between the number of filled spikelets and the total number of spikelets per panicle; filled spikelets were separated by unfilled spikelets using the airflow of a rice huller model “Hercules” and manually counted on the five-panicles used for PaniDM and RachisNum. Finally, grain yield (Yield, t ha^{-1}) was determined by weighting the harvested material and converting it in tonnes of biomass per hectare assuming a plot area of 14.4 m^2, and measuring water content with a thermogravimetric scale, model “Sartorius MA 150”. Plots were harvested with a plot combine model “Iseki”. We performed all the statistical analysis with the R statistical software (R Core Team, 2017) and the FactoMineR package for Principal Component Analysis and Hierarchical Clustering on Principal Components (Lê et al., 2008). Charts were created with the ggplot2 R package (Wickham, 2009).

3.3.1 TRAITS VARIABILITY

We analysed the variability of fourteen yield-related traits on 40 Italian rice cultivars selected to maximise sample variance. We reported the summary statistics for the analysed traits in

Table 3.2. A graphical boxplot representation is available in Supplementary Figure 3.2. Coefficient of variations were calculated as the ratio between the pseudosigma ($\widetilde{\sigma}$, robust measure of dispersion calculated as IQR / 1.35) and the median ($\tilde{x}$) (Tukey, 1977). We classified the coefficients of variation (CVs) values into low $[CV \leq (\tilde{x} - \widetilde{\sigma})]$, medium $[(\tilde{x} - \widetilde{\sigma}) < CV \leq (\tilde{x} + \widetilde{\sigma})]$, high $[(\tilde{x} + \widetilde{\sigma}) < CV \leq (\tilde{x} + 2\,\widetilde{\sigma})]$, and very high $[CV > (\tilde{x} + 2\,\widetilde{\sigma})]$, according to the median and pseudosigma of CVs distribution, as proposed by Costa *et al.* (2002) to systematically evaluate the results.

Table 3.2 – Summary statistics calculated for each of the considered traits. Min. = minimum value; 1Q = first quartile; 3Q = third quartile; max. = maximum value; SD = standard deviation; CV = coefficient of variation.

Trait	Min.	1Q	3Q	Max.	Range	Mean	SD	Median	CV	Rating
Phyl	54.4	59.4	78.0	89.9	35.5	68.4	10.6	66.3	20.8%	medium
Heading	80.0	93.0	102.0	118.0	38.0	97.5	6.7	98.0	6.8%	low
GDDflo	776.0	971.2	1098.5	1324.1	548.2	1039.1	95.5	1054.5	8.9%	medium
Maturity	37.0	46.0	58.8	74.0	37.0	52.1	8.9	50.5	18.7%	medium
GDDmat	329.1	377.7	412.2	438.8	109.7	392.4	26.7	390.3	6.6%	low
CulmLen	48.0	67.0	91.8	134.0	86.0	79.5	18.3	75.0	24.4%	medium
ShootDM	1.2	2.9	3.9	6.0	4.8	3.5	0.9	3.4	22.6%	medium
Density	174.0	309.0	419.0	784.0	610.0	376.7	105.8	361.0	22.6%	medium
FinLeafNum	10.0	12.0	13.0	13.0	3.0	12.3	0.8	12.5	5.9%	low
LAI	3.1	4.1	4.8	6.4	3.3	4.5	0.6	4.4	10.5%	medium
PaniLen	8.0	17.0	22.0	36.0	28.0	19.7	3.5	20.0	18.5%	medium
PaniDM	0.9	3.2	4.3	6.5	5.6	3.8	1.0	3.8	22.1%	medium
RachisNum	6.0	10.0	12.0	15.0	9.0	10.8	1.8	11.0	13.5%	medium
SpikesWeight	11.0	24.2	32.2	46.7	35.6	28.2	6.0	27.0	22.0%	medium
GrainNum	40.0	114.8	159.2	240.6	200.6	138.4	34.0	137.1	24.0%	medium
Sterility	0.0	0.1	0.1	0.2	0.2	0.1	0.0	0.1	45.4%	very high
Yield	2.2	5.9	7.3	9.0	6.8	6.5	1.2	6.7	15.5%	medium

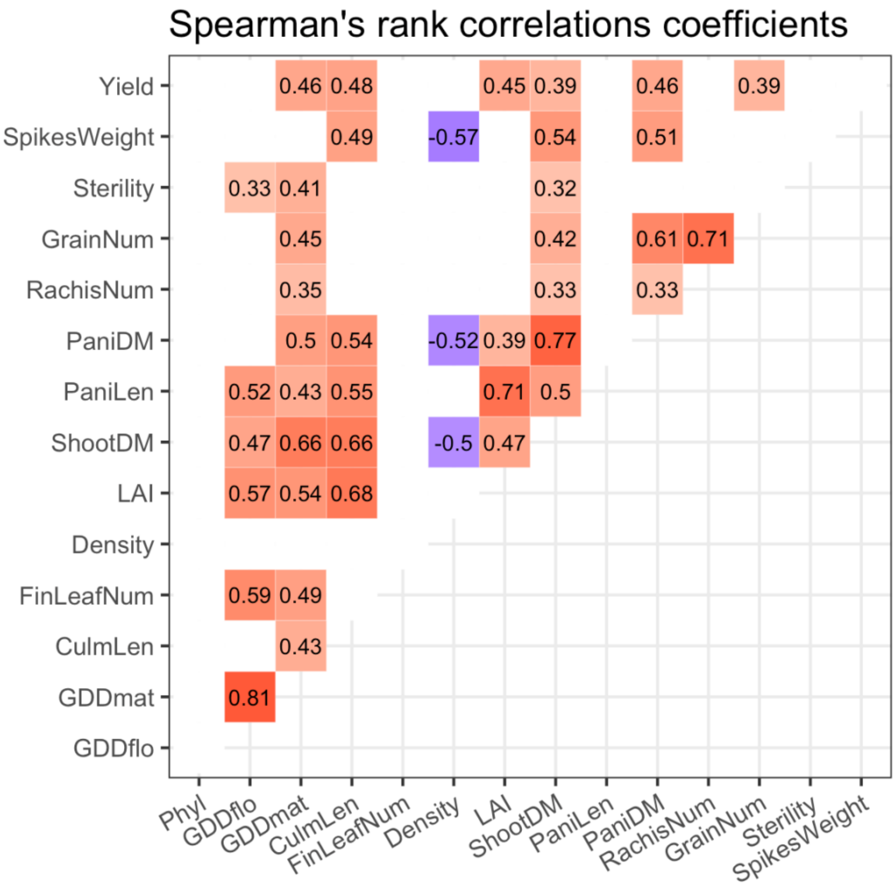
The thermal requirements for the main phenological stages (Heading - GDDflo, and Maturity - GDDmat) showed medium low relative variation (CVs of 8.9% and 6.6%, respectively); however, their small variability translated in significant differences in the duration of Heading (38 days) and Maturity (37 days). The traits associated with vegetative development (Phyll, LAI, CulmLen, ShootDM) obtained medium to high CVs (20.8%, 10.5%, 24.4%, 22.6%, respectively), except for FinLeafNo that had the lowest CV (5.9%). The CVs of the other traits associated with reproductive development were classified as medium to very high: the most variable was Sterility (CV = 45.4%) followed by Density (22.6%). We recorded a medium CV for LAI (10.5%), ranging from 3.13 m^2 m^{-2} to 6.43 m^2 m^{-2}. The grain features, i.e. GrainNum (CV = 24.0%), PaniDM (CV = 22.1%), and

SpikeWeight (CV = 22.0%) were also rated as “medium”, mostly because very different grain shapes were present in the sample.

The measured yield varied between 2.19 t ha^{-1} (Megumi) to 8.95 t ha^{-1} (Italpatna, Supplementary Figure 3.3); the CV was medium (15.5%), with a global average was 6.47 t ha^{-1} which is consistent with the official statistics (Ente Nazionale Risi, 2018).

3.3.2 TRAITS RELATIONSHIPS

We investigated the relationships among traits by firstly computing the pair-wise Spearman’s rank correlation coefficients (ρ) and then tested their significance with α = 0.05 (Fieller, Hartley, & Pearson, 1957). The correlation matrix, reporting only significant values, is provided in Figure 3.1.



	Phyl	GDDflo	GDDmat	CulmLen	FinLeafNum	Density	LAI	ShootDM	PaniLen	PaniDM	RachisNum	GrainNum	Sterility	SpikesWeight
Yield			0.46	0.48			0.45	0.39		0.46		0.39		
SpikesWeight				0.49		-0.57		0.54		0.51				
Sterility		0.33	0.41					0.32						
GrainNum			0.45					0.42		0.61	0.71			
RachisNum			0.35					0.33		0.33				
PaniDM			0.5	0.54		-0.52	0.39	0.77						
PaniLen		0.52	0.43	0.55			0.71	0.5						
ShootDM		0.47	0.66	0.66		-0.5	0.47							
LAI		0.57	0.54	0.68										
Density														
FinLeafNum		0.59	0.49											
CulmLen			0.43											
GDDmat		0.81												
GDDflo														

Figure 3.1 – Pairwise Spearman's rank correlation coefficient calculated for the considered traits. Only significant values are reported. The acronyms of the crop traits are explained in Table 3.1.

All the significant relationships were positive except for Density, that negatively correlated with SpikesWeight (ρ = -0.57), PaniDM (ρ = -0.52) and ShootDM (ρ = -0.5). Yield was significantly related with CulmLen (ρ = 0.48), GDDmat (ρ = 0.46), PaniDM (ρ = 0.46), LAI (ρ = 0.45), GrainNum (ρ = 0.39), and ShootDM (ρ = 0.39). The strongest relation was found between GDDflo and GDDmat (ρ = 0.81), suggesting that late flowering cultivars presented a long reproductive phase, and these traits were both positively correlated with LAI (ρ = 0.57 and ρ = 0.54, respectively). GDDflo was also positively correlated with FinLeafNum (ρ = 0.59), PaniLen (ρ = 0.52), and ShootDM (ρ = 0.47). GDDmat was strongly related with most traits (except SpikesWeight, Phyllochron, and Density), and especially with ShootDM (ρ = 0.66), which was in turn positively associated with CulmLen (ρ = 0.66) and PaniDM (ρ = 0.77). As expected, PaniDM was correlated with other panicle traits, i.e. GrainNum (ρ = 0.61), SpikesWeight (ρ = 0.51), RachisNum (ρ = 0.33), GDDmat (ρ = 0.5) and LAI (ρ = 0.39). Other positive relationships were found between GrainNum and RachisNum (ρ = 0.71), GrainNum and PaniLen (ρ = 0.61). ShootDM positively influenced most panicle traits, i.e. SpikesWeight (ρ = 0.54), PaniLen (ρ = 0.5), GrainNum (ρ = 0.42), and RachisNum (ρ = 0.33). Also, Sterility was positively associated with ShootDM (ρ = 0.32), even if the strongest positive relation resulted with GDDmat (ρ = 0.41). We also acknowledged the limitations of statistical test used which may be prone to residual confounding effect of phenology. We investigated the confounding effect of phenology in the relations of measured traits with yield by plotting the residuals of the linear correlation between GDDflo and Yield against the measured traits (Supplementary Figure 3.4). We did not found any strong pattern in the analysis of the residual except for a slightly increasing trends and bigger r^2 in FinalLeafNum (r^2 = 0.272), Panilen (r^2 = 0.169), and LAI ((r^2 = 0.101): except for PaniLen in which data distribution was homogeneous across its range of variation, these trends could also have resulted from the uneven distribution of data in the independent variables FinalLeafNum (only 3 observations under 12) and LAI (only 3 observations over 5).

We further explored the associations among traits with Principal Component Analysis (PCA), in order to quantify the strength and direction of correlations between the original traits and the extracted Principal Components (PCs). The first three components, explaining the 64.2% of the total variance were retained for analysis (Supplementary Figure 3.5). We adopted the methodology proposed by Husson *et al.* (2010a) which integrates “illustrative elements”, i.e. supplementary variables and individuals which are introduced after the computations of PCs. We used the days from emergence to heading (Heading) and the days from heading to maturity (Maturity) as supplementary quantitative variables, to avoid redundancy with GDDflo and GDDmat. The Italian market classification based on grain shape was introduced as a supplementary qualitative variable (i.e. “Grain shape”), as this is the primary determinants of the rice selling price. The corresponding classes were long B, round, medium, and long A (European Parliament, 2013), the latter further divided into long A for parboiling transformation (long A PB) and long A for the domestic market (long A DM, i.e. traditional cultivars suited for the preparation of risotto) because of the huge phenotypic differences between these two groups (Mongiano et al., 2018). Cv. Megumi was removed from the analysis and used as a supplementary individual since it resulted as an outlier due to extreme values for most traits (Yield, SpikesWeight, GrainNum, CulmLen, PaniLen, GDDflo, GDDmat). Principal Components were obtained via diagonalisation of the correlation matrix, and extraction of the associated eigenvectors and eigenvalues. PCA biplot of variables is reported in Figure 3.2, while the correlation coefficients calculated between traits and the extracted PCs are reported in Supplementary Table 3.2.

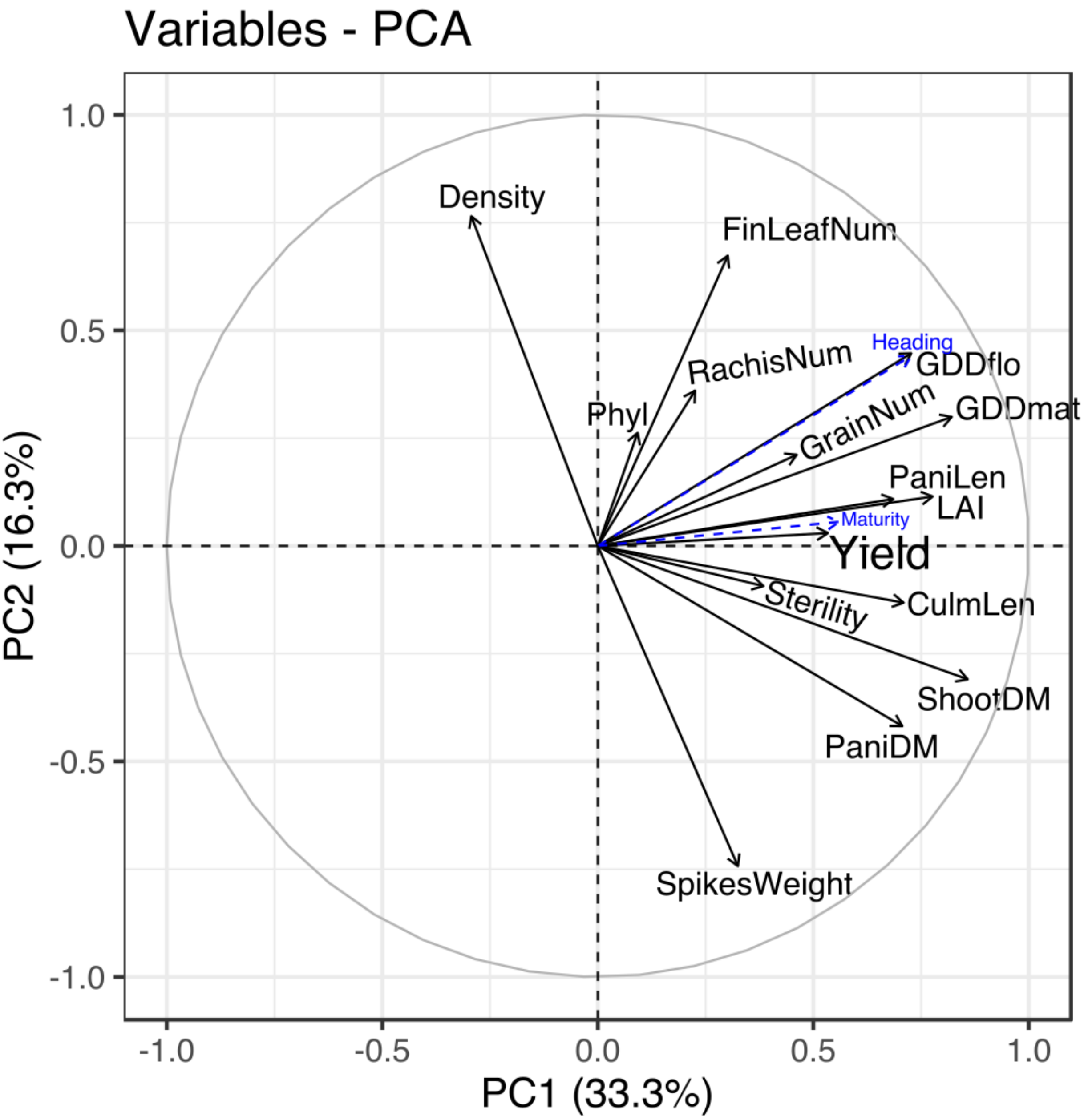


Figure 3.2 - PCA biplot of variables. List of abbreviations is reported in Table 1. Supplementary variables (i.e. illustrative elements, not taking parts in computation of principal components space) are colour-coded in blue.

PC1 synthesised the direct relations between most traits and Yield. We found the strongest correlations with PC1 for ShootDM (0.86), GDDmat (0.82), LAI (0.78), GDDflo (0.73), CulmLen (0.71), PaniDM (0.71), PaniLen (0.69), and to a lesser extent for Yield (0.53). PC2 was positively correlated with Density (0.76), FinalLeafNum (0.67), GDDflo (0.45), and RachisNum (0.36), while we found negative correlations with PaniDM (-0.42) and SpikesWeight (-0.74). Cultivars at positive coordinates on PC2 had a longer duration of vegetative stage, with a higher number of leaves and increased tillering, but lighter seeds and panicles. PC3 positively correlated with Phyll (0.46), PaniLen (0.41), SpikesWeight (0.36), and FinLeafNum (0.32), and negatively with GrainNum (-0.79), RachisNum (-0.73), and PaniDM (-0.40). This indicates a positive relation between higher final number of leaves, larger phyllochron, and increased panicle length and seeds weight with fewer secondary rachis-branches and number of seeds, resulting in lower panicle biomass (and vice-versa). The significance of the difference between average coordinates of cultivars grouped by grain shape and overall mean were tested (*t-tests*, Supplementary Table 3.3). The results indicated that long A (DM) group had different coordinates on PC1 (positive), PC2 (negative), and PC3 (positive), meaning that these cultivars showed considerable biomass accumulation during both vegetative and reproductive phases, while producing few tillers, less branched panicles and fewer but heavier grains, with an extended phyllochron and higher final leaves number. Long A (DM) opposed to long B grain cultivars on PC2, the latter presenting significantly lighter grain with a high number of grains placed on a lengthy panicle with many ramifications; furthermore, they produced more leaves and tillers.

3.3.3 CLUSTER ANALYSIS

An exploratory cluster analysis was conducted to further highlight grouping patterns among the tested cultivars and to provide a phenotypic classification. We performed a Hierarchical Clustering on Principal Components (Figure 3.3), retaining only the first three components to minimise data noise. Three main clusters were selected, in order to maximize the relative loss of inertia (Husson, Lê, & Pagès, 2010a).

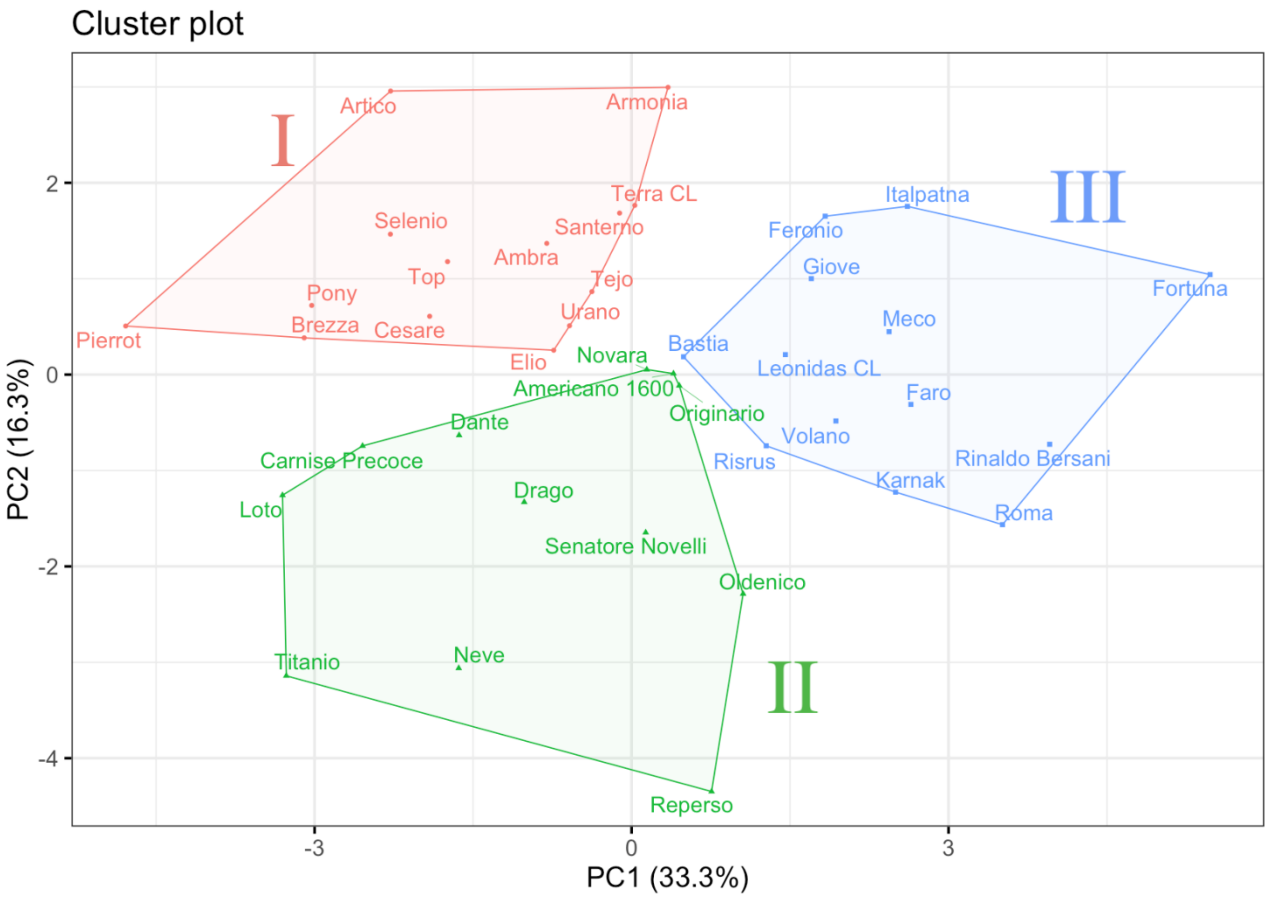


Figure 3.3 - PCA biplot of individuals with indication of the three extracted clusters resulting from Hierarchical Clustering on Principal Components (HCPC).

We assessed the between-cluster variance over the total variance explained by each trait by calculating η^2 (Supplementary Table 3.4). The variables explaining the largest amount of between-cluster variance were GDDmat ($\eta^2 = 0.59$) and GDDflo ($\eta^2 = 0.53$), ShootDM ($\eta^2 = 0.46$), and SpikesWeight ($\eta^2 = 0.42$).

Cluster I comprised all the long B cultivars, except for Giove, together with three short-height long A for parboiling (Cesare, Pony and Tejo) and several round grain cultivars (Ambra, Elio, Pierrot, Selenio, Terra CL, Top). This cluster was characterised by high-tillering, short-height cultivars that had lower than average values for SpikesWeight, PaniLen, PaniDM, and ShootsDM. The mean Yield in this cluster was slightly lower than the overall mean (6.16 t ha^{-1} compared to 6.57 t ha^{-1}), despite the presence of modern high-yielding cultivars, like Selenio and Terra CL. Terra CL (medium-height, late, round grain cultivar) was one of the farthest from the barycentre of the cluster, while cultivar Top (short-height, early, round grain) the closest.

In Cluster II most of the individuals were early long A cultivars except for three early 1900 accessions, i.e. Novara (medium), Americano 1600, and Originario (round). These cultivars presented above average SpikesWeight but lower RachisNum, Density, Sterility, GDDmat, GDDflo and FinLeafNum. Cultivar 'Senatore Novelli' (traditional low-tillering genotype with long culm and intermediate time of maturity) was one of the closest to Cluster's barycentre; on the contrary, Titanio (long A for parboiling cultivar, very early and with limited tillering ability) was at the farthest distance from the barycentre. This cluster included cultivars with short crop cycle, associated with a lower number of leaves and tillers, with less sterility and panicles with fewer secondary rachis-branches. Lowest yielding cultivars (Neve 5.58 t ha^{-1}, Carnise precoce 5.98 t ha^{-1}, Titanio 5.26 t ha^{-1}) were all characterised by the lowest thermal requirements for vegetative and reproductive stage. On the contrary, cultivars Americano 1600 and Originario (average yield above 7 t ha^{-1}) had an extended crop cycle.

Cluster III consisted of cultivars with many traits above the overall mean, i.e., GDDmat, GDDflo, ShootDM, LAI, PaniDM and PaniLen, CulmLen, FinLeafNum, and SpikesWeight. We also noted an above average Sterility (11%) compared to the overall mean (9%). Half of the long A (DC) cultivars were in this cluster, except for two round grain cultivars and one long B. The remaining cultivars were all long A (PB). Genotypes in this cluster were characterised by extended crop cycle associated with a remarkable biomass accumulation (longer stems, more leaves, bigger panicles and seeds) both in the vegetative and reproductive stage. These were all high-yielding cultivars except for Fortuna (5.4 t ha^{-1}), a genotype that has been probably imported from Louisiana during the first years of 1900 and adapted to Italian environment (Adair et al., 1973). It showed the largest thermal requirements and biomass accumulation before heading among all the cultivars. The poor performances despite its fast growth rate were probably due to the self-shading effect and the large loss of assimilates due to increased respiration (Dingkuhn, Penning de Vries, De Datta, & Van Laar, 1991; Vergara, Tanaka, Lilis, & Puranabhavung, 1966). The cultivars composition in this cluster further confirmed that increased source and sink strengths are associated with higher thermal requirements, often resulting in higher yields.

3.3.4 DISCUSSION

The variability found in the 40 Italian rice cultivars was considerable for all the analysed traits and comparable to published literature (Faivre-Rampant et al., 2010; Katsura et al., 2007; Samonte et al., 1998; Samonte, Wilson, McClung, & Tarpley, 2001; Volante et al., 2017). We selected the cultivars from a set of 351 Italian genotypes which were characterised in a previous study for traits days to heading and maturity, culm and panicle length, thousand seeds weight, caryopsis width and length (Mongiano et al., 2018). The method of cultivar selection (Kennard-Stone algorithm) proved to be efficient in providing a sample with considerable variability also for the traits measured in this study, thanks to the multiple correlations existing among traits (McClung et al., 2011; Reynolds et al., 1998; Sürek & Befier, 2003; Upadhyay, 2017; Volante et al., 2017).

The traits associations were evaluated a) via pair-wise Spearman's rank correlations coefficients, b) with Principal Components Analysis, and c) by observing patterns highlighted by Cluster Analysis. As expected, panicle weight (i.e. the sink size) was significantly associated with yield. The former depends upon the interaction of grain weight, number of grains per panicle, length of the primary axis of the panicle and the number of secondary rachis-branches, that can be considered as the "panicle components" (Hittalmani et al., 2003). Highest-yielding cultivars had different combinations of the analysed traits leading to heavy panicles (Dingkuhn et al., 2015), depending on grain shape, that leads to different weights (SpikesWeight range = 35 g).

Other than being related to grain features, higher yields were also associated with extended thermal requirements for flowering (GDDflo) and ripening (GDDmat) phases, because a longer growth duration allows intercepting larger amount of solar radiation (Katsura et al., 2007). Increased total aboveground biomass (ShootDM and PaniDM) was in fact associated with extended vegetative and reproductive phase, which were strongly inter-related and positively correlated to many other traits impacting yield, i.e. CulmLen, PaniLen, FinLeafNum, and LAI, as pleiotropic effects are also known to exists amongst the genes controlling these traits (Xue et al., 2008).

Another main outcome of our study is that rice cultivars maintained a constant balance between the biomass accumulated during the vegetative and ripening stage, as a significant association was found between ShootDM and PaniDM. This agrees with previous studies that highlighted the importance of non-structural carbohydrates accumulation before heading, which contributes to a large portion of grain carbohydrates and serve as a buffer during periods of sub-optimal radiation levels (Katsura et al., 2007; Samonte et al., 2001; Stella, Bregaglio, & Confalonieri, 2016). Moreover, compensatory mechanisms are known to modulate the balance between sink and source strengths (Kumar et al., 2016). Genotypes with short culms accumulated less biomass during the vegetative stage, had smaller leaf area, lighter panicle, and had a reduced duration of both vegetative and reproductive phase, resulting in low grain yield. Examples of these genotypes are Megumi, Pierrot, and Pony, all short-culm, high-tillering and very early genotypes that produced an average yield lower than 5 t/ha. On the contrary, most of the highest-yielding genotypes accumulated a large amount of biomass during the whole crop cycle, due to either long culms and higher number of leaves (Novara, Americano 1600), or to shorter culms but of increased weight (Meco), or to an increased number of productive tillers (Italpatna, Terra CL), adjusted according to panicle component traits, like GrainNum and SpikesWeight (Katsura et al., 2007; Peng et al., 2008). All these features were supported by an extended crop cycle, while earliness was often associated with lower yields (Vergara, Lilis, & Tanaka, 1964). One exception was the cultivar Novara that, despite being one of the genotypes with the shortest duration of the crop cycle led to an average yield of 7.5 t ha^{-1}. One of the possible explanation is the favourable ratio between the duration of the ripening stage over the whole crop cycle (28.7%), which is associated with massive biomass accumulation during the vegetative stage that characterised many of the high-yielding genotypes (Peng et al., 2000). When earliness was associated with an increased number of tillers, it showed to negatively impact yield, probably because of an increased abortion phase (normally occurring after panicle initiation) determined by the limited biomass accumulation (Kumar et al., 2016). Early-flowering genotypes presented many unproductive tillers, counterbalanced with reduced seed and panicle size as the partitioning of the assimilates to more tillers impacted biomass accumulation in individual shoots (Peng & Khush, 1994). Our results suggested that when this trait was highly expressed (Pony - Long A, Brezza - Long B, Pierrot - Round), it impacted yield (5, 5.18, and 3.68 t ha^{1} respectively). There are, however, cultivars like Ambra and Selenio (round) that produced an average yield of 7.19 t ha^{1}, despite the emission of many tillers. Contrarily to low-yielding cultivars with enhanced tillering ability, their primary differences were the duration of the vegetative and reproductive phases, which is crucial in supporting the growth of an increased number of tillers (Dingkuhn et al., 1991). Examples of this are Artico and Armonia (long B), or Tejo (long A). GDDmat was also strongly correlated with Sterility, which was more severe in late genotypes probably because of the low temperatures during grain filling, leading to not fully developed panicles. Although we cannot provide any quantitative explanation, this hypothesis is supported by the fact that most of the empty spikelets were at the base of panicle, since ripening occurs in a basipetal gradient (Counce et al., 2000). However, spikelet sterility did not significantly impacted yield in our dataset, since many high-yielding cultivars (Faro, Volano, Meco) had high Sterility values. Cluster analysis extracted three groups that can be resumed as high-tillering (Cluster I), early (Cluster II), and increased source-sink (Cluster III) genotypes. We noted that the farthest individuals from the barycentre of their respective cluster were either low or high-yielding cultivars. Lower yields were associated to genotypes at extreme coordinates (both positive or negative) on PC1 (Pierrot, Pony, Brezza, Loto, Titanio, Fortuna), while the high-yielding specific individuals were at coordinates next to origin on PC1 (Italpatna, Terra CL, Novara, Americano 1600, Originario), further confirming that when a specific “breeding strategy” is primed, a proper balance between yield components should be ensured to attain high grain production (Katsura et al., 2007).

Surprisingly, the top-yielding genotypes were four pre-mechanisation genotypes, bred before 1960. Rice growers abandoned these genotypes many decades ago because of many undesirable agronomic traits like high susceptibility to rice blast (Magnaporthe grisea - *T.T. Hebert - M.E. Barr)*, tendency to lodging, large amounts of unwanted residual straw and excessive duration of growing cycle (Faivre-Rampant et al., 2010; Titone, Mongiano, & Tamborini, 2015). Furthermore, the market demands regarding grain quality completely changed in the last century (Tamborini & Lupotto, 2006). The advanced agricultural techniques used mitigated these adverse features so that their remarkable growth rate could be fully expressed in our trial. Rice blast was efficiently controlled with the use of fungicides and appropriate nitrogen inputs as the excess favour the disease severity (Webster & Gunnell, 1992), and we did not have to account for straw management, and harvest complications due to long crop cycle. Lodging was still present, even though in our trial it did not significantly impacted yield. The variability in agricultural practices was avoided in our study since all cultivars were managed with the same practices, with a fixed sowing date. This is a simplification because alternative management strategies markedly affect the productivity. For example, late sowing could have positively affected the yields of early genotypes (Loto, Titanio), by postponing the grain filling period to more favourable conditions, as high temperatures during the ripening phase are responsible for anticipated panicle senescence, resulting in lower yields (Kim et al., 2011). However, the aim of the study required this simplification in order to focus on the genotypic component, so that we tried to minimise the impact of agricultural management by using a standard management adopted in north west Italian rice cultivation area.

One question that remains unanswered in the present study is whether the measured ranges of traits could be used to determine the physiological limits of the traits in the actual germplasm, even if we assumed that our sample is representative of the maximum variance in the measured traits of Italian cultivars.

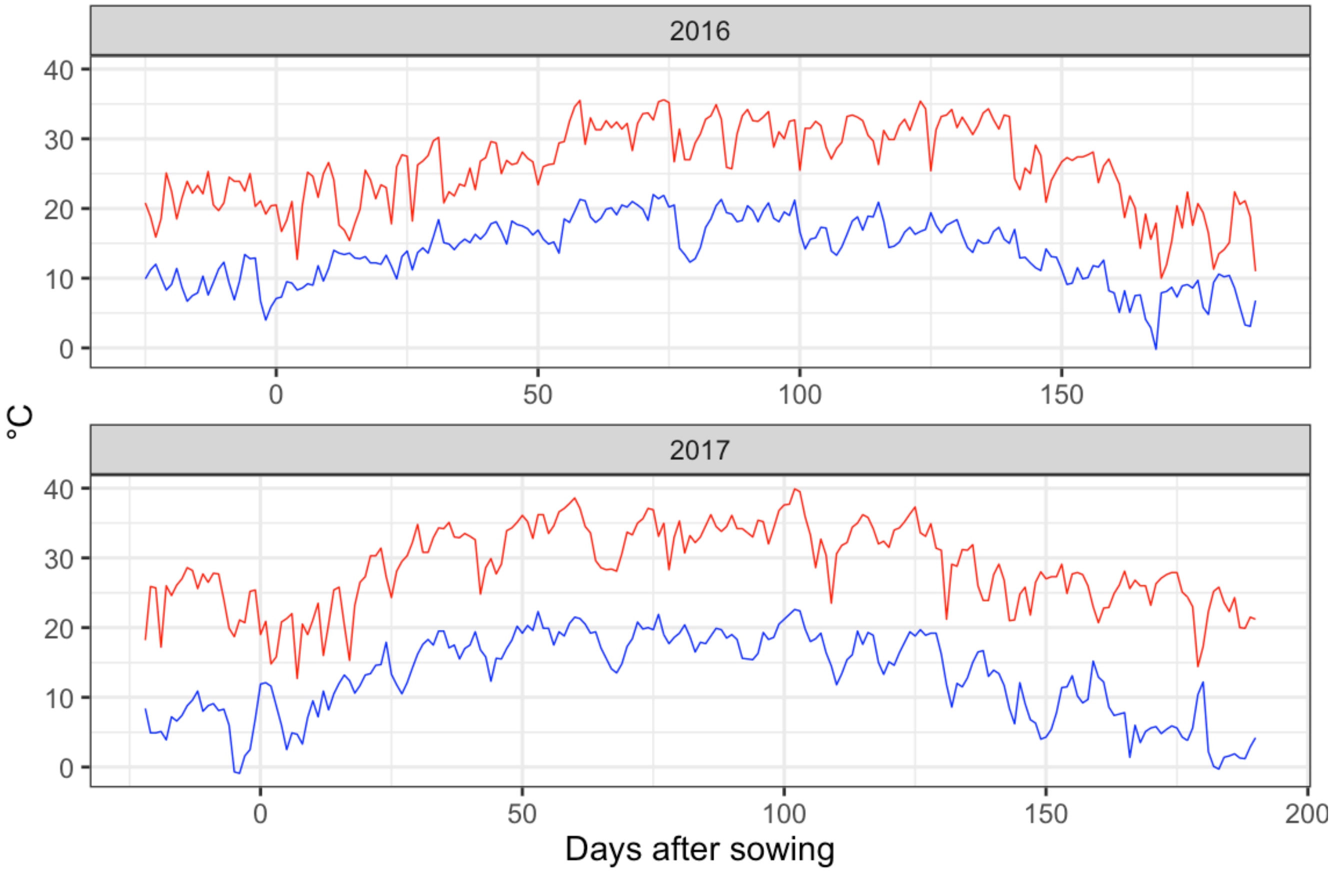
3.3.5 CONCLUSION

This study delivers the range of variations of most critical yield-related traits in Italian rice cultivars and shed lights on their associations. Our results can be used in modelling studies targeting the performances of the Italian rice varietal landscape, by providing phenotypic data associated to plant processes currently implemented in crop models, together with their range of variations and correlations. This effort could be the base of an effective usage of crop models to support rice breeding programs, as it allows defining new ideotypes to face the future challenges of Italian rice agriculture.

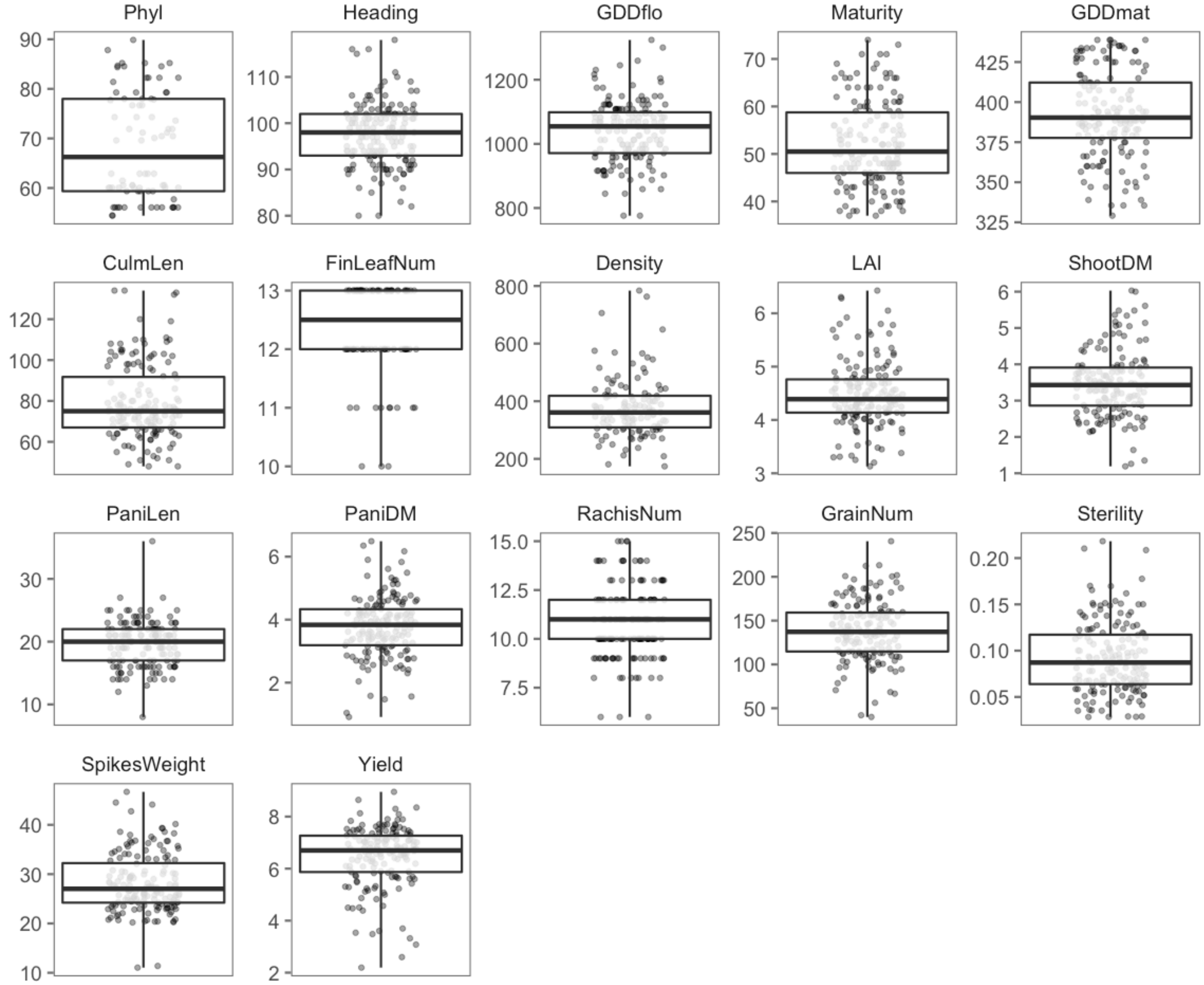
3.4 SUPPLEMENTARY MATERIAL

Supplementary Table 3.1 – List of field management practices performed during the execution of field trials during both seasons.

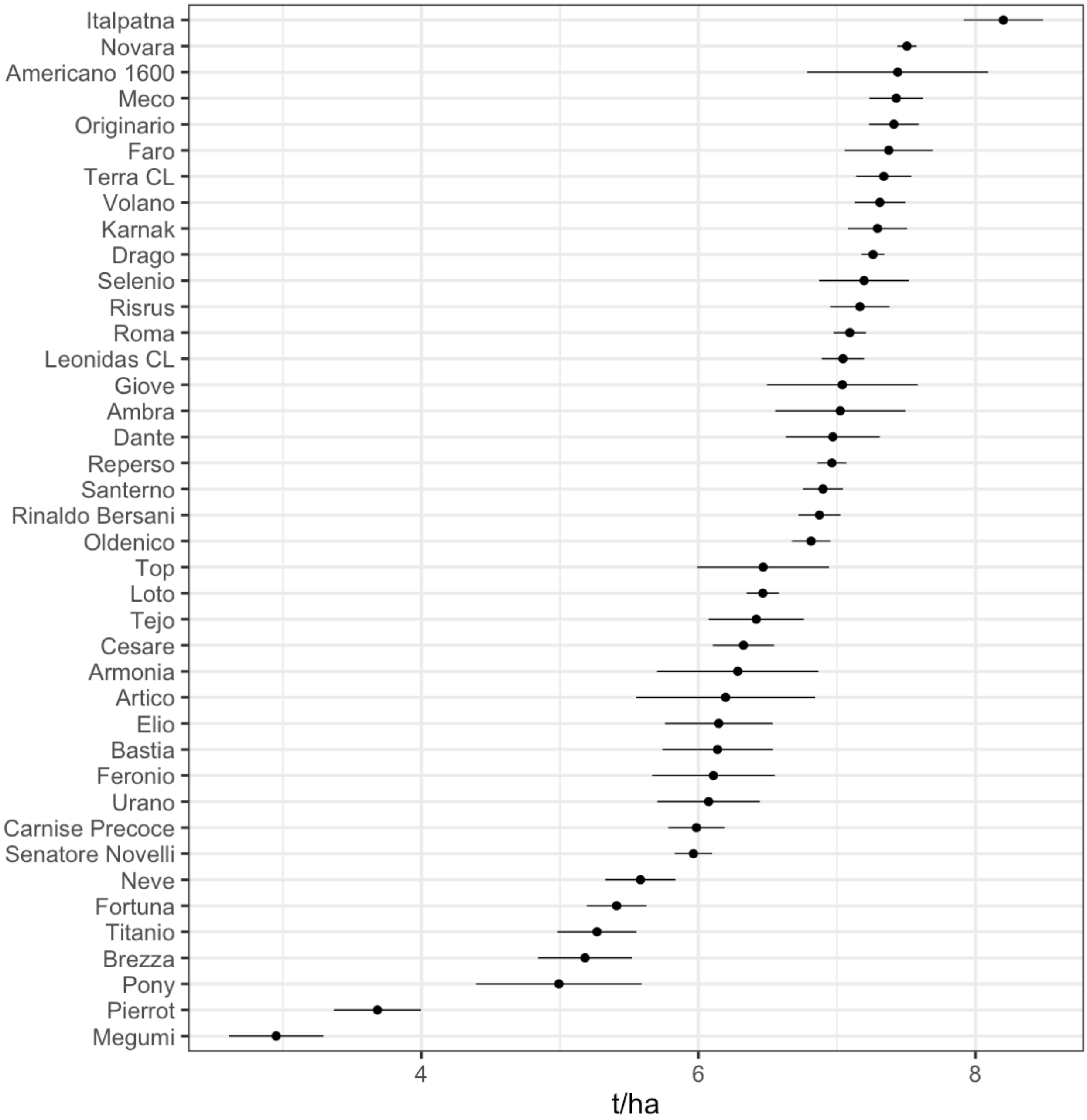
Date	Operation	Notes
2016-03-24	Organic fertiliser application (3-1-2)	15 g m^{-2}
2016-03-29	Ploughing (~ 0.3m)	
2016-04-05	Levelling of the paddy	
2016-04-13	Harrowing	
2016-04-16	Chemical weeding (pre-sow)	cycloxydim, bensulfuron-methyl, oxadiazon
2016-04-29	Chemical weeding (pre-emergence)	clomazone, pendimethalin
2016-06-03	Mineral fertiliser application (urea, 46-0-0)	13 g m^{-2}
2016-07-15	Mineral fertiliser application (23-0-30)	18.2 g m^{-2}
2016-07-26	Fungicide application	tryciclazole, azoxystrobin
2017-02-08	Organic fertiliser application (3-1-2)	15 g m^{-2}
2017-04-04	Ploughing (~ 0.3m)	
2017-04-12	Levelling of the paddy	
2017-04-22	Chemical weeding (pre-sow)	cycloxydim, bensulfuron-methyl, oxadiazon
2017-06-09	Chemical weeding (post-emergence)	penoxsulam, quinlorac, bensulfuron-methyl, lambda-cyhalothrin
2017-06-21	Mineral fertiliser application (urea, 46-0-0)	13 g m^{-2}
2017-07-03	Chemical weeding (post-emergence)	cyhalofop-butyl
2017-07-18	Mineral fertiliser application (23-0-30)	21 g m^{-2}
2017-08-10	Fungicide application	tryciclazole, azoxystrobin



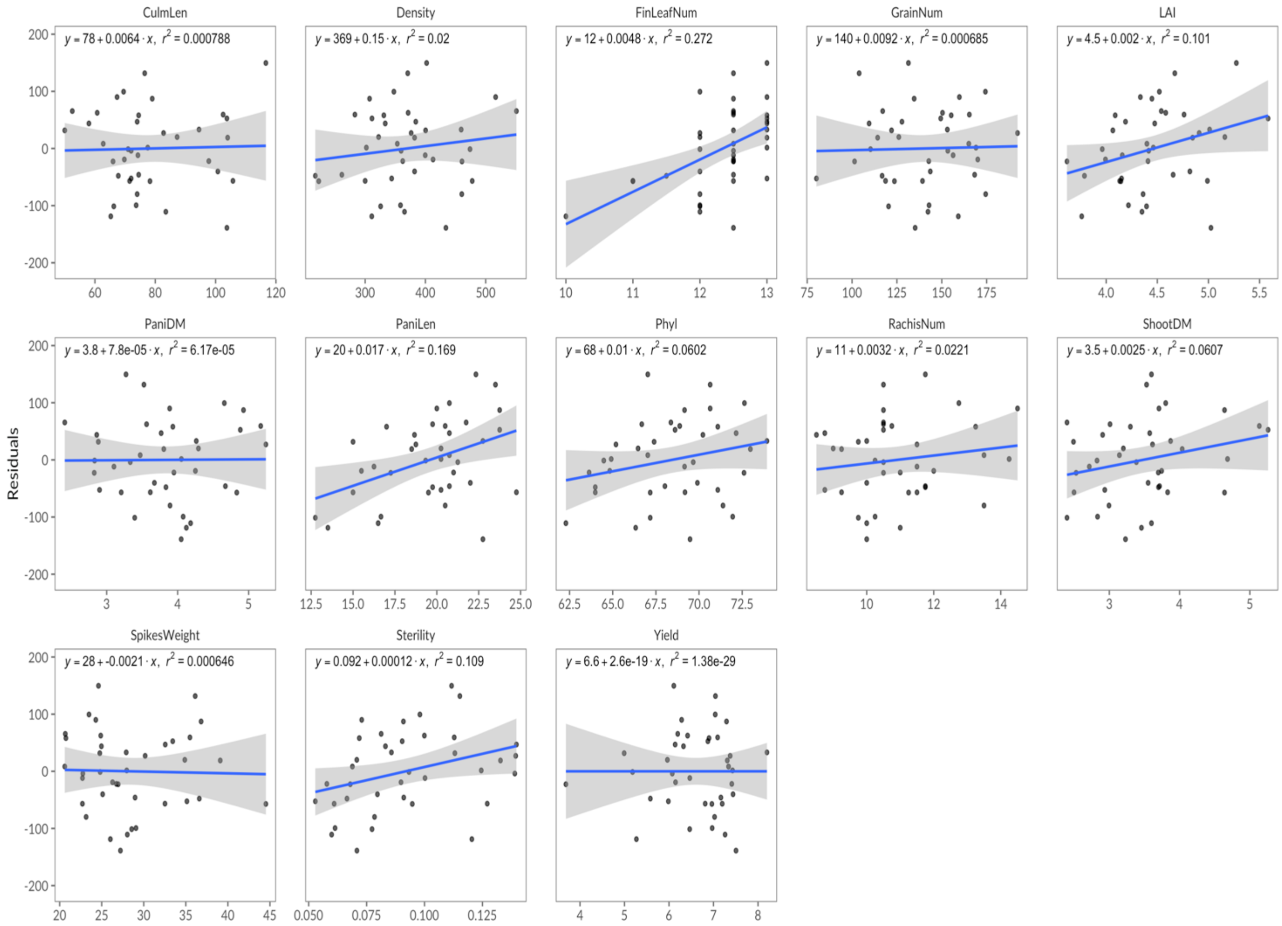
Supplementary Figure 3.1 – Minimum (blue) and maximum (red) daily temperatures (°C) recorded in the two seasons during the growing cycle.



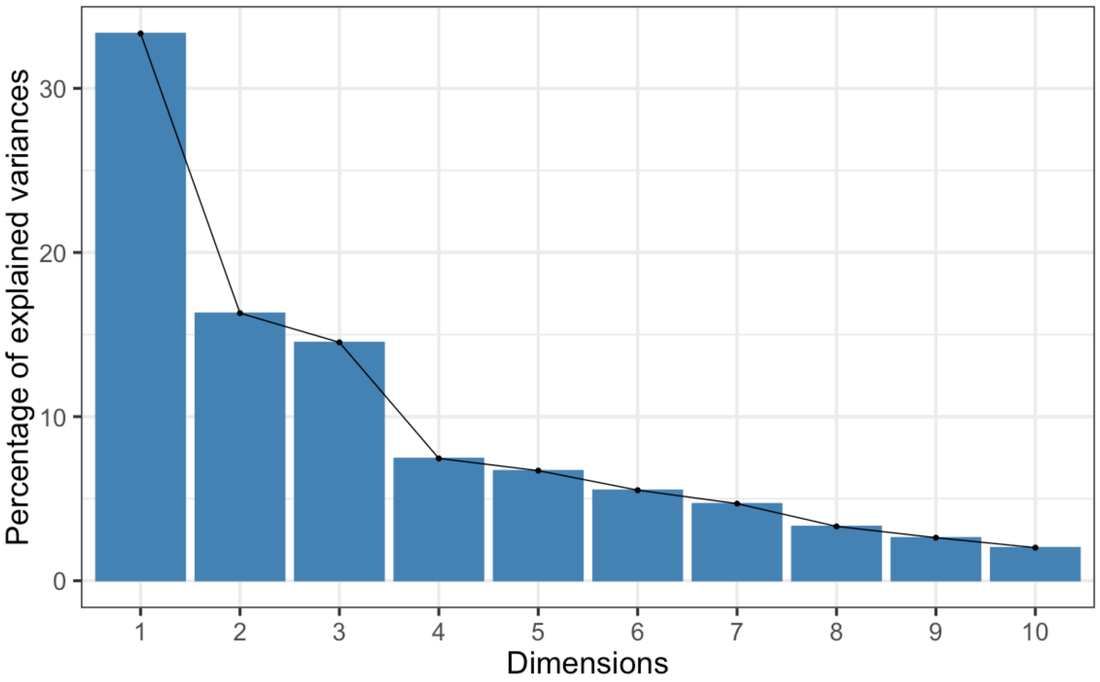
Supplementary Figure 3.2 – Visual representation of traits data distribution with boxplot combined with data points. Points are horizontally adjusted to minimise overlapping).



Supplementary Figure 3.3 – Plot showing mean yield (point) and standard deviation (errorbars) measured in the two seasons expressed in tonnes per hectare.



Supplementary Figure 3.4 –Analysis of the residual relations between yield and phenological traits (GDDflo, Heading, GDDmat, and Maturity) was carried out to check the absence of patterns in residuals. r^2 and model equation are also reported. Measure units of the dependant variables are reported in Table 3.1.



Supplementary Figure 3.5 – Percentage of variance explained by each dimension resulting from Principal Component Analysis

*Supplementary Table 3.2 – Correlation coefficient calculated between variables and the first three Principal Components, tested for significance (Sig). Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.*

Trait	PC1	Sig	PC2	Sig	PC3	Sig
Phyl	0.09		0.26		0.46	* *
Heading	0.73	* * *	0.44	* *	0.17	
GDDflo	0.73	* * *	0.45	* *	0.17	
Maturity	0.56	* * *	0.06		-0.31	
GDDmat	0.82	* * *	0.30		-0.09	
CulmLen	0.71	* * *	-0.13		0.26	
ShootDM	0.86	* * *	-0.31		-0.07	
Density	-0.29		0.76	* * *	0.12	
FinLeafNum	0.30		0.67	* * *	0.32	*
LAI	0.78	* * *	0.11		0.29	
PaniLen	0.69	* * *	0.11		0.41	* *
PaniDM	0.71	* * *	-0.42	* *	-0.40	*
RachisNum	0.23		0.36	*	-0.73	* * *
SpikesWeight	0.33	*	-0.74	* * *	0.36	*
GrainNum	0.46	* *	0.21		-0.79	* * *
Sterility	0.38	*	-0.09		0.05	
Yield	0.53	* * *	0.03		-0.21	

*Supplementary Table 3.3 – Coordinate estimates of the categories describing the grain shape of cultivars on each Principal Components, tested for significance (Sig) with t-test. Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.*

Category	PC1	Sig	PC2	Sig	PC3	Sig
long A (dc)	1.442	* *	-1.557	* * *	0.1703	
long A (pb)	-0.724		-0.1131		-0.668	
long B	-0.847		1.427	* *	-0.8166	
medium	-0.0306		-0.109		0.5551	
NA	1.281		0.0470		1.717	
round	-1.122		0.3050		-0.9581	

*Supplementary Table 3.4 – η^2 values calculated for the considered traits and tested for significance (Sig). Significance codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

Trait	η^2	Sig
GDDmat	0.5906	* * *
GDDflo	0.5387	* * *
Heading	0.5333	* * *
ShootDM	0.4679	* * *
SpikesWeight	0.4213	* * *
PaniDM	0.397	* * *
CulmLen	0.3652	* * *
FinLeafNum	0.3596	* * *
LAI	0.3121	* *
Density	0.3100	* *
Sterility	0.3011	* *
Maturity	0.2881	* *
PaniLen	0.2206	*
Yield	0.1494	
RachisNum	0.1315	
GrainNum	0.1165	
Phyl	0.0711	

CHAPTER 4 ADVANCING CROP MODELLING CAPABILITIES VIA CULTIVAR-SPECIFIC PARAMETER SETS FOR THE ITALIAN RICE GERMPLASM.

Gabriele Mongiano, Patrizia Titone, Luigi Tamborini, Roberto Pilu, Simone Bregaglio

4.1 ABSTRACT

Rice is one of the excellences of Italian agriculture. In recent years, many studies developed advanced technological techniques to support rice growers and local stakeholders in the main rice harvested area of Northern Italy. Crop modelling is among the most prominent, as proved by the various model-based services that flourished in the last decade. Here we propose a methodology to derive cultivar-specific model parameter sets using a minimum dataset of field observations collected on the primary rice cultivars currently grown in the area. The definition of cultivar-specific parameter sets was based on the calibration of the crop model WOFOST_GT using long-term (2004-2015) experimental data collected in two sites (167 experiments). The results revealed a good ability of the crop model in reproducing reference observations of heading (average RRMSE 5.96% ± 1.41%) and maturity date (22.8% ± 4.52%), canopy height (10% ± 1.39%) and yield (11.2% ± 3.95%) across cultivars and years. The analysis of the derived cultivar-specific parameter sets allowed to highlight the internal relations between model parameters and to find similarities with the available classifications of grain shape, phenological development and stem length. Our study is meant to deepen the modelling knowledge used to characterise the vast differences in the Italian rice varietal landscape, fostering improvements of current crop model applications at different spatial and temporal resolutions.

Keywords

WOFOST_GT, sensitivity analysis, automatic optimisation, multi-start simplex, equifinality

4.2 INTRODUCTION

Rice is the most important source of staple food globally, along with wheat. Around 480 million metric tons of rough rice are produced annually, half of which in China and India only (Muthayya, Sugimoto, Montgomery, & Maberly, 2014). Although being predominantly grown in the Asian continent, rice is also cultivated in Europe (EU), where the total annual production amounted to 1.83 million milled-equivalent tons in 2017 (European Commission, 2017). European rice production does marginally contribute to world production (0.4%), despite sustaining the safeguard of domestic demand (Krahemer, Thomas, & Vidotto, 2017) and playing a fundamental role in the maintenance of equilibrium and biological richness of flooded ecosystems (Fasola et al., 1996; Longoni, 2010).

Italy is the leading rice producing country in EU, with about 230,000 ha of cultivation area and 1.55 Mt of total grain production in 2017 (Ente Nazionale Risi, 2017). Italian rice area is concentrated in the north-western provinces of Pavia, Vercelli, and Novara (184,195 ha distributed over an area of 4000 km^2), which contribute to around 90% of the national production. Here, paddy rice cultivation is a high-input specialised agricultural activity, with homogenous agro-ecological conditions and management, i.e. direct seeding, continuous flooding with two or three drainages to allow rooting and top dress fertilisation (Sacco et al., 2006). Primary crop protection practices consist of chemical weed control (*Echinochloa* spp. and weedy rice) and disease management, mostly against *Magnaporthe grisea* (T.T. Hebert) M.E. Barr. The autochthonous rice varieties mostly belong to the *temperate japonica* ecotype and have been locally adapted through multiple breeding programs leading to an extensive, very rich, and diversified varietal landscape (Cai et al., 2013; Mongiano et al., 2018).

The social, cultural and economic relevance of Italian rice has stimulated numerous research programs aimed at providing decision-making support at each level of the rice supply chain, from rice growers to local and national stakeholders, including regional agencies and private companies (Basso, Cammarano, & Carfagna, 2013). Simulation modelling is among the most used research techniques in recent years, and its application in the area focused on, e.g., the comparative assessment of different crop models in current (Confalonieri et al., 2009) and future weather scenarios (Bregaglio, Hossard, et al., 2017a), the impacts of different water management strategies (Bocchiola, 2015), the assessment of the impact of rice blast disease on yield (Bregaglio et al., 2016), the forecasting of qualitative aspects of productions (Cappelli et al., 2018) and the development of *in silico* ideotyping studies (Paleari et al., 2015; Paleari, Bregaglio, Cappelli, Movedi, & Confalonieri, 2016). A distinctive trait of Italian rice agriculture is the richness of cultivated varieties (Tamborini, 2016b) encompassing a vast diversity of grain types (i.e., long B, long A, round, medium) anthocyanin colourations (i.e., black, purple, brown, orange, bronze), and aromatic rice. From a crop model perspective, the genotypic differences regulating phenological attributes (e.g., thermal requirements to reach vegetative and reproductive phases), physiological plant traits (e.g., specific leaf area, light extinction coefficient, radiation use efficiency) or morphological features (e.g., maximum plant height, root depth) are embedded into parameters, which are used in sub-models of plant processes concurring to determine the synthetic model outputs, e.g., yield (Bregaglio, Titone, et al., 2017b). Model parameters can thus be considered the mathematical representations of the gene effects under different environmental conditions, i.e. the "genetic coefficients" of the crop (Boote et al., 2001). It follows that crop models could be used profitably to analyse the non-linear G×E×M interactions occurring at yield level (Hammer et al., 2006).

Rice modellers managed the large heterogeneity of the Italian germplasm by developing parameters sets specific to groups of rice varieties sharing the same plant type (*indica* or *japonica* that, in this case, do not indicate the real ecotype) and time of maturity (Bocchi et al., 2006; Confalonieri et al., 2009; Confalonieri & Bocchi, 2005), using data from field or greenhouse experiments collected on few varieties (Bregaglio, Titone, et al., 2017b; Paleari et al., 2015). Nonetheless, this simplified approach does not take into account the large variability in genetic makeup (Faivre-Rampant et al., 2010; Mantegazza et al., 2008; Spada et al., 2004), yield potential (Ben Hassen et al., 2017) and economic value (Ferrero, 2007) of Italian rice varieties, with implications on the crop model applicability at regional or even at a larger scales (Angulo et al., 2013; Teixeira et al., 2017). The lack of morpho-physiological data to characterize the different cultivars and the high costs associated to experimental field trials to collect detailed data for model calibration hinder further improvements (Mavromatis et al., 2001), especially considering the large number of new rice cultivars annually released in Italy and their usually short commercial life (Tamborini, 2016b). Reducing the gap between the varietal traits and their model representation could also foster the implementation of advanced breeding strategies supported by crop models, as already accomplished in many studies (Collins et al., 2008; Hammer et al., 2006; Reymond et al., 2003; Tardieu & Tuberosa, 2010; YIN et al., 2003; 2005).

Here we propose and evaluate a methodology to develop cultivar-specific model parameter sets, requiring the availability of crop traits commonly measured in cultivar field trials (or value of cultivation use, VCU), i.e. phenology, canopy height, and yield. We used long-term VCU trials data to perform model (WOFOST-GT Stella et al., 2014) calibration and validation, and then we correlated the resulting parameter sets with common morphological descriptors conventionally used in the European rice area. The present study, whose methodology is meant to be transferable to other crops and crop models, releases thirteen model parameter sets representing the most cultivated Italian rice varieties in the period 2004-2015.

4.3 MATERIALS AND METHODS

The methodological workflow followed in this paper is summarised in Figure 4.1.

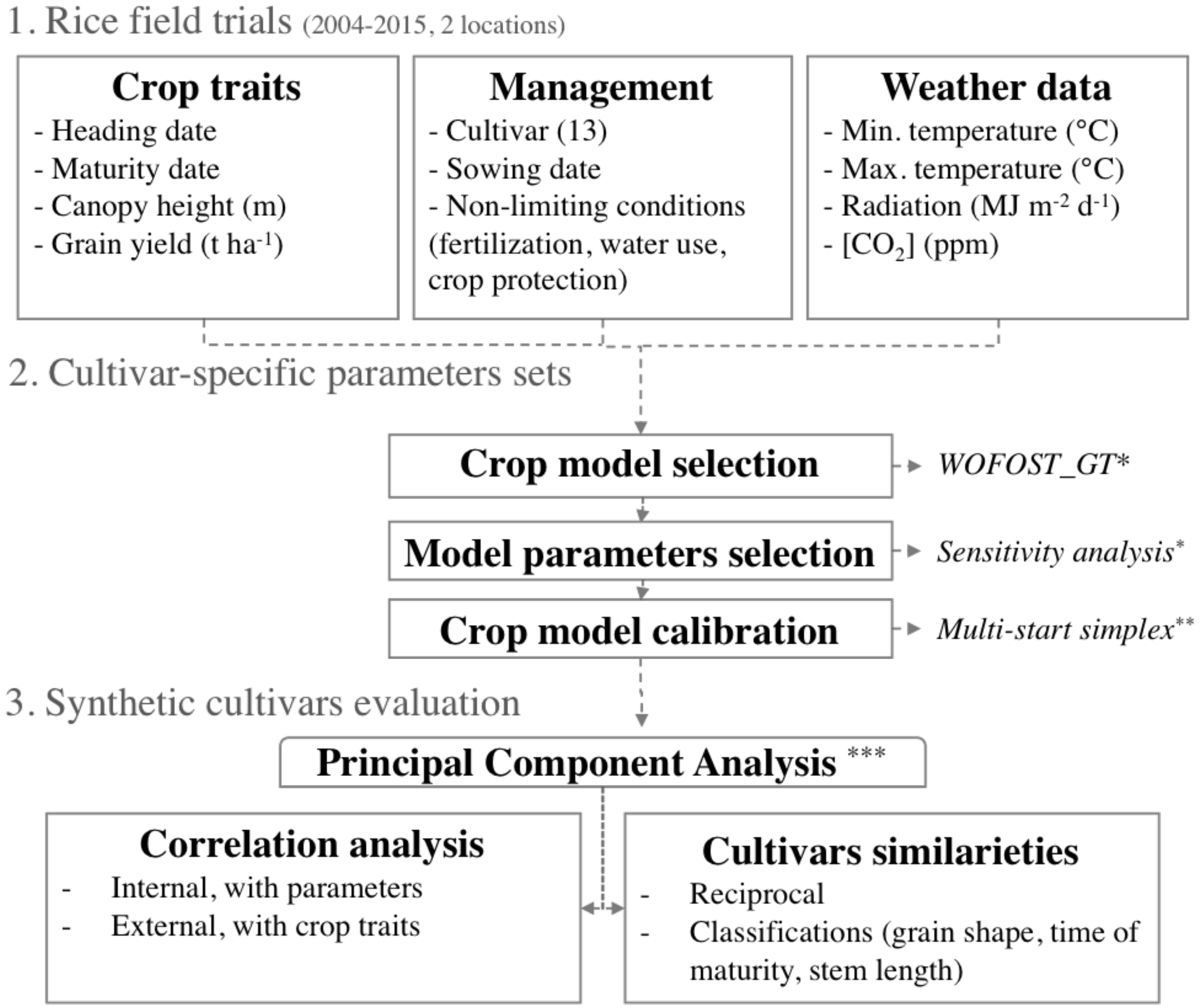


*Figure 4.1 – Graphic summary of the study workflow. * Revised version of WOFOST and sensitivity analysis taken from Stella et al. (2014); ** Downhill multi-start simplex algorithm used for the optimisation, as proposed by Nelder & Mead (1965); *** Principal Component Analysis performed with FactoMineR R Package, as illustrated in Lê et al. (2008).*

We used four crop traits collected during rice Value of Cultivation and Use trials in 2004-2015 at two sites, as reference data to characterise the thirteen Italian rice cultivars. Crop traits were the date of heading (HEADING, days from sowing to heading) and maturity (MATURITY, days from heading to maturity), the canopy height (CANOPY, m) and the grain yield (YIELD, t ha^{-1}). We assumed that the experimental trials were conducted in non-limiting conditions for the crop, as prescribed by VCU protocols. The seasonal weather data were recorded by stations placed in proximity with the field. The WOFOST_GT crop model was chosen because it was parameterized in the area, and its sensitivity to parameters changes was already assessed (Stella et al., 2014). We selected the most relevant parameters highlighted by the sensitivity analysis as the "genetic coefficients" to develop cultivar-specific model parameter via automatic optimisation of their values. The optimisation algorithm was targeted at minimising the crop model error in reproducing field data. A Principal Component Analysis was applied on the developed parameters sets allowing a multivariate assessment of the associations between parameters and with the original crop traits. Principal Components Analysis was also used to investigate the similarities between the synthetic cultivars and to compare our results with available classifications of grain shape, time of maturity and stem length.

4.3.1 LONG-TERM EXPERIMENTAL DATA

EXPERIMENTAL DESIGN

The field data used for model calibration and validation came from the VCU tests performed during 2004-2015 by the Research Centre for Plant Protection and Certification (CREA-DC) of the Council for Agricultural Research and Economics, which is the national authority for the registration of new agricultural varieties to the Common Catalogue. VCU tests aim at comparing the agronomic performance of the candidate varieties to well-established reference varieties, by testing them in typical environments for the crop of interest in the country of origin. The design was a complete randomised block with three replications, with an experimental unit consisting of a 45 m^2 plot sown at a fixed plant density. Rice sowing was performed by manual broadcast in flooded paddies, with specific seed rates for each cultivar, adjusted according to thousand seeds weight and sample germinability, to reach a density of 500 plants m^{-2}. The experiments were selected to comprise the same cultivar for at least two years in the two sites of Collobiano and Garbagna Novarese, placed in the main Italian rice district. Collobiano trial (Vercelli Province) was located at 45°24'12.2"N 8°21'48.4"E, the soil was medium loam with 2.76% organic matter, high CEC, and rich in phosphorous. The soil texture in Garbagna Novarese (Novara province, 45°23'31.8"N 8°39'59.3"E) was similar but silty loam and with slightly lower content of organic matter (2.16%). The protocols and guidelines for the executions of VCU tests prescribe optimal growing conditions targeted at limiting biotic and abiotic stress (Ministero delle Politiche Agricole Alimentari e Forestali, 2014), therefore we performed simulations at potential production level (de Wit et al., 2018), assuming the absence of water and nutrients limitations to crop growth. The rice cultivars used in this study were used during the period 2004-2015 in VCU tests as reference varieties (Supplementary Table 4.1).

167 field experiments (cultivars × site × year combinations) were used for model calibration. Some experiments were removed from the dataset as suggested by CREA-DC field technicians due to abiotic damages determining non-potential conditions to rice growth and development.

FIELD DATA COLLECTION

Field samplings aimed at collecting the most relevant agronomic crop traits as indicated in the guidelines and technical protocols used in VCU tests (Ministero delle Politiche Agricole Alimentari e Forestali, 2014). These traits were coded as HEADING, MATURITY, CANOPY, and YIELD. HEADING is the number of days from the date of sowing to the date of heading: visual assessments were performed weekly on the whole plot, and the day of heading was set when more than 50% of the plants showed emerging panicles. MATURITY is the number of days from the date of heading to the date of maturity, determined when grain moisture reached 22% of relative humidity. This trait was determined either by field samplings of grain moisture in plots (using a portable OGA "TA-5" digital grain moisture analyser) or by laboratory analysis with a thermogravimetric scale (Sartorius "MA 150"). CANOPY (m) was measured on 20 plants per plot with a ruler, summing the stem and the panicle length, the former corresponding to the plant elongation from the root crown to the panicle insertion and the latter from panicle insertion to its tip. This variable was considered as a proxy of the real canopy height, besides the lack of consideration of the panicle bearing. YIELD (t ha^{-1}) was determined by harvesting the plot one week after maturity with a plot combine. Plot edge (1 m) was removed to avoid interferences with neighbouring plots. The grain biomass was weighted, and moisture determined to estimate dry weight. Appropriate calculations were applied to determine the equivalent yield expressed as t ha^{-1} of dry matter. All the experimental methodology used in the field trials complied with the standard protocol detailed in national regulations regarding registration of new cultivars (Ministero delle Politiche Agricole Alimentari e Forestali, 2014).

4.3.2 CROP MODEL APPLICATION

The crop model used in this study was WOFOST_GT (Stella et al., 2014), which revised the original WOFOST model (Van Diepen et al., 1989) by reducing the number of parameters and providing a more detailed representation of the canopy architecture. This crop model simulates rice development and growth as a function of primary plant eco-physiological processes, including phenological development, light interception, gross photosynthesis, transpiration, growth and maintenance respiration losses, and partitioning of assimilates to plant organs. The choice of this model was driven by the availability of a dedicated sensitivity analysis assessment, followed by the model calibration for rice crop in the same study area (Stella et al., 2014). The sensitivity analysis drove the selection of parameters that could differentiate rice varieties (Table 3 of the seminal paper). The simulation of vegetative and reproductive phases was performed using average daily temperature as the input of a non-linear function, with 12 °C as the base, 28° C as the optimum and 42° C as the maximum critical temperature for phenological development. The accumulation of thermal time was normalized to a development stage code (DVS), with integers representing key phenological phases, each characterised by degree-days thresholds defining the thermal requirements from emergence to anthesis (DVS = 2, GDDant, °C days^{-1}) and from anthesis to maturity (DVS = 3, GDDmat, °C day^{-1}). Gross photosynthesis has been estimated three times per day, using intercepted radiation as input for the light response curve of individual leaves, driven by the maximum leaf CO_2 assimilation (A_{max}, kg ha^{-1} h^{-1}). The canopy light interception was estimated considering the total incoming radiation, a dynamic canopy extinction coefficient decreasing from emergence ($KDIF_{max}$, unitless) and tillering, and the photosynthetically active leaf area. Daily expansion of leaf area index was estimated using an exponential function driven by temperature and its maximum relative increase (RGRLAI, m^2 m^{-2} day^{-1}) during early stages, then multiplying the daily increase in leaves dry weight by specific leaf area. The dynamic of specific leaf area during the growing season was defined by a function decreasing from a maximum value at emergence (SLA_{em}, m^2 kg^{-1}) to a lower asymptote after tillering (SLA_{till}, m^2 kg^{-1}). The decrease in leaf area after flowering has been simulated considering the effect of self-shading and senescence processes, the latter triggered by physiological age (SPAN, days). The canopy was split into a dynamic number of layers depending on DVS, assuming two layers from emergence to early stages and maximum layer emission rate at tillering (DVS ~ 0.25-0.35), while it starts decreasing during stem elongation, to reach 20 layers at flag leaf emission stage. Plant height was computed as a function of DVS, using the maximum plant height (H_{max}, m) to drive a logistic function. Maintenance and growth respiration reduced the daily gross carbon assimilation, the former derived by the dry weight ratios of the different plant organs modulated by a fixed Q10. Remaining carbohydrates were converted to structural carbohydrates and partitioned to plant organs with different efficiencies (e.g., CVO, the efficiency of conversion into storage organs, unitless), corresponding to the growth respiration expenses. The WOFOST_GT model was further modified in this study by setting the thermal requirements of rice growth according to Sánchez, B., Rasmussen, A., & Porter, J. R. (2014) who derived them by literature search and assuming no differences among the tested varieties. Cardinal temperatures in the different phases were separately defined for the emergence-tillering period (DVS<1.25, T_{min} = 10.5 °C, T_{opt} = 29.7 °C, T_{max} = 40 °C), tillering-panicle initiation (1.25<DVS<1.6, T_{min} = 16.4 °C, T_{opt} = 28.4 °C, T_{max} = 35.3 °C), panicle initiation-heading (1.6<DVS<1.9, T_{min} = 14.9 °C, T_{opt} = 26.7 °C, T_{max} = 33.1 °C), heading-anthesis (1.9<DVS<2.1, T_{min} = 14 °C, T_{opt} = 24.3 °C, T_{max} = 36.9 °C) and anthesis-grain filling (DVS>2.1, T_{min} = 17.9 °C, T_{opt} = 21.2 °C, T_{max} = 31.3 °C). The CO_2 response function originally developed in the STICS model and used in a recent rice crop modelling study in the area (Bregaglio, Hossard, et al., 2017a) was also included in WOFOST_GT to consider the fertilisation effect of atmospheric carbon dioxide, to prepare the model for future applications in climate change scenarios.

4.3.3 DEVELOPMENT OF CULTIVAR-SPECIFIC PARAMETER SETS

The cultivar-specific parameter sets were derived by the optimisation of the most relevant model parameters emerging from the sensitivity analysis carried out by Stella *et al.* (2014). The first step of model calibration targeted the phenological development of the tested cultivars, as suggested by (2018). We fixed the thermal requirement to reach emergence phase at 100 °C day^{-1} for all the varieties tested, due to lack of availability of observations for this phenological phase. The two parameters GDDant and GDDmat were subjected to automatic optimization using the version of the multi-start downhill simplex algorithm (Nelder & Mead, 1965), proposed by Acutis and Confalonieri (2006). This widely used optimization method (Confalonieri et al., 2016; Pagani et al., 2018; Wallach et al., 2001) explore the *p*-dimensional parameters space, with *p* as the number of the parameters under evaluation, following the gradient of the objective function, which was the relative root mean square error between predictions and observations (RRMSE, %). We used ten simplexes at each optimization run to minimize the risk of avoiding global *minima*, with 1000 as the maximum number of iterations for each simplex, and a RRMSE tolerance of 10^{-6}. Once the phenological parameters were adjusted, the parameters connected to growth processes were optimized with the same methodology, using the CANOPY (m) and YIELD (t ha^{-1}) as reference variables. The ranges used in the automatic optimisation, together with the calibrated values for the thirteen rice varieties are listed in Supplementary Table 4.2.

We decided to use all the available datasets for parameters optimization, without performing a separated calibration and evaluation of model performances, in order to take advantage of the maximum variability captured in field experiments. Model performances after parameter optimisation were evaluated using RRMSE plus additional evaluation metrics widely used in the assessment of crop models performances.

4.3.4 CORRELATIONS IN MODEL PARAMETER SETS AND ASSOCIATIONS WITH MORPHOLOGICAL CLASSIFICATION.

We performed a Principal Component Analysis with a twofold purpose: a) analysing crop model internal (i.e. among parameter sets) and external (i.e. between parameters sets and the crop traits) correlations and b) evaluating the similarities in the developed parameters sets compared to cultivar characteristics by investigating associations with common morphological descriptors. PCA was performed on the centred and scaled parameter sets, by diagonalization of the correlation matrix and extraction of the associated eigenvector and eigenvalues. We adopted the procedure proposed by Husson *et al.* (2010b) that allows the introduction of supplementary variables in the analysis, i.e. "illustrative elements" (Lê et al., 2008). Contrarily to the variables targeted by PCA, supplementary variables did not participate in the computation of Principal Components (PCs) and are used as supporting information. We used both quantitative and qualitative supplementary variables in PCA. Supplementary quantitative variables were the averages of the four crop traits (HEADING, MATURITY, CANOPY, and YIELD), introduced to highlight their associations with crop model parameters. The simultaneous evaluation of both active and supplementary quantitative variables has been performed by examining the correlations between the variables and the PCs.

The supplementary qualitative variables were the three common morphological classifications used to group rice varieties in Italy, i.e. grain shape, time of maturity, and stem length. The cultivars were categorised by grain shape (according to EU Regulation 1308/2013 rice is classified on the basis of the length/width ratio of the grain in groups “long A”, “long B”, “medium”, and “round”, (European Parliament, 2013)), time of maturity (early, medium, and late), and stem length (short, intermediate, long, very long), the last two according to their official morpho-physiological description (Community Plant Variety Office, 2012; Ministero delle Politiche Agricole Alimentari e Forestali, 2014). These classifications were adopted to check whether the model complied to reproduce the marked phenotypic differences among the groups (Mongiano et al., 2018). To accomplish this, several one-way ANOVA models were formulated to see if the categorical variables could predict the coordinates of individuals on each PCs. Then, the significance of each category was tested (t-test) to see if its mean coordinates on the PCs was different from the overall mean. The values of all the supplementary variables (crop traits and morphological classifications) are reported in Supplementary Table 4.1. All the analyses were performed in the R software environment (R Core Team, 2017), making use of the functions contained in the "FactoMineR" R package (Lê et al., 2008), while graphical representations relied on the "ggplot2" (Wickham, 2009) R package.

4.4 RESULTS

4.4.1 MODEL PERFORMANCES IN REPRODUCING FIELD REFERENCE DATA.

We developed thirteen parameters sets of the WOFOST_GT model, corresponding to the most cultivated Italian rice cultivars of the last twenty years. The overview of model performances in reproducing phenological development, canopy height and yield of the thirteen cultivars is reported in Table 4.1.

*Table 4.1 – Summary of model performances in reproducing field observations of phenological development (HEADING and MATURITY, days), CANOPY (m) and YIELD (t ha^{-1}). For each reference variable the average value ($\overline{x}$) and coefficient of variation (CV), of observed (obs) and simulated (sim) is reported, along with Pearson r and associated significance (Sig.), mean bias error (MBE), root mean square error (RMSE) and relative root mean square error (RRMSE). Significance codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

Crop trait	Unit	$\overline{x}_{obs}$	CV_{obs}	$\overline{x}_{sim}$	CV_{sim}	r	Sig.	MBE	RMSE	RRMSE
HEADING	days	88	7.45 %	86	7.85 %	0.69	* * *	-1.90	5.62	6.37 %
MATURITY	days	32	24.48 %	33	16.62 %	0.47	* * *	0.21	7.19	22.25 %
CANOPY	m	0.83	15.69 %	0.82	15.27 %	0.79	* * *	-0.01	0.08	10.00 %
YIELD	tha^{-1}	6.17	13.93 %	6.20	11.27 %	0.46	* * *	0.02	0.82	13.28 %

WOFOST_GT predicted field observations with RRMSEs ranging between 6.37% (HEADING) and 22.25% (MATURITY), generating lower variance for traits MATURITY and YIELD (46.3% and 23.2% less, respectively) than in the reference dataset. The correlation coefficients between observed and simulated data were highly significant for each crop trait ($p < 0.001$).

The simulated average duration of the growing cycle was very similar to the observations ($\bar{x}_{sim}$ = 119 days, $\bar{x}_{obs}$ = 120 days), split in 86 days for vegetative stage (sowing – heading, HEADING) and 33 for ripening stage (heading – maturity, MATURITY), the latter being much more variable than the vegetative stage (CVs of 7.45% and 24.48% respectively). The variability of HEADING in simulations matched field measurements (CV_{obs} = 7.85%, CV_{sim} = 7.45%), with a systematic model underestimation of two days (Mean Bias Error, MBE = -1.9 days). The correlation coefficient between observed and simulated data (r = 0.69) was highly significant ($p < 0.001$), with 5.62 days of RMSE (RRMSE = 6.37%). The simulated variability for the trait MATURITY was larger than in observations, with RMSE equal to 7.19 days (RRMSE = 22.25%) and low bias (MBE = 0.21 days).

The thirteen cultivars were largely different in CANOPY, with a very similar CV in observations (15.69%) and consistently in simulations (15.27%). This trait was the most efficiently predicted by the model, with highly significant correlation (r = 0.79, $p < 0.001$) and very low RMSE (0.08 m), RRMSE (10%) and MBE (-0.01 m). A considerable inter-cultivar (and seasonal) yield variability was present in field observations (CV_{obs} = 13.93%), but the model slightly underestimated it (CV_{sim} = 11.27%), despite observed and simulated data were significantly correlated ($r = 0.46$, $p < 0.001$) with small associated error (RMSE = 0.82 t ha^{-1}, RRMSE = 13.28%). The distributions of reference and simulated data for the thirteen cultivars are compared in Figure 4.2.

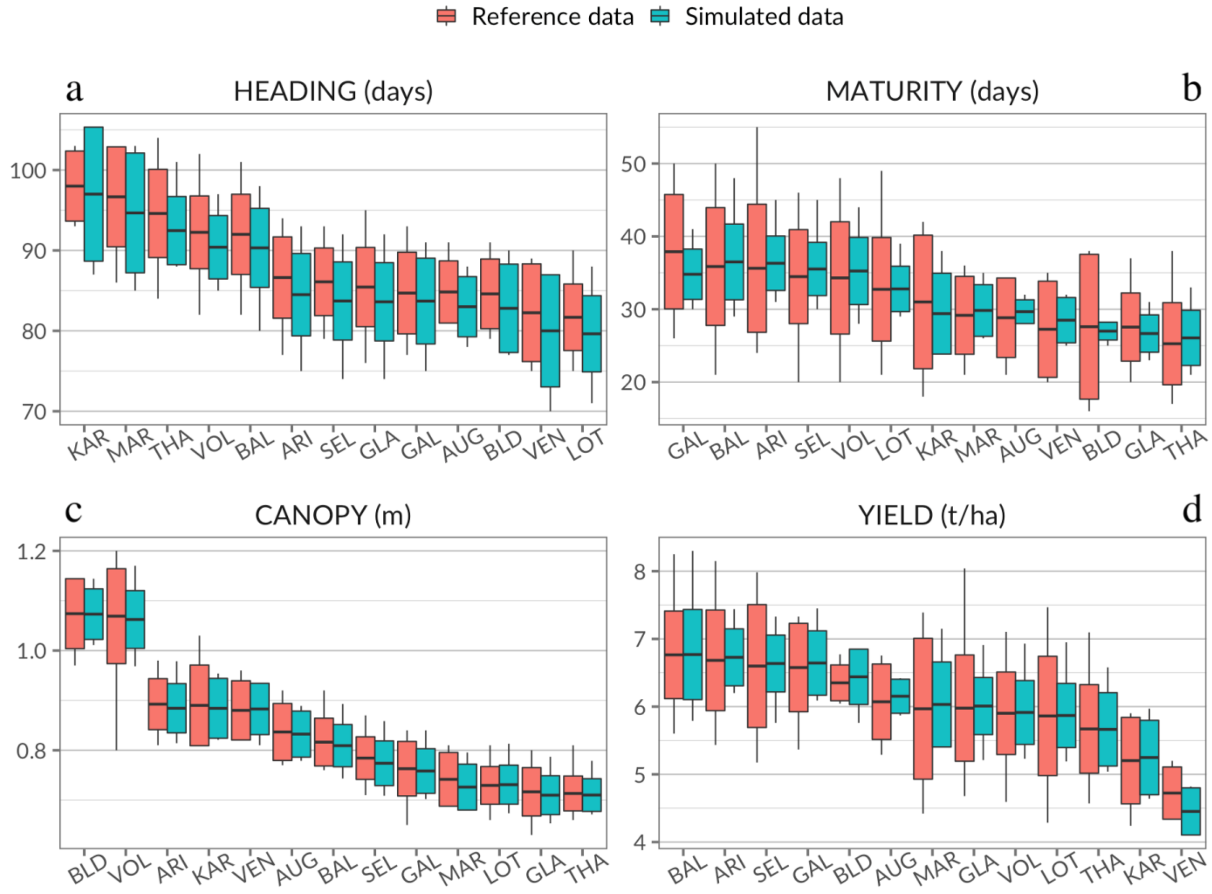


Figure 4.2 – Observed (red boxplots) and simulated (grey boxplots) variability of the traits Sowing-Heading (a, HEADING), Heading-Maturity (b, MATURITY), Canopy height (c, CANOPY) and yield (d, YIELD) in the thirteen rice cultivars, whose acronyms are explained in Supplementary Table 4.1.

The parameters values obtained from the automatic optimisation proven to be sufficient in differentiating cultivar trends of the considered traits (Figure 4.2) with RRMSE ranging between 4.1% (AUG, HEADING) and 31.7% (BLD, MATURITY) depending on the trait and the number of experiments (Supplementary Table 4.3).

The observed duration of vegetative stage (HEADING, Figure 4.2a) ranged from mean values of 80 days (LOT) to 102 days (KAR), and resulted as the most accurately predicted with RRMSE varying from 4.05% (AUG) to 8.07% (BLD) and MBE ranging from -1 (GAL and KAR) to -2.38 (SEL).

We can hypothesise that the lower model performance in reproducing heading - maturity period (MATURITY, Figure 4.2b) could be due to the larger variability generated by the effect of water availability on the dry-down processes of the grains during the ripening stage (De Datta, 1981), as trial paddies were discharged at different times after milk stage. Consequently the model underestimated the variance of the observed duration of the ripening stage, with RRMSE ranging from 16.2% (AUG) to 31.7% (BLD). The simulations denoted a high accuracy in reproducing mean canopy height and associated variability (CANOPY, Figure 4.2c) among cultivars (min. THA, RRMSE 7.92%; max. KAR, RRMSE 12.9%), which were short to medium height (less than 0.8 m), except for VOL and BAL ($\bar{x}_{obs}$ = 1.07 m). During field experiments, the round grain cultivars BAL and SEL were the most productive (average YIELD 6.68 t ha^{-1}), while VEN (the only medium grain) was the least productive, with maximum observed yields of 5.2 t ha^{-1}. GAL (long A for domestic market) and ARI (long A for parboiling) also obtained similar yields than round grain varieties. This wide range in the productivity levels (Figure 4.2d) was reproduced by WOFOST_GT with variable accuracy, leading to RMSEs comprised between 0.28 and 1.1 t ha^{-1}. Lower prediction errors (RMSE lower than 0.65 t ha^{-1}) were associated to cultivars presenting a small number of available experiments (VEN, 4: BLD and KAR, 5; AUG and MAR, 6) and to BAL and VOL (22 and 20 experiments), which obtained RMSE of 0.84 and 0.63 t ha^{-1}, respectively (RRMSE 12.5% and 10.6%); on the contrary, the model did not completely reproduce the whole yield variability for LOT and SEL (19 and 21 associated experiments), which were characterised by larger model errors (0.95 and 1.1 t ha^{-1}, respectively). The thirteen cultivar-specific parameters sets, each composed by ten parameters (Stella et al., 2014), are presented in Table 4.2. The ranking of cultivars with respect to GDDflo, GDDmat and Hmax values were consistent to the observations of HEADING, MATURITY, and CANOPY, respectively, suggesting a direct relationship of these parameters with the observed traits.

Table 4.2 – Cultivar-specific parameter sets resulting from automatic optimisation of model parameters using field observations on cycle length (HEADING, MATURITY), canopy height (CANOPY) and yield (YIELD) as reference data. The acronyms of the cultivars are explained in Supplementary Table 4.1, whereas the ones of parameters are reported in Supplementary Table 4.2, together with units and description.

Variety	GDDflo	GDDmat	Amax	RipL0	CVO	SLAem	KDIFmax	SPAN	RGRLAI	Hmax
ARI	855	475	22.16	0.89	0.77	23.0	0.51	42.4	0.0058	0.78
AUG	822	363	26.05	0.87	0.68	25.2	0.57	22.9	0.0100	0.85
BAL	937	434	20.05	0.84	0.76	23.0	0.50	29.6	0.0075	0.68
BLD	810	348	19.47	0.90	0.77	29.7	0.50	27.4	0.0098	1.00
GAL	852	451	23.06	0.89	0.76	22.0	0.56	44.9	0.0060	0.67
GLA	842	347	26.44	0.55	0.71	23.6	0.50	41.0	0.0068	0.65
KAR	1036	331	20.96	0.75	0.77	25.6	0.58	23.0	0.0081	0.63
LOT	786	427	20.95	0.55	0.69	29.9	0.50	38.8	0.0076	0.71
MAR	1004	340	27.68	0.90	0.75	27.8	0.50	24.0	0.0085	0.69
SEL	842	452	22.10	0.84	0.71	25.9	0.53	24.0	0.0100	0.77
THA	969	315	25.77	0.77	0.77	22.3	0.50	20.8	0.0093	0.80
VEN	778	371	16.91	0.90	0.77	29.9	0.60	44.0	0.0062	0.79
VOL	920	420	21.39	0.81	0.76	22.1	0.50	21.0	0.0089	1.03
Mean	881	390	22.54	0.81	0.74	25.4	0.53	31.1	0.0080	0.77
StDev	84	54	3.15	0.12	0.04	3.1	0.04	9.6	0.0015	0.13

The thermal requirement for the heading stage (GDDflo, °C day^{-1}) varied in the optimised parameters sets from 777 (VEN) to 1036 °C day^{-1} (KAR), while from 314 (THA) to 474 °C day^{-1} (ARI) for the ripening stage (GDDmat, °C day). The relative contribution of the ripening stage to the whole cycle length was largely variable and ranged from 24% (KAR) to 36% (ARI). The average value of Amax in cultivar-specific parameter sets was 22.5 kg ha^{-1} h^{-1} with substantial variation among cultivars (CV = 14%) and ranged between 16.9 (VEN) and 27.7 (MAR) kg ha^{-1} h^{-1}. The parameter CVO, corresponding to the efficiency of assimilates conversion into storage organs presented two distinct clusters of calibrated values around 0.7 and 0.76 kg kg^{-1}. A similar situation occurred for SPAN (lifespan of leaves, days), presenting a bi-modal distribution with peaks at 26.76 and 33.75 days, and RipL0 (partitioning of assimilates to leaves at emergence, unitless) which showed two clusters, with optimized values near to the superimposed limits (e.g. GAL, ARI, MAR, BLD, VEN at the upper limit of 0.9; GLA and LOT at the lower limit of 0.55). Calibrated values close to the lower limit (0.5) also emerged in KDIFmax (maximum extinction coefficient for diffuse visible light) for VOL, THA, BLD, BAL, MAR, LOT, GLA, ARI; on the contrary, VEN had a calibrated value of 0.6, corresponding to the upper limit. RGRLAI (maximum relative increase in LAI), SLAem (specific leaf area at emergence), and Hmax (maximum plant height) were more distributed within their optimisation range, with only a few values converging at the constraints. The automatic optimisation was repeated multiple times (20 repetitions with ten simplexes and 1000 iterations) to evaluate the robustness and the replicability of the obtained results: the outcomes of repeated optimisations are presented in Supplementary Figure 4.1 – Mean and standard error of the calibrated values resulting from the repetition 40 independent optimisation of growth.. The parameters sets obtained were consistent among runs of optimisation, with small differences for most cultivars. The broader variability was associated with parameters SLAem and CVO, especially for varieties with a very high or low number of experiments (e.g. GLA, LOT, SEL).

4.4.2 MULTIVARIATE EVALUATION OF PARAMETERS SETS.

CORRELATIONS BETWEEN PRINCIPAL COMPONENTS AND MODEL PARAMETERS

We performed a Principal Component Analysis (PCA) on the cultivar-specific parameters sets to evaluate (i) the linear relationships among parameters and (ii) the similarities between cultivars, considering the classifications of grain shape, time of maturity, and stem length as supplementary variables (Figure 4.1, step 3). Figure 4.3 reports the PCA biplot of variables including model parameters (black colour) and crop traits (grey), the latter considered as supplementary variables (‘supplementary’ meaning that these variables were introduced after the computation of Principal Components space to support the analysis, see Methods section).

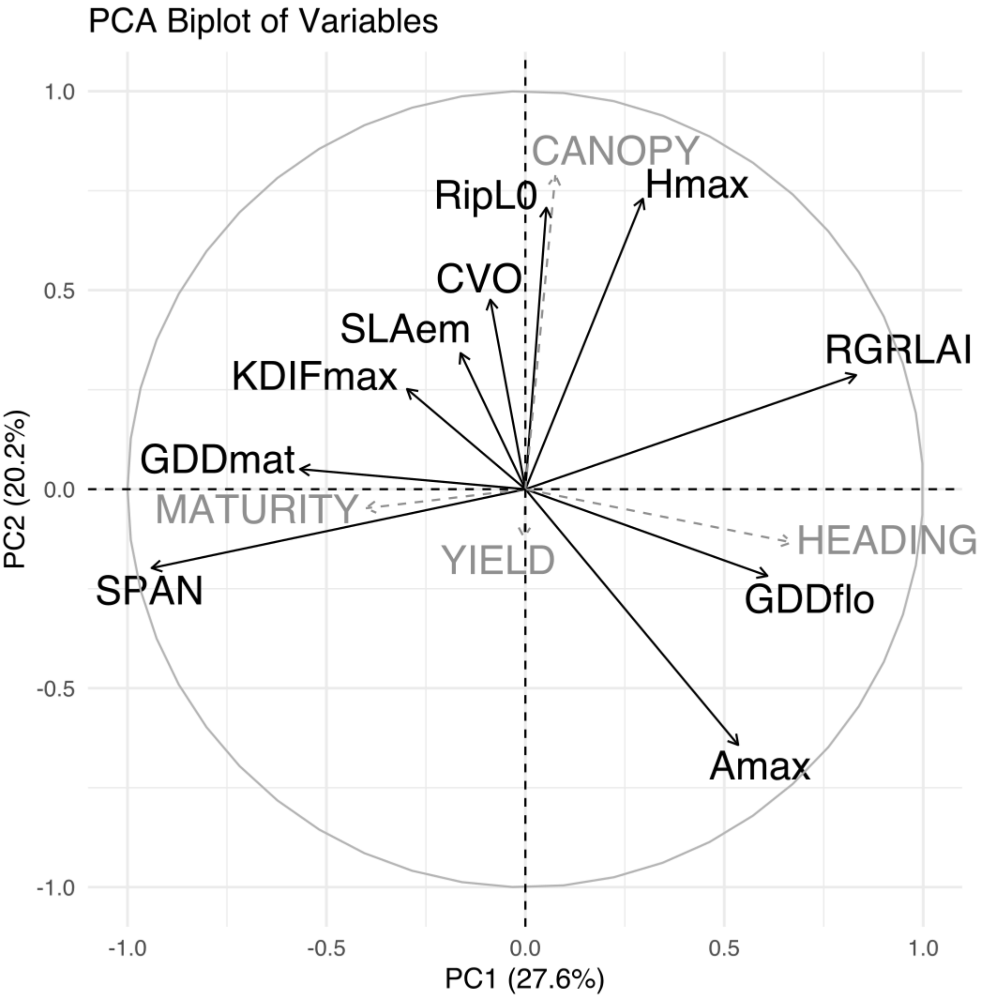


Figure 4.3 – PCA biplot of variables showing active variables (i.e., model parameters, coloured in black) and supplementary variables (i.e. crop traits, coloured in gray).

We retained the first four components for analysis (79.57% of the total variance) which were then correlated and tested for significance with the model parameters and the supplementary variables (Supplementary Table 4.4). Principal Components result as the multilinear combination of the variables from which they are calculated, therefore, testing the significance of their correlation has no meaning, but it was used here to rank the relevance of the variables (Husson, Lê, & Pagès, 2010a). The Principal Component 1 (PC1) was mostly correlated with SPAN (r = -0.94) and RGRLAI (r = 0.83), and to a lesser extent with GDDflo (r = 0.6) and GDDmat (r = -0.57). The crop traits associated with the phenological parameters, HEADING and MATURITY, obtained similar and higher correlation coefficients (r = 0.67, p = 0.01; r = -0.75, p = 0.003). Along PC1, the cultivars with high coordinates presented high values of RGRLAI, short ripening duration (low GDDmat) and long vegetative development (high GDDflo). PC2 was positively associated to Hmax (r = 0.73), and RipL0 (r = 0.71), and negatively with Amax (r = -0.64); PC2 was also significantly correlated with CANOPY (r = 0.79, p = 0.001). These results indicated that the cultivars with higher Amax were in general short (low Hmax) and with low partitioning to leaves at emergence (low RipL0). Hmax and the associated trait 'canopy height' had similar correlation coefficient with PC2, similarly to what observed for phenological parameters and PC1. PC3 primarily correlated with CVO (r = 0.77) and SLAem (r = -0.64), highlighting an inverse relation between those traits in calibrated parameter sets; GDDflo (r = 0.64) and Heading (r = 0.61, p = 0.02) were also positively correlated with PC3, suggesting that synthetic cultivars with high CVO had lower SLAem and long duration of the vegetative period (high GDDflo). PC4 highlighted the inverse relation between KDIFmax (r = 0.685, p = 0.01) and GDDmat (r = -0.66, p = 0.01); YIELD was also inversely related with PC4 indicating that high-yielding cultivars presented longer duration of the ripening stage.

COMPARISON OF CULTIVAR-SPECIFIC PARAMETERS SETS WITH AVAILABLE CLASSIFICATIONS

We assessed the similarities among cultivars by analysing their reciprocal distance on the PC biplot (Figure 4.4).

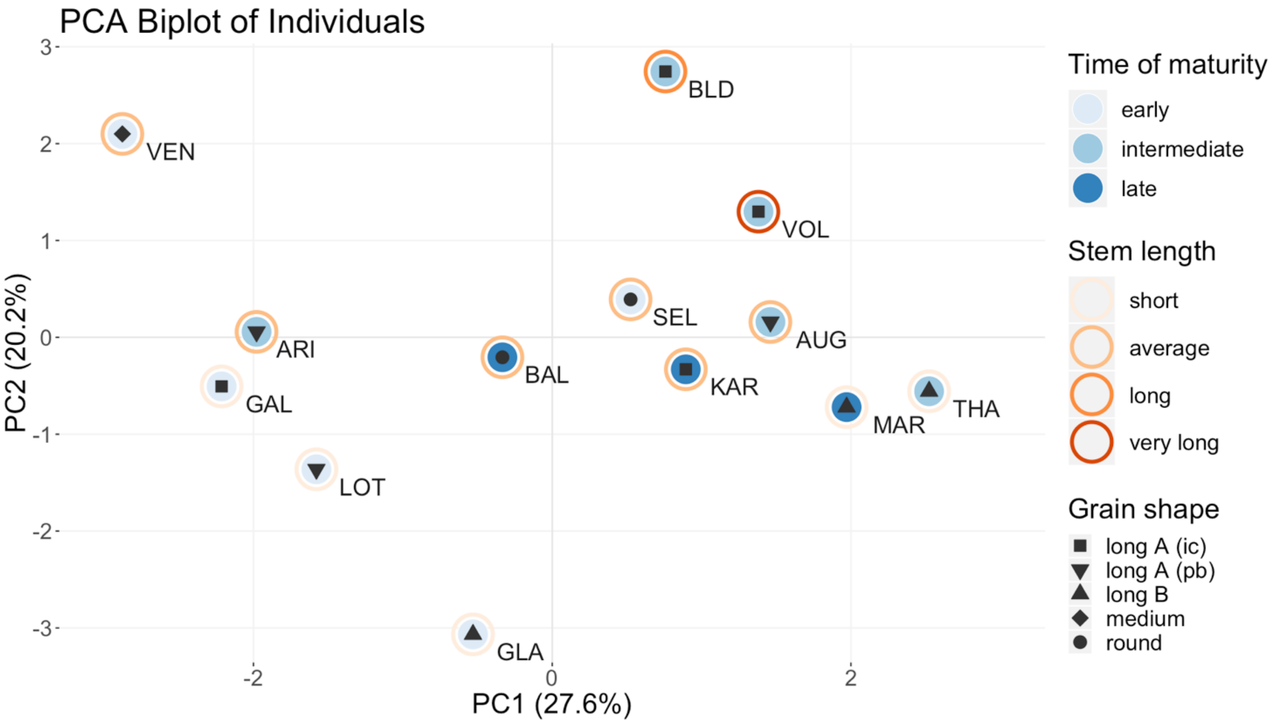


Figure 4.4 – PCA biplot presenting the coordinates of the rice varieties in the Principal Components space. Each variety is labelled with three graphical elements characterizing the grain shape (symbols), the length of the growing cycle (blue shade) and the stem length (red circles).

Available classifications of rice cultivars based on grain shape, time of maturity, and stem length were overlapped to the biplot, to unravel patterns of similarieties within the optimised parameter space (Figure 4.4 and Figure 4.5). The average values of optimised parameters grouping the cultivars by grain shape, time of maturity, and stem length were also calculated to compare our results with available rice modelling studies (Supplementary Table 4.7).

Long B grain varieties, i.e. MAR, THA, and GLA, were clearly separated from the other grain shapes on PC2 (negative values) and PC1 (positive values, except for GLA). These cultivars were characterised by high Amax (mean 26.63 kg ha^{-1} h^{-1}), low KDIFmax (mean 0.501) and reduced plant height (mean Hmax = 0.71 m). The cultivars MAR and THA were very close on the biplot, consistently with their phenotype (i.e., time of maturity, plant height, grain shape, yield potential). These two varieties were characterised by high thermal requirements for the heading stage (968 and 1002 °C day^{-1}, respectively) and low ripening duration (324 and 331 °C day^{-1}); also, they obtained low SPAN (20.1 and 23.9 days) and large RGRLAI (0.00941 and 0.00991 m^2 m^{-2} day^{-1}). The other long B cultivar, GLA, positioned quite far on the biplot due to its smaller thermal requirement for heading stage (GDDflo 841 °C day^{-1}) and longer duration of the ripening period (GDDmat 350 °C day^{-1}), besides a longer life-span of leaves (SPAN 44.7 days), and lower value of RGRLAI (0.00677 m^2 m^{-2} day^{-1}) and RipL0 (0.560).

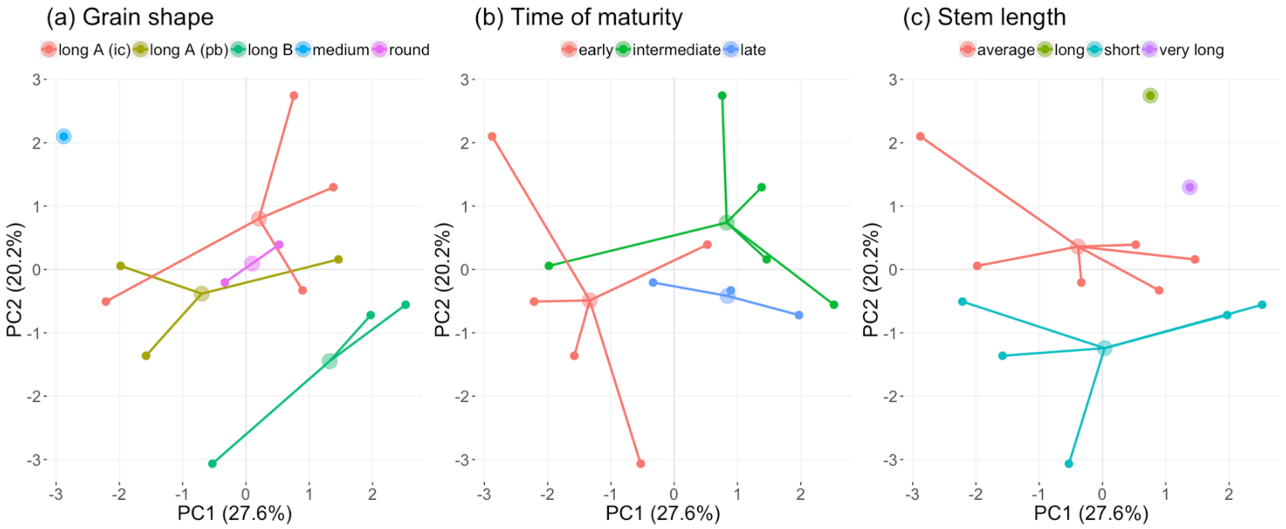


Figure 4.5 – PCA biplots highlighting clusters of rice varieties based on grain shape (a), time of maturity (b) and stem length (c). Each point in the space corresponds to a rice cultivar, which has been characterized in Figure 4.4.

'Long A' grain varieties were scattered along PC1 and PC2, with coordinates ranging from -2 to 2 and from -1 to 3, respectively. Specifically, 'Long A' cultivars intended for the Italian market (BLD, KAR, VOL, and GAL, i.e. mostly sold in the Italian market and intended for the preparation of traditional dishes like risotto) positioned on the positive side of PC1, except GAL. The similarities between these cultivars were limited to low Amax (mean 21.22 kg ha^{-1} h^{-1}) and high CVO (mean 0.7668). The phenological development of these varieties markedly varied in this group, with BLD (GDDflo 809.9 °C day^{-1}, GDDmat 348.3 °C day^{-1}) and KAR (GDDflo 1036 °C day^{-1}, GDDmat 331.5 °C day^{-1}) considered as early and late, respectively. Stem length was also very variable within Long A cultivars, covering all the possible classes of expression from 'very long' (VOL) to 'short' (GAL); Italian traditional cultivars were very tall (like VOL and BLD) but their size has been greatly reduced in recent years (Mongiano et al., 2018), even with the use of chemical mutagenesis (KAR). Our results were congruent, since the parameter Hmax largely varied, from 0.62 to 1.03 m. Coherently, RGRLAI was high for tall cultivars (BLD and VOL, 0.00981 and 0.00894 ha ha^{-1}), and lower for short cultivars (KAR and GAL, 0.00808 and 0.00598 ha ha^{-1}).

AUG, ARI, and LOT are also classified as Long A grain, but they were bred for the production of parboiled rice, resulting over the years in a phenotypically distinct group compared to cultivars for domestic consumption. These cultivars were characterised by small thermal requirements for heading (GDDflo 821 °C day^{-1}) and reduced Hmax (0.78 m) compared to Long A for Italian market. The related parameter sets were heterogeneous, coherently with their considerable variability in phenology, morphology, physiology, and productivity attitude. However, ARI and LOT placed at similar coordinates on the biplot, likely due to their similar ripening duration (GDDmat 475 and 427 °C day^{-1}, respectively). On the contrary, AUG which had lower thermal requirements for the completion of vegetative and ripening cycle was at opposite coordinates on PC1. The cultivar VEN, the only representative of ’medium’ grain shape, was isolated on the biplot with positive coordinates on PC1 and negative on PC2. The associated parameter set presented low thermal requirements (GDDflo = 778 °C day^{-1}, GDDmat = 371 °C day^{-1}) and limited photosynthetic capacity (Amax = 16.9 kg ha^{-1} h^{-1}) and canopy expansion (RGRLAI = 0.00618 m^2 m^{-2} day^{-1}), with long life-span of the leaves (SPAN = 44 days), high specific leaf area at emergence (SLAem = 29.93 m^2 kg^{-1}), efficient conversion into storage organs (CVO = 0.775), and the highest canopy light extinction coefficient (KDIFmax = 0.6).

Round grain cultivars (BAL and SEL) obtained high yield levels in simulations and field trials and were characterised by a long growing cycle and medium stem length. Their synthetic description in the parameters sets reflected these features, particularly on GDDflo (889 °C day^{-1}), GDDmat (443 °C day^{-1}), and Hmax (0.72 m). They were adjacent on the biplot and close to axes origin, according to their phenotypic similarity, except for slight differences in the crop cycle.

The cultivars within the same ‘time of maturity’ class clustered in the biplot (Figure 4.5b). The barycentre of late and intermediate cultivars were at the same coordinates on PC1 although slightly different on PC2; on the contrary, early cultivars clustered at negative coordinates on PC1, clearly separated from the other classes. As expected, the most substantial differences among the three maturity groups concerned their thermal requirements: early varieties presented low average GDDflo values (820 °C day^{-1}), and high GDDmat (410 °C day^{-1}); on the contrary, late varieties were characterized by a longer vegetative cycle (992 °C day^{-1}) and a shorter ripening duration (368 °C day^{-1}). The three groups did not differ much regarding other parameters, except for SPAN (38.55 days for early varieties, and 26.88 and 25.55 days for intermediate and late varieties, respectively), RGRLAI (0.007294 for early, 0.008776 and 0.008012 m^2 m^{-2} day^{-1} for intermediate and late) and Hmax (early 71.88, medium 89.14, late 66.45 s)

The variable ‘Stem length’ explained a significant amount of variance on PC2 (R^2: 0.67, $p = 0.01$, Supplementary Table 4.5) since CANOPY and Hmax were the most correlated variables on PC2. In fact, short varieties were at negative coordinates on PC2 while average to very long cultivars were mostly at positive coordinates (Figure 4.5c).

4.5 DISCUSSION

4.5.1 MODEL ACCURACY IN SIMULATING ITALIAN RICE CULTIVARS

We propose a methodology to develop cultivar-specific crop model parameter sets, starting from experimental field data including phenological observations and agronomic traits commonly recorded during variety trials. The feasibility of our methodology, which encompasses the use of multi-experiment automatic calibration of the WOFOST_GT model, was tested on long-term rice variety trials of thirteen cultivars using data from the VCU tests.

The reproduction of the vast genetic diversity of the Italian rice varietal landscape (Faivre-Rampant et al., 2010; Mongiano et al., 2018) into crop models would bring benefits for the various model-based studies and services in the area, notably when complex process-based models with several parameters are applied at regional scale (Angulo et al., 2013; Pagani et al., 2018; Teixeira et al., 2017). The relevance in the selected case study is amplified in some Italian settings were few high-quality varieties are cultivated in vast areas (e.g. “Vialone nano” in Verona province) (Ente Nazionale Risi, 2017).

The prerequisite for the crop models application in operational monitoring and forecasting services is the availability of reliable experimental data to perform calibration and validation activities (Basso et al., 2013; Cappelli et al., 2018; Pagani et al., 2018). At the same time, the high costs associated with field trials markedly limit their execution, complicating the maintenance and update of crop modelling tools (Grassini et al., 2015; Mavromatis et al., 2001). Also, the high release rate of new varieties requires their continuous characterisation in field trials for a sufficient number of years before relying on crop model predictions (Mavromatis et al., 2001). Our methodology, whose limits are discussed in the following section, limits the data requirement to the standard agronomic traits collected in variety trials or VCU trials, i.e. phenology observations, the height of canopy, and grain yield.

The thirteen parameter sets developed here led to satisfactory model performances in reproducing the marked diversity of the corresponding rice cultivars for the four crop traits of interests, even if the model systematically generated less variability. This could be explained by the limited sources of agro-environmental variability used as input in our modelling study compared to those in play under real conditions, which we neglected by assuming the absence of stresses (KROPFF:1993gl; Van Wart et al., 2013). The accuracy of the WOFOST_GT model was in line with available simulation studies on rice and other crops, even if the number of reference experiments used in our study was extraordinary large (Archontoulis, Miguez, & Moore, 2014; Boote et al., 2001; Boschetti et al., 2017; Li et al., 2017; Makowski, Hillier, Wallach, Andrieu, & Jeuffroy, 2011; Martre et al., 2014; Mavromatis et al., 2001). The high number of reference experiments contributed to increase the complexity of the model calibration, while improving the robustness of the derived parameter sets for further applications (Angulo et al., 2013; Martin et al., 2018). However, in some cases, the cultivar-specific calibration led to poor model performances for some traits (MATURITY, average RRMSE 22.8% ± 4.52%). This could be again due to the agronomic management in the field trials, as variable management strategies were adopted during the execution of the field trials despite ensuring the absence of biotic and abiotic stresses for the whole crop cycle. Among the factors contributing to the variability in agricultural management, the fertilisations timing (which can promote growth during ripening stages; Nayak, 2017), the weed management plans (with herbicides molecules which could alter phenological development; European and Mediterranean Plant Protection Organization, 2014), and the associated water management (De Datta, 1981) could have played a major role.

4.5.2 INCREASING THE LEVEL OF DETAIL IN THE REPRESENTATION OF RICE CULTIVARS.

The cultivar-specific parameter sets were characterised by a considerable dissimilarity in phenological development (GDDflo and GDDmax) and growth parameters (Amax, RipL0, CVO, SLAem, KDIFmax, SPAN, RGRLAI, Hmax), while presenting values consistent to other studies performed with the same model in the same area (Stella et al., 2014), and also with different models and crops (Archontoulis et al., 2014; Mavromatis et al., 2001). We found considerable variability in model parameter sets within the available cultivar classifications (grain shape, maturity class, and stem length), despite the consideration of few representative varieties for each group. A main outcome of the present study is the execution of a cultivar-specific calibration aimed at capturing the heterogeneity of Italian rice germplasm. The need of specify unique parameters sets for each cultivar is required considering their large variability within the same morphological groups, which was traditionally neglected by using the same parameter set for all varieties in the same group.

Confalonieri and Bocchi (2005) developed sets of model parameters (Stöckle et al., 2003) specific to coarser classifications, leading to three parameter sets targeting cultivar groups based on plant type (*japonica* or *indica* type) and time of maturity (early or medium-late). These authors used data collected on representative cultivars in each group, i.e. *japonica*-early (Loto), *japonica* medium-late (Ariete, Drago, and Cripto) and *indica*-type (Sillaro and Thaibonnet). Despite the limited number of varieties shared with our study (Loto –LOT, Ariete – ARI, Thaibonnet - THA), the thermal requirements for heading and ripening are in accordance, particularly for long B grain cultivars. However, we observed large intra-group differences between Thaibonnet (THA) and Mare CL (MAR) compared to Gladio (GLA) both for phenology and growth parameters consistently with their phenotypic differences, thus justifying a further detail in their model representation.

The thermal time needed to reach the flowering stage (GDDflo) for Loto (japonica early) is coherent with Confalonieri and Bocchi (2005), while the calibrated length of the ripening period in our study is much larger (427 °C day^{-1} compared to 262 °C day^{-1}). Also, the thermal requirements of japonica medium-late group from another modelling study (2005) based on one round grain (Cripto) and two long A (Ariete and Drago) cultivars are systematically larger than in our study (+ 100 °C day-1).

Synthetic cultivars from the same group of maturity (early, medium, and late) shared a similar length of the ripening period, even if mediated by different combinations of phenological parameters (GDDflo and GDDmat). Early varieties generally had minimal thermal requirements for completing the vegetative phase while being characterised by an extended ripening; on the contrary, late varieties required up to 200 extras °C day^{-1} for the completion of the vegetative phase and presented a shorter ripening phase. According to this classification, the rice cultivars in the same group were characterised by similar average values of parameters (except for SPAN, RGRLAI and Hmax), suggesting that it might be possible to define parameter sets based on the maturity class. Several authors used this approach on soybean improving the simulation of phenological development (Setiyono et al., 2007; Torrion et al., 2011, from Archontoulis 2014)

4.5.3 CRITICALITIES AND PERSPECTIVES OF OUR APPROACH

The main criticalities associated with this study concern the use of an automatic optimisation algorithm to develop cultivar specific model parameter sets, which modulate biophysical processes not fully characterised by the reference data. Several methodologies are available to perform crop models calibration which can be carried out manually by manipulating parameters based on field observations of phenology, growth, and yield (Boote, Jones, Batchelor, Nafziger, & Myers, 2003). Other authors proposed the use of automatic optimisation algorithms, to derive model parameters using field observations from a wide range of phenotypes under different environments (Wallach et al., 2001). These iterative algorithms, made accessible by the availability of cheap computational power, try to minimise prediction errors by performing multiple runs with different values of model parameters (Nelder & Mead, 1965). Crop models typically include a large number of parameters, and it would be unreasonable try to optimise them all together (Jeuffroy, Casadebaig, Debaeke, Loyce, & Meynard, 2013). The main associated risks are the inability of the optimisation algorithm to converge, falling in local *minima* instead of the global minimum, and the possible condition of equifinality, i.e. when different parameters sets led to same results in predicting the studied phenomena (Savenije, 2001). However, the application of automatic optimisation algorithms along with procedures aimed at minimising the complexity of the problem *a priori* (e.g. reduced number of optimised parameters, stepwise approach, imposed constraints) has proven to limit the uncertainties in parameters estimation and to reduce the risks of equifinality or inconsistent representations (Makowski et al., 2011). In our study, the differences in the parameters values across multiple optimisations procedures were consistent, with average CVs below 6% in all calibrated parameters and cultivars (Supplementary Figure 4.1).

Another potential source of uncertainty in our study is the subjectivity in the selection of the crop model parameters to design the cultivar-specific parameters sets, which should be able to efficiently represent the genotypic differences among cultivars (Boote et al., 2003; Mavromatis et al., 2001). We managed the parameters selection by using the results of a model sensitivity analysis study performed in the same growing condition on rice crop (Stella et al., 2014) in order to select the most relevant model parameters. The stepwise calibration of phenological and growth parameters has also contributed to limit the uncertainty of their determination (Makowski et al., 2011), although a broader availability of reference crop traits (e.g., date of emergence, tillering, leaf area index, grain number, aboveground biomass) would have increased the reliability of our results. This process will be surely fostered by the increased release of cost-effective methods to perform field assessments and the recent advances in remote sensing and high throughput phenotyping technologies (Andrade-Sanchez et al., 2014; Boschetti et al., 2017; Manfron, Crema, Boschetti, & Confalonieri, 2012).

However, the use of long-term data obtained from the evaluation of agronomic performance needed for the registration of new cultivars (Van Waes, 2009) proved to be a sufficient source of information to derive robust cultivar-specific parameters sets, at least for phenological development. This is crucial to improve the reliability of crop model predictions, as phenology alone (flowering and maturity dates) plays a significant role in determining yield potential (Archontoulis et al., 2014; Van Wart et al., 2013). Our methodology could be easily incorporated in current VCU testing to develop a cultivar-specific parameter set as one of the standard outcomes provided by such trials, along with their morpho-physiological description and value of cultivation and use assessment. This quantitative information could be then easily exported to enhance the modelling based services to support the agricultural rice chain at multiple levels, from forecasting activities (Pagani et al., 2018) up to plant breeding programs (Paleari, Movedi, & Confalonieri, 2017). The formalisation of a methodological procedure also allows its recursive application to improve the definition of the parameter sets of the reference cultivar used to evaluate the agronomic performances of new candidate varieties.

4.6 CONCLUSION

Although perfectible in many aspects, the methodology proposed here proved to be adequate in translating the extensive genetic differences characterising the Italian rice varietal landscape in a crop modelling study. The release of cultivar-specific parameter sets is a ready-to-use information, which could be both implemented in current model-based services and used to reduce the uncertainty connected to climate change impacts assessment. The latter aspect will also favour the design of improved adaptation strategies to sustain Italian rice production, by allowing the *in silico* test of current elite cultivars in future climatic scenarios.

4.7 SUPPLEMENTARY MATERIAL

4.7.1 LIST OF CULTIVARS

Supplementary Table 4.1 – List of cultivars considered in the study with indication of the year of release, the cultivated area in hectares in the period 2004-2015, grain shape. Time of maturity and stem length are the morphological descriptors taken from their official description.

Cultivar	Code	Year of release	Cultivated area	HEADING (days)	MATURITY (days)	CANOPY (m)	YIELD (t ha^{-1})	Grain shape	Time of maturity	Stem length
Ariete	ARI	1985	4595	86.62	35.62	0.8925	6.683	long A (pb)	intermediate	average
Augusto	AUG	2002	59607	84.83	28.83	0.8367	6.072	long A (pb)	intermediate	average
Baldo	BLD	1964	120786	84.6	27.6	1.074	6.351	long A (ic)	intermediate	long
Balilla	BAL	1934	96246	92	35.86	0.8164	6.764	round	late	average
Galileo	GAL	2002	36609	84.7	37.9	0.763	6.577	long A (ic)	early	short
Gladio	GLA	1998	330762	85.44	27.56	0.7167	5.978	long B	early	short
Karnak	KAR	2002	45844	98	31	0.89	5.203	long A (ic)	late	average
Loto	LOT	1988	115241	81.68	32.74	0.7295	5.862	long A (pb)	early	short
Mare CL	MAR	2012	12746	96.67	29.17	0.7417	5.968	long B	late	short
Selenio	SEL	1987	184516	86.1	34.48	0.7843	6.599	round	early	average
Thaibonnet	THA	1992	39571	94.6	25.27	0.7133	5.671	long B	intermediate	short
Venere	VEN	1997	2072	82.25	27.25	0.88	4.723	medium	early	average
Volano	VOL	1972	195696	92.25	34.3	1.069	5.902	long A (ic)	intermediate	very long

4.7.2 LIST OF PARAMETERS

Supplementary Table 4.2 – List of parameteres selected for the representation of cultivars in the study. Choice of parameter was driven by a previous sensitivity analysis (see Methods section of the paper).

Parameter code	**M.U.**	**Range min.**	**Range max.**	**Description**
GDDflo	°C day^{-1}	600	1300	Thermal treshold triggering anthesis stage
GDDmat	°C day^{-1}	300	650	Thermal threshold to reach maturity
Amax	kg ha^{-1} ha^{-1}	15	28	Maximum rate of CO_2 assimilation in the leaves
RipL0		0.55	0.9	Partitioning of assimilates to leaves at emergence
CVO		0.68	0.775	Efficiency of conversion into storage organs
SLAem	ha kg^{-1}	22	30	Specific leaf area at emergence
KDIFmax	-	0.5	0.6	Maximum extinction coefficient for diffuse visible light
SPAN	days	20	45	Life span of leaves growing at 35 °C
RGRLAI	m^2 m^{-2} day^{-1}	0.005	0.01	Maximum relative growth of leaf area index
Hmax	m	0.6	1.2	Maximum plant height

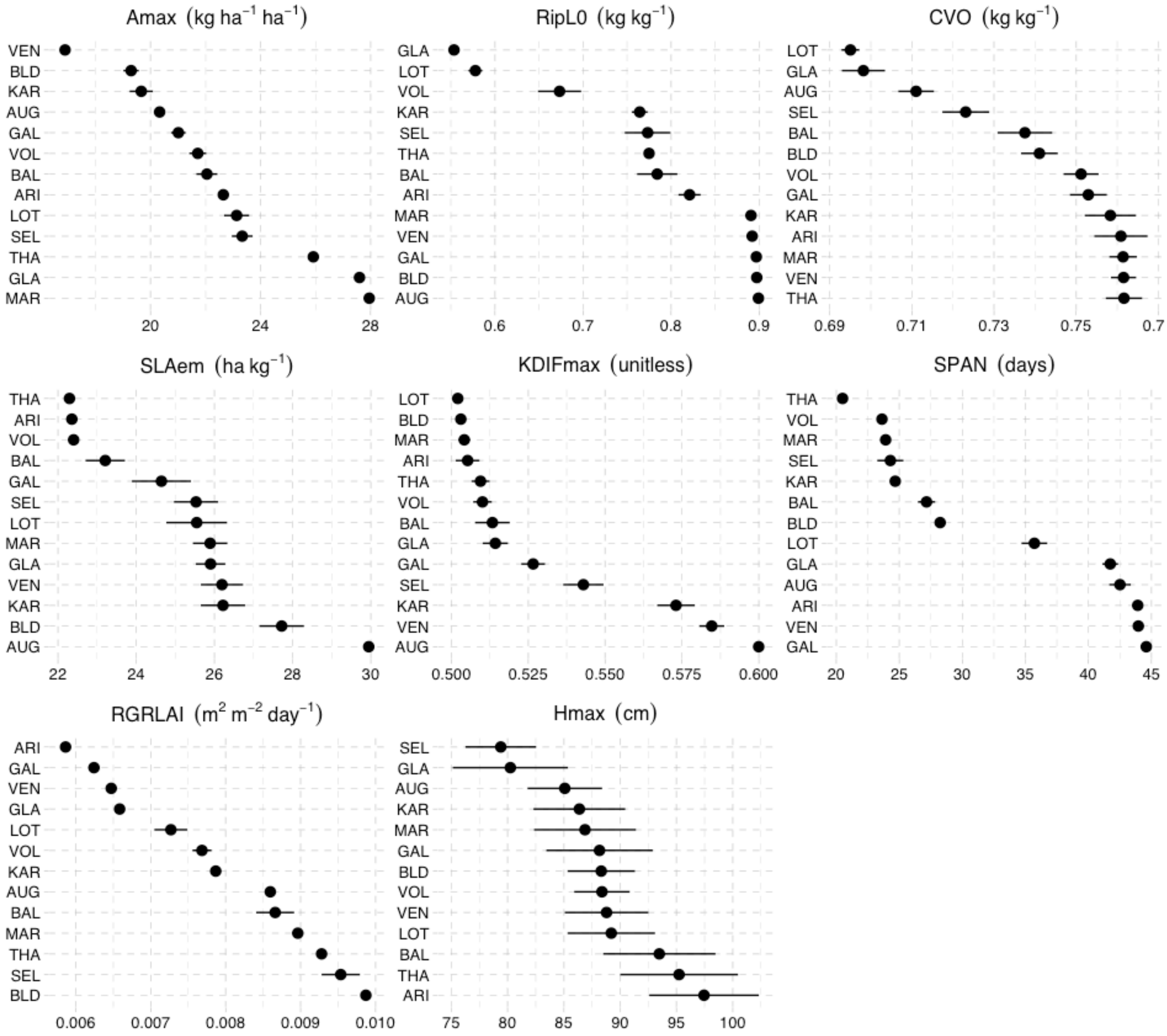
4.7.3 PERFORMANCES IN SIMULATING CULTIVARS

Supplementary Table 4.3 – Model performances divided per cultivar and per crop trait. Observed vs Simulated data mean, coefficient of variation, mean bias error, correlation coefficient and its associated significance ($\alpha = 0.05$), root mean square error, and relative root mean square error. Significance codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Cultivar	Trait	M.U.	$\bar{x}_{obs}$	CV_{obs}	$\bar{x}_{sim}$	CV_{sim}	MBE	r	Sig.	RMSE	RRMSE
ARI	HEADING	days	87	5.84%	84	6.07%	-2.1	0.14		5.9	0.068
ARI	MATURITY	days	36	24.7%	36	10.3%	0.69	0.026		9.8	0.28
ARI	CANOPY	days	0.89	5.74%	0.88	5.59%	-0.0082	0.34	*	0.087	0.098
ARI	YIELD	days	6.7	11.1%	6.7	6.25%	0.044	0.027		0.77	0.11
AUG	HEADING	days	85	4.56%	83	4.51%	-1.8	0.42		3.4	0.041
AUG	MATURITY	days	29	18.9%	30	5.5%	0.83	0.16		4.7	0.16
AUG	CANOPY	days	0.84	6.83%	0.83	5.55%	-0.0043	0.7	*	0.091	0.11
AUG	YIELD	days	6.1	9.17%	6.2	4.09%	0.082	0.00053		0.56	0.092
BAL	HEADING	days	92	5.42%	90	5.44%	-1.7	0.072		6.1	0.066
BAL	MATURITY	days	36	22.5%	36	14.2%	0.64	0.36	* *	6.4	0.18
BAL	CANOPY	days	0.82	5.88%	0.81	5.24%	-0.0071	0.24	*	0.077	0.094
BAL	YIELD	days	6.8	9.56%	6.8	9.83%	0.0047	0.018		0.84	0.12
BLD	HEADING	days	85	5.13%	83	6.64%	-1.8	0.012		6.8	0.081
BLD	MATURITY	days	28	36%	27	4.54%	-0.6	0.042		8.8	0.32
BLD	CANOPY	days	1.1	6.54%	1.1	4.73%	-0.00093	0.1		0.088	0.082
BLD	YIELD	days	6.4	4.16%	6.4	6.34%	0.089	0.46		0.28	0.045
GAL	HEADING	days	85	6%	84	6.37%	-1	0.41	*	4.3	0.051
GAL	MATURITY	days	38	20.7%	35	9.94%	-3.1	0.0075		8.9	0.24
GAL	CANOPY	days	0.76	7.18%	0.76	5.91%	-0.0046	0.64	* *	0.09	0.12
GAL	YIELD	days	6.6	9.93%	6.6	7.15%	0.067	0.053		0.68	0.1
GLA	HEADING	days	85	5.76%	84	5.82%	-1.8	0.038		6.3	0.074
GLA	MATURITY	days	28	17%	27	9.63%	-0.89	0.00045		5.2	0.19
GLA	CANOPY	days	0.72	6.77%	0.71	5.5%	-0.0068	0.22	*	0.073	0.1
GLA	YIELD	days	6	13.1%	6	7.02%	0.03	0.016		0.91	0.15
KAR	HEADING	days	98	4.45%	97	8.59%	-1	0.78	*	4.5	0.046
KAR	MATURITY	days	31	29.6%	29	18.9%	-1.6	0.28		7.2	0.23
KAR	CANOPY	m	0.89	9.09%	0.88	6.79%	-0.0058	0.43		0.12	0.13
KAR	YIELD	m	5.2	12.3%	5.2	10.5%	0.045	0.61		0.36	0.07
LOT	HEADING	m	82	5.06%	80	5.95%	-2.1	0.071		5.6	0.069
LOT	MATURITY	m	33	21.7%	33	9.51%	0.053	0.0025		7.7	0.24
LOT	CANOPY	m	0.73	5.18%	0.73	5.36%	0.0016	0.2		0.064	0.087
LOT	YIELD	m	5.9	15%	5.9	8.07%	0.0066	0.004		0.95	0.16
MAR	HEADING	m	97	6.43%	95	7.87%	-2	0.71	*	4.2	0.043
MAR	MATURITY	m	29	18.3%	30	11.9%	0.67	0.78	*	7.9	0.27
MAR	CANOPY	m	0.74	7.26%	0.73	6.34%	-0.016	0.1		0.076	0.1

Cultivar	Trait	M.U.	$\overline{x}_{obs}$	CV_{obs}	$\overline{x}_{sim}$	CV_{sim}	MBE	r	Sig.	RMSE	RRMSE
MAR	YIELD	m	6	17.4%	6	10.4%	0.063	0.58		0.64	0.11
SEL	HEADING	m	86	4.89%	84	5.8%	-2.4	0.17		5.4	0.062
SEL	MATURITY	m	34	18.7%	36	10.3%	1	0.00027		7.3	0.21
SEL	CANOPY	m	0.78	5.45%	0.77	5.8%	-0.01	0.18		0.073	0.093
SEL	YIELD	tha^{-1}	6.6	13.8%	6.6	6.33%	0.038	0.12		1.1	0.17
THA	HEADING	tha^{-1}	95	5.8%	92	4.59%	-2.1	0.014		7.4	0.078
THA	MATURITY	tha^{-1}	25	22.3%	26	14.5%	0.8	0.27	*	4.8	0.19
THA	CANOPY	tha^{-1}	0.71	4.9%	0.71	4.62%	-0.0032	0.23		0.056	0.079
THA	YIELD	tha^{-1}	5.7	11.5%	5.7	9.57%	-0.0067	0.027		0.89	0.16
VEN	HEADING	tha^{-1}	82	7.39%	80	8.72%	-2.2	0.73		3.8	0.047
VEN	MATURITY	tha^{-1}	27	24.2%	28	10.9%	1.2	0.15		7.3	0.27
VEN	CANOPY	tha^{-1}	0.88	6.75%	0.88	5.83%	0.0028	0.57		0.09	0.1
VEN	YIELD	tha^{-1}	4.7	8.16%	4.5	7.82%	-0.27	0.96	*	0.28	0.059
VOL	HEADING	tha^{-1}	92	4.91%	90	4.36%	-1.9	0.26	*	4.5	0.049
VOL	MATURITY	tha^{-1}	34	22.5%	35	13.1%	0.95	0.19		7	0.2
VOL	CANOPY	tha^{-1}	1.1	8.91%	1.1	5.46%	-0.0067	0.0069		0.11	0.11
VOL	YIELD	tha^{-1}	5.9	10.3%	5.9	7.98%	0.012	0.098		0.63	0.11

4.7.4 ASSESSMENT OF UNCERTAINTIES RELATED TO OBTAINED PARAMETERS SETS.



Supplementary Figure 4.1 – Mean and standard error of the calibrated values resulting from the repetition 40 independent optimisation of growth.

4.7.5 CHARACTERISATION OF THE PRINCIPAL COMPONENTS

Supplementary Table 4.4 – Correlations between variables (model parameters and crop traits) and the first four Principal Components, tested for significance ($\alpha = 0.05$). Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.

Parameter / Trait	PC1	Sig.	PC2	Sig.	PC3	Sig.	PC4	Sig.
GDDflo	0.6083	*	-0.2177		0.6424	*	0.2543	
GDDmat	-0.5664	*	0.05047		0.1144		-0.6569	*
Amax	0.5349		-0.6425	*	0.02147		-0.07256	
RipL0	0.05272		0.7071	* *	0.409		-0.0005805	
CVO	-0.08805		0.4761		0.7681	* *	0.1216	
SLAem	-0.1629		0.3419		-0.64	*	0.438	
KDIFmax	-0.2974		0.2517		-0.04493		0.685	* *
SPAN	-0.9394	* * *	-0.1981		0.01389		-0.003106	
RGRLAI	0.8313	* * *	0.2867		-0.3984		-0.09519	
Hmax	0.2953		0.7297	* *	-0.2192		-0.4263	
CANOPY	0.07539		0.789	* *	0.06584		-0.1781	
HEADING	0.6692	*	-0.1337		0.6075	*	0.2286	
MATURITY	-0.4011		-0.04782		0.3418		-0.5149	
YIELD	-0.003093		-0.1214		0.1328		-0.7492	* *

*Supplementary Table 4.5 – P*ercentage of variance explained (Coefficient of determination R^2) by the supplementary categorical variables and related significance ($\alpha = 0.05$) on each of the extracted Principal Components. Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.

Classification	PC1	Sig.	PC2	Sig.	PC3	Sig.	PC4	Sig.
Grain shape	0.4223		0.5218		0.1873		0.345	
Time of maturity	0.4031		0.1695		0.2375		0.2507	
Stem length	0.094		0.6737	*	0.07135		0.2185	

Supplementary Table 4.6 – Categories coordinates tested for significance of difference from the overall mean ($\alpha = 0.05$) on each of the extracted Principal Components. Significance codes: * p < 0.05, ** p < 0.01, *** p < 0.001.

Category	PC1	Sig.	PC2	Sig.	PC3	Sig.	PC4	Sig.
long A (pb)	-0.3081		-0.6151		-0.8848		-0.7254	
long A (ic)	0.596		0.5686		0.6369		-0.1512	
long B	1.712		-1.681	*	0.3462		0.1105	
round	0.4866		-0.1399		0.1749		-0.9809	
medium	-2.487		1.867		-0.2732		1.747	
early	-1.448	*	-0.4329		-0.8161		-0.03727	
intermediate	0.7162		0.7959		-0.08709		-0.7476	
late	0.7314		-0.363		0.9031		0.7849	
short	-0.4127		-2.033	* *	0.04977		0.4922	
average	-0.8321		-0.4284		0.2171		0.7164	
long	0.311		1.954	*	-0.9901		0.1814	
very long	0.9338		0.5072		0.7232		-1.39	

4.7.6 CULTIVARS CLASSIFICATIONS: MEANS OF PARAMETERS VALUES.

Supplementary Table 4.7 – Average parameters values calculated per each category of the morphological descriptors considered in the study. These classifications are the most commonly used in the Italian rice area.

Descriptor	Class	HEADING	MATURITY	CANOPY	YIELD	GDDflo	GDDmat	Amax	RipL0	CVO	SLAem	KDIFmax	SPAN	RGRLAI	Hmax
Grain shape	long A (ic)	89.89	32.7	0.949	6.008	904.5	387.9	21.22	0.8379	0.7668	24.88	0.5339	29.07	0.008204	83.06
Grain shape	long A (pb)	84.38	32.4	0.8195	6.206	821	421.5	23.05	0.771	0.7144	26.05	0.5274	34.7	0.007795	78.01
Grain shape	long B	92.24	27.33	0.7239	5.872	938.2	334.1	26.63	0.7392	0.7419	24.57	0.5013	28.59	0.008174	71.27
Grain shape	medium	82.25	27.25	0.88	4.723	777.9	371.4	16.91	0.9	0.775	29.93	0.5999	44	0.006177	79.38
Grain shape	round	89.05	35.17	0.8003	6.682	889.3	443	21.08	0.8421	0.7321	24.45	0.5144	26.82	0.008744	72.49
Time of maturity	early	84.03	31.98	0.7747	5.948	820	409.8	21.89	0.7471	0.7284	26.28	0.5379	38.55	0.007294	71.88
Time of maturity	intermediate	88.58	30.32	0.9171	6.136	875.2	384.2	22.97	0.8492	0.7522	24.48	0.5159	26.88	0.008776	89.14
Time of maturity	late	95.56	32.01	0.816	5.978	992.2	368.7	22.9	0.8282	0.7575	25.47	0.5271	25.55	0.008012	66.45
Stem length	short	88.62	30.53	0.7328	6.011	890.6	376.1	24.78	0.7316	0.7358	25.13	0.5124	33.9	0.007611	70.3
Stem length	average	88.3	32.17	0.85	6.007	878.2	404.4	21.37	0.849	0.7432	25.44	0.548	30.99	0.007929	74.98
Stem length	long	84.6	27.6	1.074	6.351	809.9	348.3	19.47	0.9	0.775	29.75	0.5	27.36	0.009814	99.99
Stem length	very long	92.25	34.3	1.069	5.902	920	420.4	21.39	0.814	0.7625	22.13	0.5	21.01	0.008943	103.1

CHAPTER 5 EXTENDING THE APPLICATION OF A HAZELNUT SIMULATION MODEL TO DIFFERENT GROWING ENVIRONMENTS

Simone Bregaglio, Laura Giustarini, Eloy Suarez, Gabriele Mongiano, Tommaso De Gregorio

5.1 ABSTRACT

Global hazelnut market and trade are steadily increasing last decades, with Turkey leading the export and producing 75% of the total world amount of fruits. Current production levels are no longer able to sustain the rising hazelnuts demand by the private sector, which is promoting the establishment of new plantations in alternative environments. New hazelnut orchards are managed with high farmer inputs, claiming the need of supporting tools to improve yield levels and stability, while promoting environmental sustainability. Here we propose a methodology to foster the application of a hazelnut simulation model in new environments, and we test it with experimental data collected in 2015-2017 growing seasons in four orchards placed in Italy, Chile and Georgia. Our workflow articulates in four steps, starting from (i) the screening of the most relevant parameters in explaining model errors on leaf area index, fruit yield and soil water content via sensitivity analysis (Morris method). The selected parameters are further analyzed (ii) via a quantitative sensitivity analysis (Sobol method) to rank their importance and (iii) are then subjected to automatic calibration (multi-start simplex method), to increase model accuracy in reproducing field data. At the final step, (iv) the distributions of model parameters and of simulated outputs are correlated (Spearman method) to analyse their relationships. The model sensitivity varied according to the model output considered, with parameters associated to leaf area expansion and plant dimension gaining highest relevance on leaf area index. The photosynthetic process and the root water uptake resulted the most important processes in modulating fruit yield per plant and soil water content simulation, respectively. The model performances after calibration were quantified and compared to model runs with original parameter sets, revealing a high accuracy in simulating leaf area index (RRMSE = 19.3%), fruit yield (RRMSE =16.3%) and soil water content (RRMSE =12.5%). This study demonstrated the feasibility of the extension of the hazelnut model to contrasting growing conditions while highlighting areas for model improvement and providing indications to guide new data collection.

Keywords: process-based modelling, Morris screening method, Sobol total order, multi-start simplex

5.2 INTRODUCTION

The total value of the global hazelnut market is projected to reach $5,755 billion in 2021, with 7% of compounded average annual growth rate from 2017 (Technavio, 2018). This tremendous expansion has numerous causes ranging from the steady increase in hazelnut worldwide production (+59% from 1984 to 2014; FAOSTAT, 2018), to the rising economic value of hard-shelled fruits (Fallico et al., 2003; Alasalvar et al., 2009), and up to the global rising hazelnut demand due to augmented awareness about health and nutrition (Sonnenberg et al., 2013).

The hazelnut harvested area and production is intensely concentrated in Turkey, which is the leadinf world producer and exporter, contributing to ~75% of the global amount, despite a very low average yield per hectare (0.64 t ha^{-1}) compared to more productive environments as the United States (average yield of 2 t ha^{-1}, FAOSTAT, 2016). Nevertheless, Turkish hazelnut cultivation is traditionally placed in marginal hilly lands, with low agronomic inputs and lack of mechanization, thus posing at high-risk the sustainability of the hazelnut economic sector, as shortage supply problems could be even exacerbated by late frost events (Ustaoğlu, 2012) and water shortage during ripening (Mingeau et al., 1992). Hazelnut production is also subjected to large inter-annual fluctuations, due to the marked alternate bearing of the hazelnut tree (Boubaker et al., 2015), characterized by a high production season (on year) followed by one or several years of low yields (off year). The main private players in the global hazelnut market are thus promoting the diversification of production, including the expansion of the cultivated area in other key producing countries, as well as by funding research programs to promote new plantations in different environments. Worldwide hazelnut growers are positively responding to these incentives, as proved by the national project *'Progetto Nocciola Italia'*, aiming at 30% increase of production within 2021 in Italy, the second world larger producer (10% of global production, FAOSTAT, 2018), and by the massive increase of harvested area in Chile, whose extension reached 17,000 ha in 2017, with an increase of 2,500 ha $year^{-1}$ from 1990 (Food and Agriculture Organization of the United Nations, 2018).

The intensification of hazelnut cultivation has been accompanied by the implementation of improved management plans, including advancements in the technological and agricultural management of the orchards. New hazelnut plantations are mostly established in plain up to hilly areas, with a medium-large extension (> 10 ha) and planting density in the range 360-600 plants ha^{-1}; they are managed with high level of mechanization (Tous et al., 1994) including chemical inputs as compound mineral fertilizers (Cristofori et al., 2015), herbicide applications (Kaya-Altop et al.,

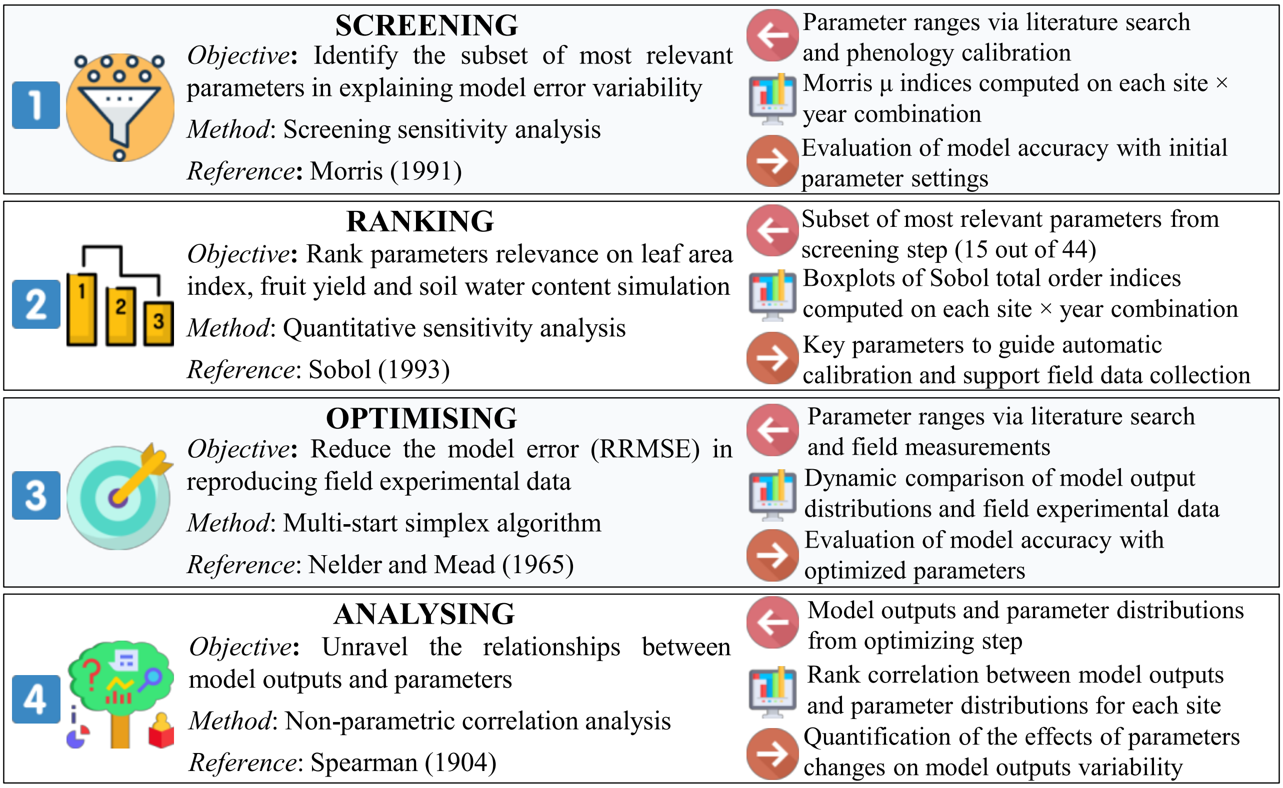
2016) and phytosanitary treatments (Farrar et al., 2016), as well as mechanical harvesting and irrigation systems (Mačkić et al., 2016).

The development of supporting tools to assist hazelnut growers in the design and management of the new orchards, as well as to inform stakeholders on land suitability and to forecast hazelnut yield trends would bring essential information to foster the sustainability of hazelnut production in the new growing environments. Among available tools, crop yield forecasting systems based on simulation models are increasingly adopted to provide in-season estimates of the production of various food crops (de Wit and van Diepen, 2007; Kogan et al., 2013; Bregaglio et al., 2015). Most of these systems rely on the projections carried out by process-based simulation models, which can dynamically reproduce the non-linear interactions between crop physiology and agro-pedo-climatic conditions (Singh, 1994; Archontoulis and Miguez, 2014). Crop yield forecasting system can also be coupled with databases at different spatial resolution like, e.g., the General Large-Area Model for annual crops (Challinor et al., 2004) or the Crop Growth Monitoring Systems of the European Commission (Supit et al., 2010). Despite the availability of simulation models for main orchard tree species (e.g., for peach, Lopez et al., 2010; orange, Pereira et al., 2017; apple, Pallas et al., 2016), a hazelnut model-based yield forecasting system is not yet available.

The first step towards its realisation is the model adaptation to the targeted growing conditions, which is usually performed via model calibration and validation using experimental field data. Here we propose a methodology to foster the application of a hazelnut simulation model (Bregaglio et al., 2016) on different orchards, which includes the assessment of the model sensitivity to parameter changes to support the refinement of its accuracy in reproducing observed data. Our work, which relies on a three-year field experiment carried out on four orchards placed in Italy, Georgia and Chile, provides a reproducible workflow to increase model performances, while supporting model improvement and new data collation activities, towards the realisation of an operational hazelnut yield forecasting system.

5.3 MATERIALS AND METHODS

5.3.1 THE METHODOLOGICAL WORKFLOW



1	**SCREENING** *Objective*: Identify the subset of most relevant parameters in explaining model error variability *Method*: Screening sensitivity analysis *Reference*: Morris (1991)	← Parameter ranges via literature search and phenology calibration 🖥 Morris μ indices computed on each site × year combination → Evaluation of model accuracy with initial parameter settings
2	**RANKING** *Objective*: Rank parameters relevance on leaf area index, fruit yield and soil water content simulation *Method*: Quantitative sensitivity analysis *Reference*: Sobol (1993)	← Subset of most relevant parameters from screening step (15 out of 44) 🖥 Boxplots of Sobol total order indices computed on each site × year combination → Key parameters to guide automatic calibration and support field data collection
3	**OPTIMISING** *Objective*: Reduce the model error (RRMSE) in reproducing field experimental data *Method*: Multi-start simplex algorithm *Reference*: Nelder and Mead (1965)	← Parameter ranges via literature search and field measurements 🖥 Dynamic comparison of model output distributions and field experimental data → Evaluation of model accuracy with optimized parameters
4	**ANALYSING** *Objective*: Unravel the relationships between model outputs and parameters *Method*: Non-parametric correlation analysis *Reference*: Spearman (1904)	← Model outputs and parameter distributions from optimizing step 🖥 Rank correlation between model outputs and parameter distributions for each site → Quantification of the effects of parameters changes on model outputs variability

Figure 5.1 – Description of the methodological workflow developed in this study, which articulates in four steps (screening, ranking, optimising and analysing). Each step is presented along with specific objectives, main methods and supporting reference. The green left arrow icon indicates the input data requirement, the screen icon highlights the primary results and the red right icon corresponds to the key information provided.

The workflow presented in this paper (Figure 5.1) begins with (i) a screening sensitivity analysis (sSA, Campolongo et al., 2007), aimed at identifying the subset of the most relevant parameters in influencing the model error in simulating fruit yield per plant (FY, kg plant^{-1}), leaf area index (LAI, m^2 m^{-2}) and average soil water content in rooted zone (SWC, m^3 m^{-3}). The relative root mean square error (RRMSE) was chosen as the target metric to evaluate the variability of model accuracy. Parameters related to vegetative and reproductive phenological development were calibrated in the four orchards and set as fixed in sSA simulations, as in other studies on crop (e.g., Stella et al., 2014) and tree (e.g., Leolini et al., 2018) models, in order to focus on parameters related to growth processes only. Before model calibration, we compared the distributions of model outputs with reference data and evaluated model performances with the original parameter sets. The model sensitivity to most relevant parameters was further investigated via (ii) a quantitative sensitivity analysis (qSA, Sobol, 1993), implying a larger number of simulations to provide a more robust parameters ranking. At the next step, (iii) we performed an automatic calibration of the most relevant parameters from sSA aimed at minimising the errors on FY, LAI and SWC (multi-start simplex method, Nelder and Mead, 1965) with plant height and crown dimensions set according to field measurements. The model accuracy in explaining observations was re-evaluated, and the associated improvements with respect to sSA simulations were quantified. Finally, (iv) the distributions of simulated LAI (maximum value) and FY after calibration were correlated via Spearman rank analysis with the parameters distributions, in order to evaluate the sign of their relationships and the strength of their association.

5.3.2 FIELD DATA COLLECTION

Field experimental data used in this study were collected in four hazelnut orchards (Table 5.1) placed in Italy (Baldissero d'Alba, BAL and Viterbo, VIT), Georgia (Chitaskari, CHI) and Chile (Camarico, CAM).

Table 5.1 – Geographic information (latitude, longitude, altitude), agronomic data (hazelnut variety, planting density, training system, age of the orchard in 2015, irrigation and fertilization), soil texture and plant dimensions (plant height, crown radius, crown height) of the four experimental hazelnut orchards where experimental trials were carried out. TGDL = Tonda Gentile delle Langhe, TG = Tonda Giffoni.

Data	Variable	Experimental site			
		Baldissero	Camarico	Chitaskari	Viterbo
Geographic	Country	Italy	Chile	Georgia	Italy
	Latitude (°)	44.76 N	35.19 S	42.17 N	42.27 N
	Longitude (°)	7.92 E	71.22 W	41.51 E	12.13 E
	Altitude (m)	360	658	66	404
Agronomic	Variety	TGL	TG	TG	TG
	Planting density	5×4.5	5×3	5×3	4.5×4.5

	(m×m)				
	Training system	Bush	Bush	Bush	Bush
	Age (years)	11	11	7	12
	Irrigation	No	Yes	No	No
	Fertilization	Yes	Yes	Yes	Yes
Soil	Sand (%)	38.5	56.7	46.1	53.5
	Clay (%)	8.8	24.3	25.2	16.4
	Silt (%)	52.3	19	28.7	30.1
Plant dimension	Plant height (m)	3.4-5.8	4-5.7	3.3-4.7	3.9-4.4
	Crown radius (m)	1.5-2.2	1.3-2	1.1-1.7	1.5-2
	Crown height (m)	0.2-0.5	0.7-1.1	0.13-0.61	0.9-1.1

Two hazelnut varieties were grown in the experimental orchards, i.e., Tonda Gentile delle Langhe (BAL) and Tonda Giffoni (VIT, CAM, CHI). CHI was the only lowland orchard, whereas the other three were located in hilly areas, with CAM at the highest altitude (658 m). Three out of four orchards were at full production in 2015 (11-12 years, BAL, VIT and CAM), whereas in CHI the trees were still in the active growth phase (7 years). The planting density in CAM and CHI were 5 m × 3 m, whereas in Baldissero d'Alba the width inter-row was 4.5 m. VIT was planted with a standard density of 4.5 m × 4.5 m. According to USDA classification (Soil Survey Staff, 1999), the soil in BAL was silty loam. CAM and VIT soils were classified as sandy clay loam and sandy loam, respectively, whereas the soil texture in CHI was loam. The only site with drip irrigation was CAM.

The same experimental protocol to guide field data collection was adopted in the four orchards. Ten plants were randomly selected in four adjacent rows in a representative area of each field and labelled by an identification number. In each growing season starting from 2015, the tree height, the crown diameter inter-row, and the distance between the ground and the crown were measured at bud break (V4, Italian Phenological Gardens network, IPG, Malossini, 1993, Supplementary Table

5.1). The bush training system was adopted in the four orchards, with plant height ranging from 3.3 m (CHI) to 5.8 m (BAL), with the maximum variability in BAL (2.4 m) and the minimum in VIT (0.5 m). The crown radius inter-row was similar in BAL, CAM and VIT (1.5-2.2 m) and lower in CHI (1.1-1.7 m). The distance between the ground and the crown was lower in BAL (0.2-0.5 m) and CHI (0.13-0.61 m), whereas CAM (0.7-1.1 m) and VIT presented higher values (0.9-1.1 m).

Vegetative and reproductive phenological development were assessed every two weeks, and the corresponding stages were reported according to the IPG scale (Supplementary Table 5.1). LAI was measured using the smart app PocketLAI (Confalonieri et al., 2013), which was already tested on orchard species (Orlando et al., 2015). The fresh and dry weight of hazelnuts was measured when fruits were visible and immature (R12, three plants), and during fruit fall (R14-R16, seven plants). Fresh fruits were weighted at collection and after oven drying until constant weight. Volumetric water probes were placed at different soil depths (depending on the site) to measure SWC. Average daily SWC was then derived considering 1 m as representative of hazelnut root depth (Marsal et al., 1997). A summary of the experimental data collected is provided in Supplementary Table 5.2 of the Supplementary Material. The average thermal conditions and pluviometric regimes occurred during field experiments are presented in Table 5.2.

Table 5.2 – Average weather conditions in the experimental sites (average air maximum and minimum temperature, °C, and cumulated precipitation, mm) during hazelnut vegetative growth period (March to October in the boreal hemisphere, September to April in the austral hemisphere).

Variable	Year	Experimental site			
		Baldissero	Camarico	Chitaskari	Viterbo
Air maximum temperature (°C)	2015	24.09	23.12	24.45	24.18
	2016	24.03	25.25	24.37	26.3
	2017	24.48	23.88	24.51	27.72
Air minimum temperature (°C)	2015	9.58	7.63	13.3	12.52
	2016	8.36	7.84	12.96	10.03

	2017	8.53	7.5	12.93	9.84
	2015	561.2	585.4	787.2	729.8
Precipitation (mm)	2016	499.6	526.8	1025.1	720.2
	2017	338.4	267.8	832.8	231.03

Weather stations placed close to the experimental orchards (< 5 km) collected daily air maximum and minimum temperature (°C) and precipitation (mm) used as input in the hazelnut model. The average minimum temperature in the hazelnut vegetative growth period in CAM was the lowest among the experimental sites, ranging from 7.5 °C in 2017-2018 to 7.84 °C in 2016-2017, whereas the highest minimum temperature was recorded in CHI, ranging from 12.93 °C in 2017 to 13.3 °C in 2015. Low variability in average air maximum temperature was registered across sites, with VIT-2017 resulting as the warmest growing season (27.7 °C) and CAM-2015 the coldest (23.1 °C). The total precipitation amount markedly varied, with 2017 as the driest season in BAL (338.4 mm), CAM (267.8 mm, where irrigation water was applied) and VIT (231 mm). The largest precipitation amount was recorded in VIT-2015 (1058.6 mm) and CHI-2016 (1025.1 mm), the latter being the wettest site (average total precipitation of 881.7 mm).

5.3.3 MODEL APPLICATION

THE HAZELNUT SIMULATION MODEL

The simulation of the hazelnut phenology and growth was carried out with the best performing modelling solution proposed by Bregaglio et al. (2016), who calibrated and evaluated the model in BAL using field measurements of LAI, SWC and specific leaf area (SLA, m^2 kg^{-1}) collected on the same orchard than in this study. A synthetic description of the model workflow follows as the full algorithmic description is provided in the seminal paper. The model run starts at the end of the previous vegetative season (September in boreal, March in austral hemisphere) with chilling hours accumulation (base temperature 7 °C) until the end of the endo-dormancy period, when hourly thermal time starts driving forcing units accumulation. Bud break is triggered when a user-defined threshold of degree days is reached, and initial LAI is computed using plant dimensions and leaf area. The time evolution of vegetative and reproductive phenological development is reproduced via model parameters, representing thermal time thresholds to trigger the next phases. Light interception is simulated considering the shading effects of tree crowns, as a function of plant height and width. Canopy photosynthetic rate is computed by a decoupled stomatal conductance and gross photosynthesis model. Daily carbon assimilation is reduced considering the maintenance and growth respiration costs of the tree organs. The assimilate partitioning to the plant organs is dynamic during the growing season, with user-defined parameters modulating functions of vegetative (for leaves and branches) and reproductive (for fruits) phenological development. The impact of soil water availability on hazelnut growth is simulated using a stress index computed as the ratio between root water uptake and crop potential transpiration. Soil water dynamics is simulated by a tipping-bucket approach, splitting the soil into ten layers with a total depth equal to 1 m. At the end of the time step, the plant dimensions and the canopy area are updated. The only model modification introduced in this study targeted the alternate bearing of hazelnut trees, which was reproduced by an empirical coefficient reducing the partitioning of assimilates to fruits in off years. We set this coefficient to 28%, according to the average yield gap in Chilean production environments (unpublished data) in years of low production, which were determined in our experiment by visual assessments of the number of male and female flowers during winter.

SENSITIVITY ANALYSIS AND AUTOMATIC CALIBRATION

The model sensitivity to parameter changes was assessed twice in this study (Figure 5.1), (i) to screen the most relevant sources of model error at a low computational cost (sSA) and then (ii) to rank them according to their impact on FY, LAI and SWC (qSA). The RRMSE in reproducing measured field data were used as target variables to evaluate the influence of parameter changes on model outputs. The distribution of model parameters for sensitivity analyses were derived applying ±10% to each parameter value in Bregaglio et al. (2016), and are reported in Supplementary Table 5.3.

The sSA was performed according to Campolongo et al. (2007), who extended the Morris method (Morris, 1991). The method consists in resuming the overall influence of each parameter on the output in the metric μ^*, computed using the absolute value of the elementary effect (i.e., the ratio between the variation of the model output and the parameter at two different points of the parameter space) to manage non-monotonic relationships. This widely used method in ecological modelling (Song et al., 2012; Pianosi et al., 2016) creates a parameter space by dividing the range of model parameters into l discrete levels, with the model evaluated for t trajectories with random start. Each model parameter p is changed within each trajectory, and the corresponding effect of the parameter belonging to the p-dimensional vector on the output of interest is computed. 44 model parameters were investigated by sSA, by setting $t = 10$ and $l = 8$, leading to a total number of 450 runs for each site × year combination.

The subset of the most relevant parameters from sSA (15) was then analysed with qSA (Sobol, 1993), to investigate their relevance further and to rank their impact on model errors. This method, recognised as a standard in global sensitivity analysis applied to biophysical simulation models (Varella et al., 2010; Herman et al., 2015), highlights the portion of the total variance explained by the contribution of single parameters, including parameters interactions (Tang et al., 2007). The output variance is decomposed into terms of increasing dimension, representing the contribution of parameters to the uncertainty of the model output (i.e., partial variances). A quasi-Monte Carlo sampling was used to simultaneously explore the parameters hyperspace, using the total-effect index (S_{Ti}) to quantify the contribution to the output variance of each parameter, including all variance caused by its interactions, of any order, with any other input variables (Homma and Saltelli, 1996). The total number of simulations in qSA was 2048 for each site × year combination. All sensitivity analyses were performed using SimLab library (https://ec.europa.eu/jrc/en/samo/simlab).

The 15 parameters resulting from sSA were then subjected to automatic calibration to reduce the average RRMSE on LAI, FY and SWC using the multi-start downhill simplex algorithm (Nelder and Mead, 1965), as modified by Acutis and Confalonieri (2006). This algorithm explore the p-dimensional parameters space, with p as the number of the parameters under evaluation, following the gradient of the objective function. A simplex with p+1 vertexes is initialised when optimisation starts, and the average RRMSE evaluated after the model run at each simplex vertex, which moves towards the minimisation of RRMSE. Ten simplexes were used at each optimisation run, setting 1000 as maximum number of iterations and a RRMSE tolerance of 10^{-6}, leading to a variable number of simulations ranging from 261 in BAL to 422 in CAM. The two sensitivity analyses were performed using the same settings for model parameter ranges, which are reported in Supplementary Table 5.3, along with the calibrated parameters values in the four orchards.

5.4 RESULTS

5.4.1 MODEL SENSITIVITY TO PARAMETER CHANGES

The results of sSA are presented in Table 5.3, where Morris μ* is reported for the 44 parameters under evaluation, with corresponding mean rank, obtained as the average ranking of the parameter in explaining the variability of RRMSE for LAI, FY and SWC.

Table 5.3 – Results obtained in the screening sensitivity analysis performed with the Morris method on the 44 parameters for leaf area index (m^2 m^{-2}), fruit yield (kg $plant^{-1}$) and soil water content (m^3 m^{-3}), with corresponding mean rank (average parameter rank for the three simulated outputs). The parameters selected for the quantitative sensitivity analysis are marked by an asterisk. The acronym of model parameters, with unit and description are reported in Supplementary Table 5.3.

Parameter	Leaf area index		Fruits biomass		Soil water content		Mean rank
	μ*	rank	μ*	rank	μ*	rank	
LeavesRip*	37.13	1	11.24	1	0.003	2	1.3
iniHeight*	26.47	2	7.47	3	0.002	3	2.7
SLAmax*	21.48	3	7.63	2	0.002	4	3
WoodDens*	16.04	4	5.07	8	0.002	6	6
iniRadius*	15.2	5	6.74	7	0.002	7	6.3
BranchVol*	14.46	6	4.83	9	0.002	5	6.7
GrowthEff*	11.96	7	6.94	5	0.001	8	6.7
PhotoTmax*	11.95	8	7.36	4	0.001	11	7.7
PhotoTopt*	10.54	9	2.15	12	0.001	9	10
WoodC*	9.16	10	2.66	11	0.001	10	10.3
Vcmax*	5.38	11	3.84	10	5E-04	15	12
iniCrown*	4.32	12	1.41	13	5E-04	14	13
RootDepth*	0.74	20	0.67	16	0.005	1	12.3
FruitRip*	1.29	17	6.75	6	2E-04	21	14.7
WSthresh*	0.96	18	0.81	14	7E-04	12	14.7
NSCtarget	2.7	14	0.64	17	5E-04	13	14.7

Parameter	Leaf area index		Fruits biomass		Soil water content		Mean rank
	μ*	rank	μ*	rank	μ*	rank	
LeafSen	3.1	13	0.54	18	2E-04	17	16
iniCabove	2.18	15	0.37	22	4E-04	16	17.7
ImbThr	1.43	16	0.28	24	2E-04	19	19.7
Q10	0.95	19	0.3	23	2E-04	20	20.7
LeafMass	0.58	21	0.12	27	1E-04	22	23.3
NSCfract	0.58	22	0.17	26	2E-04	18	22
RootFResp	0.54	23	0.38	21	6E-05	27	23.7
LeavesResp	0.49	24	0.71	15	4E-05	28	22.3
LeafDens	0.49	25	0.11	31	7E-05	24	26.7
LeafC	0.46	26	0.11	30	6E-05	25	27
FastSen	0.44	27	0	44	3E-05	32	34.3
PhotoTmin	0.4	28	0.12	28	7E-05	23	26.3
BranchResp	0.37	29	0.38	20	6E-05	26	25
SLAmin	0.32	30	0.04	32	4E-05	31	31
SLAws	0.31	31	0.04	33	4E-05	30	31.3
RootHalfLife	0.24	32	0.17	25	4E-05	29	28.7
FruitsResp	0.2	33	0.39	19	1E-05	33	28.3
CondTmax	0.06	34	0.12	29	3E-06	35	32.7
StemsResp	0.03	35	0.02	38	4E-06	34	35.7
CondVPDmin	0.02	36	0.03	34	1E-06	36	35.3
MaxDailyNSC	0.02	37	0	40	1E-06	37	38
CondTMax	0.01	38	0.02	36	1E-06	38	37.3
CondTopt	0.01	39	0.02	35	7E-07	39	37.7
WSmult	0.01	40	0.02	39	7E-07	40	39.7

Parameter	Leaf area index		Fruits biomass		Soil water content		Mean rank
	μ*	rank	μ*	rank	μ*	rank	
WSexp	0.01	41	0.02	37	3E-07	41	39.7
LightCoeff	9E-04	42	2E-03	41	7E-08	42	41.7
CondTmin	5E-04	43	8E-04	42	4E-08	43	42.7
CondVPDmax	2E-04	44	2E-04	43	2E-08	44	43.7

We selected 15 out of 44 parameters for the qSA step. These were the 12 top ranked in explaining the variability of RRMSE on LAI, plus the maximum partitioning to fruits (FruitRip, unitless), which ranked 6th in explaining model errors on FY: the maximum root depth (RootDepth, m) and the threshold to activate water stress (WSthresh, unitless), which classified 1st and 12th in explaining the errors in simulating SWC, respectively. Two of the most relevant parameters from sSA were directly linked to the the development of leaf area, i.e., the partitioning to leaves at bud burst (LeavesRip, unitless, mean rank 1.3) and the maximum specific leaf area (SLAmax, m^2 kg^{-1}, mean rank 3). The initial plant dimension resulted as one of the primary source of variability of model error, as represented by four parameters, i.e. the initial plant height (iniHeight, m, mean rank 2.7), the crown radius (iniRadius, m, mean rank 6.3), the distance between the ground and the beginning of the crown (iniCrown, m, mean rank 13), and the ratio between branches and crown volume (BranchVol, unitless, mean rank 6.7). The photosynthetic process also emerged as a critical process in influencing model accuracy, being represented by four parameters. Three of them were related to gross photosynthesis, i.e., the maximum (PhotoTmax, °C, mean rank 7.7) and optimum (PhotoTopt, °C, mean rank 10) cardinal temperatures and the maximum carboxylation rate (VCmax, mean rank 12), while the other synthesized the cost of growth respiration (GrowthEff, unitless, mean rank 6.7). The last two parameters selected for qSA were the wood density (WoodDens, m^3 m^{-3}, mean rank 6) and the wood carbon content (WoodC, unitless, mean rank 10.3), which modulate the carbon pools at model initialisation. qSA results are shown in Figure 5.2 and Table 5.4 presenting boxplots of S_{Ti} values computed on every site × year combination and the average S_{Ti} values, respectively.

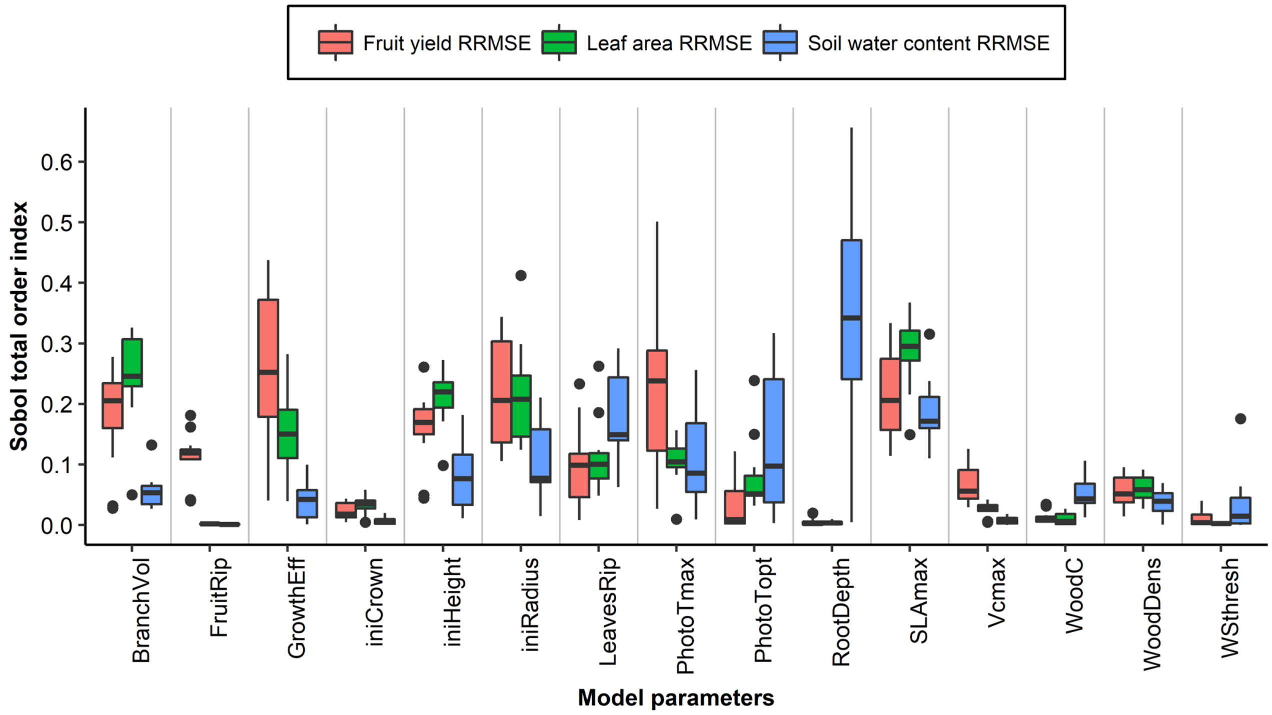


Figure 5.2 – Boxplots computed on Sobol total order indices obtained by the most relevant model parameters in the sensitivity analysis performed on the model error in reproducing fruits yield, leaf area index and soil water content data in four hazelnut orchards × three growing seasons (total 12 values per boxplot). The acronym of model parameters, with unit and description are reported in Supplementary Supplementary Table 5.3.

The rankings of parameters resulting from qSA performed on LAI, FY and SWC errors were discordant. Six parameters (i.e., GrowthEff, PhotoTmax, SLAmax, iniRadius, BranchVol and iniHeight) obtained a similar and large impact on model error variability in FY prediction (Figure 5.2), with average S_{Ti} ranging from 0.159 for iniHeight to 0.254 for GrowthEff (Table 5.4). The associated S_{Ti} variability was the largest among the analysed model outputs, especially for iniRadius (ranging from 0.105 in CHI-2015 to 0.344 in BAL-2015), PhotoTmax (ranging from 0.026 in CAM-2018 to 0.501 in VIT-2016) and GrowthEff (ranging from 0.041 in CAM-2018 to 0.437 in BAL-2015). The variability of model error on LAI was mostly due to variations in SLAmax (S_{Ti} = 0.286, sd = 0.058), BranchVol (S_{Ti} = 0.247, sd = 0.075), iniRadius (S_{Ti} = 0.216, sd = 0.082) and iniHeight (S_{Ti} =0.21, 0.044), with small interannual and site variability (Figure 5.2). RootDepth confirmed to be the most relevant parameter in explaining the model accuracy for SWC (Table 5.4), with very large variability (S_{Ti} = 0.325, sd = 0.209), followed by SLAmax (S_{Ti} = 0.172, sd = 0.076) and LeavesRip (S_{Ti} = 0.166, sd = 0.090). On average, the parameter SLAmax also resulted the most important source of variability of model error (mean rank 2.0) obtaining the highest S_{Ti} for LAI, the 3rd rank for FY and the 2nd for SWC. The qSA confirmed the large impact of initial plant dimensions on model error variability, with iniRadius (average rank 4.3), BranchVol (average rank 5.0) and iniHeight (average rank 5.7) ranked 2nd, 4th and 5th (Table 5.4). The top explaining parameter related to the photosynthetic process was PhotoTmax (average rank = 4.7), which ranked 2nd in explaining FY errors, followed by GrowthEff (average rank 6, while being the first parameter explaining FY error), and PhotoTopt, which ranked 8th according to the three model outputs considered.

Table 5.4 – Results obtained in the quantitative sensitivity analysis performed on the most relevant parameters in explaining model error (RRMSE) in simulating leaf area index (m^2 m^{-2}), fruit yield (kg $plant^{-1}$) and soil water content (m^3 m^{-3}), with mean rank (average value of the ranking for the three model variables). The three top-ranked parameters for each simulated variable are highlighted in grey. S_{Ti} = Sobol total order index. The acronym of model parameters, with unit and description are reported in Supplementary Table 5.3.

Parameter	Leaf area index		Fruits biomass		Soil water content		Mean
	S_{Ti}	Rank	S_{Ti}	Rank	S_{Ti}	Rank	rank
SLAmax	0.286	1	0.218	3	0.172	2	2.0
iniRadius	0.216	3	0.216	4	0.097	6	4.3
PhotoTmax	0.105	7	0.233	2	0.103	5	4.7
BranchVol	0.247	2	0.183	5	0.050	8	5.0
iniHeight	0.21	4	0.159	6	0.068	7	5.7
LeavesRip	0.112	6	0.096	8	0.166	3	5.7
GrowthEff	0.151	5	0.254	1	0.017	12	6.0
PhotoTopt	0.078	8	0.036	10	0.132	4	7.3
RootDepth	0.000	12	0.000	13	0.325	1	8.7
WoodDens	0.06	9	0.055	9	0.019	11	9.7
WoodC	0.004	10	0.010	11	0.047	9	10.0
FruitRip	0.002	11	0.114	7	0.000	15	11.0
WSthresh	0.000	12	0.003	12	0.031	10	11.3
Vcmax	0.000	12	0.000	13	0.005	13	12.7
iniCrown	0.000	12	0.000	13	0.000	14	13.0

5.4.2 MODEL ACCURACY IN REPRODUCING EXPERIMENTAL DATA

The performances of the hazelnut model in reproducing experimental data are presented in Table 5.5, which reports the values of various evaluation metrics computed between simulated and observed phenological development, LAI, FY and SWC in the four hazelnut orchards in 2015-2017.

Table 5.5 – Model performance evaluation indexes calculated before (PRE) and after model calibration (CAL) in the four experimental orchards. RRMSE = relative root mean square error, MAE = mean absolute error, EF = modelling efficiency, CRM = coefficient of residual mass, R^2 = coefficient of determination. Data used to compute the evaluation metrics refer to the three years of field trials (2015-2017).

			Experimental site							
Variable	Index	Unit	Baldissero		Camarico		Chitaskari		Viterbo	
			PRE	CAL	PRE	CAL	PRE	CAL	PRE	CAL
Leaf area index	RRMSE	%	17.39	17.42	34.11	29.13	33.88	13.77	21.35	16.91
	MAE	m^2 m^{-2}	0.60	0.59	1.01	0.91	1.23	0.46	0.58	0.49
	EF	-	0.80	0.80	-0.44	-0.05	-1.87	0.52	0.62	0.76
	CRM	-	0.02	0.02	0.13	-0.08	0.01	0.02	-0.09	-0.03
	R^2	-	0.80	0.80	0.07	0.18	0.23	0.55	0.71	0.77
Fruits biomass	RRMSE	%	52.03	22.89	39.36	21.45	80.06	12.26	16.80	8.60
	MAE	kg plant^{-1}	1.41	0.69	2.23	1.09	2.13	0.32	0.61	0.27
	EF	-	-0.79	0.65	-2.60	-0.07	-1.78	0.93	0.85	0.96
	CRM	-	-0.41	0.04	0.38	0.12	-0.67	-0.06	-0.07	0.03
	R^2	-	0.75	0.77	0.85	0.86	0.81	0.95	0.95	0.97
Soil water content	RRMSE	%	17.18	15.56	8.71	9.02	17.98	15.13	9.85	10.33
	MAE	m^3 m^{-3}	0.04	0.03	0.02	0.02	0.04	0.03	0.03	0.03
	EF	-	-0.23	-0.01	0.12	0.05	0.52	0.66	0.54	0.50
	CRM	-	-0.11	-0.08	-0.03	0.03	-0.12	-0.05	0.01	0.04
	R^2	-	0.57	0.57	0.68	0.47	0.76	0.79	0.64	0.65
Vegetative phases	RRMSE	%	6.48		6.33		3.53		6.63	
	MAE	days	7.26		9.14		5.43		10.00	

	EF	-	0.99	0.96	1.00	0.99
	CRM	-	-0.01	0.04	-0.01	0.00
	R^2	-	0.99	0.98	1.00	0.99
Reproductive phases	RRMSE	%	7.17	10.23	7.16	6.90
	MAE	days	7.18	10.35	8.07	8.54
	EF	-	0.98	0.98	0.99	0.99
	CRM	-	-0.02	0.00	-0.02	0.02
	R^2	-	0.98	0.98	0.99	0.99

Except for phenology, whose calibration was preliminary to sSA, the evaluation metrics were computed twice, before and after the model calibration to evaluate (i) model errors with original parameter settings and (ii) the impact of automatic optimisation on model performances. The timings of hazelnut phenological phases were correctly reproduced by the model, with RRMSE ranging from 3.53% in CHI to 6.63% in VIT for vegetative phases and from 6.9% in VIT to 10.23% in CAM for reproductive development. No systematic bias was present in the simulations, with coefficient of residual mass (Loague and Green, 1991; optimum = 0, if positive indicates model underestimation) values ranging from -0.02 (BAL and CHI, both for reproductive phases) to 0.04 (CAM for vegetative phases), and mean absolute error (Schaeffer, 1980; 0 to +∞, optimum 0) ranging between 5.43 days in CHI (vegetative development) to 10.35 days in CAM (reproductive phases). The automatic calibration led to a marked improvement of model performances especially for FY in all sites. RRMSEs after calibration ranged between 8.6% in VIT to 22.9% in BAL, with MAE in the range 0.27-1.09 kg plant^{-1} (VIT and CAM, respectively). Model application in CAM led to the largest model errors in sSA runs (MAE = 2.23 kg plant^{-1}, RRMSE = 80.1%, R^2 = 0.86), whereas best results were achieved in VIT (MAE = 0.61 kg plant^{-1}, RRMSE = 16.8%, R^2 = 0.95).

The accuracy in reproducing LAI was less affected by model calibration, with best results achieved in BAL (RRMSE = 17.39%, MAE = 0.59 m^2 m^{-2}, R^2 = 0.8) and worst performances in CAM (RRMSE = 29.13%, MAE = 0.91 m^2 m^{-2}, R^2 = 0.18). The original parameter sets used in sSA led to worst results for leaf area index in CAM (RRMSE = 34.11%, MAE = 1.01 m^2 m^{-2}, R^2 = 0.07) and CHI (RRMSE = 33.88%, MAE = 1.23 m^2 m^{-2}, R^2 = 0.23), and best performances in BAL, where the model was originally calibrated and evaluated (RRMSE = 17.42%, MAE = 0.6 m^2 m^{-2}, R^2 =

0.80). In general, SWC was correctly reproduced by the model, with similar performances after calibration in all sites, i.e., VIT (RRMSE = 10.33%, MAE = 0.03 $m^3 m^{-3}$, $R^2 = 0.65$), BAL (RRMSE = 15.56%, MAE = 0.03 $m^3 m^{-3}$, $R^2 = 0.57$), CHI (RRMSE = 15.13%, MAE = 0.03 $m^3 m^{-3}$, EF = 0.76) and CAM (RRMSE = 9.02%, MAE = 0.02 $m^3 m^{-3}$, $R^2 = 0.47$). The only cases in which the model performances were better in SA runs were the simulations of SWC in CAM and VIT, where slightly similar results were obtained in sSA and calibrated runs. The simulated LAI distributions in the four hazelnut orchards in the period 2015-2017, considering SA and calibration runs as separated are reported in Figure 5.3 along with field measurements.

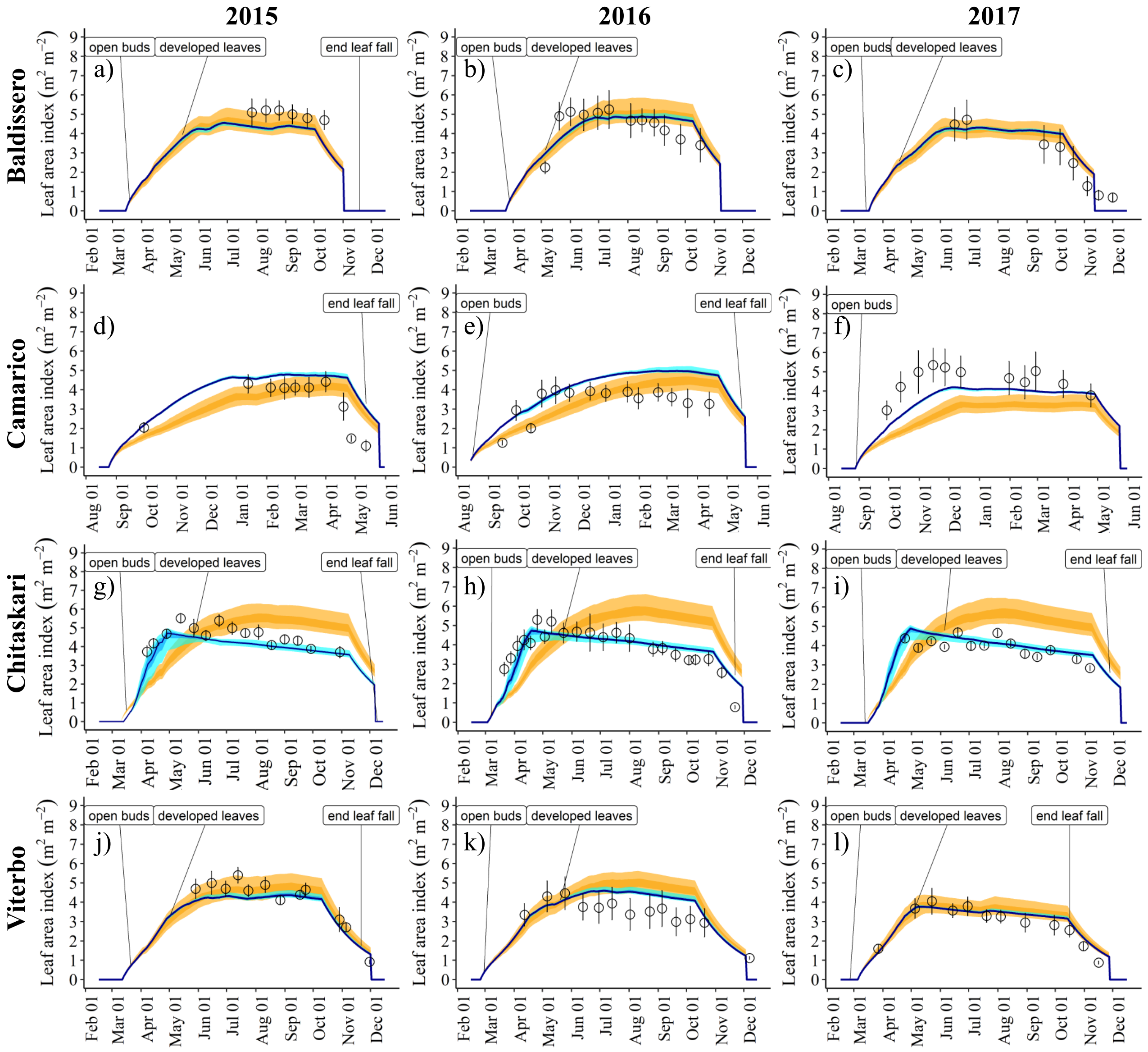


Figure 5.3 – Dynamic simulation of leaf area index in screening sensitivity analysis runs (orange shades, dark orange corresponds to the 40th-60th percentile and light orange the 25th-75th percentile) and in calibrated runs (blue shades, the line is the median, dark blue corresponds to the 40th-60th percentile and cyan to the 25th-75th percentile) in the four orchards of Baldissero (a, b, c), Camarico (d, e, f), Chitaskari (g,

h, i) and Viterbo (j, k, l) in the three years of field trials. Unfilled points indicate the average leaf area index value in measurements, with error bar corresponding to ± one standard deviation. Main vegetative phenological phases are labelled in the charts.

Simulated dynamics from sSA were closer to field measurements in BAL (Figure 3a,b,c) and VIT (Figure 3j,k,l), where the median of LAI in calibrated runs felt into the 25-75th percentile of sSA model runs (Figure 3). The field measurements in these sites depicted similar LAI shapes along the hazelnut growing season, with a smoother dynamic in the first stages after bud break, recorded between March 14th-19th in BAL and February 25th-Mar 21st in VIT (Supplementary Table 5.2). The measured and simulated LAI values increased up to completely unfolded leaves (V7), where similar maximum LAI values were measured, ranging from 4.7-5.2 m^2 m^{-2} in BAL and 4.1-5.4 m^2 m^{-2} in VIT (Supplementary Table 5.2). This phase corresponded to the start of a steep LAI decline during leaves senescence and fall, which was recorded on November 18th in BAL (2015, only available date) and between October 16th-November 21st in VIT. Simulated LAI patterns in CAM (Figure 5.3d,e,f) presented a smoother increase after bud break (around August 15th) both in SA and calibrated runs, followed by a plateau with higher values in 2017 (maximum LAI in the range 4-5.4 m^2 m^{-2} in 2017) until the start of leaves fall (May 16th-22nd), when it rapidly dropped. The calibration of model parameters led to increasing simulated LAI with respect to sSA runs, with the latter showing better results in 2015 (Figure 3d) and 2016 (Figure 5.3e). In CHI (Figure 5.3g,h,i), the simulated LAI dynamics after calibration were very close to field observations, reproducing a steeper increase in the leaves unfolding phases (bud break between March 7th-13th), with maximum values in the range 4.7-5.4 m^2 m^{-2}, followed by a smooth decline until the end of leaf fall, recorded between November 21st and December 9th in the three years of field experiments. The impact of calibration was particularly evident here, with sSA runs leading to a systematic overestimation of field data in the three growing seasons, especially after leaves unfolding.

The comparison of measured and simulated data of FY (kg $plant^{-1}$) in sSA and calibrated runs in the four hazelnut orchards in 2015-2017 is reported in Figure 5.4.

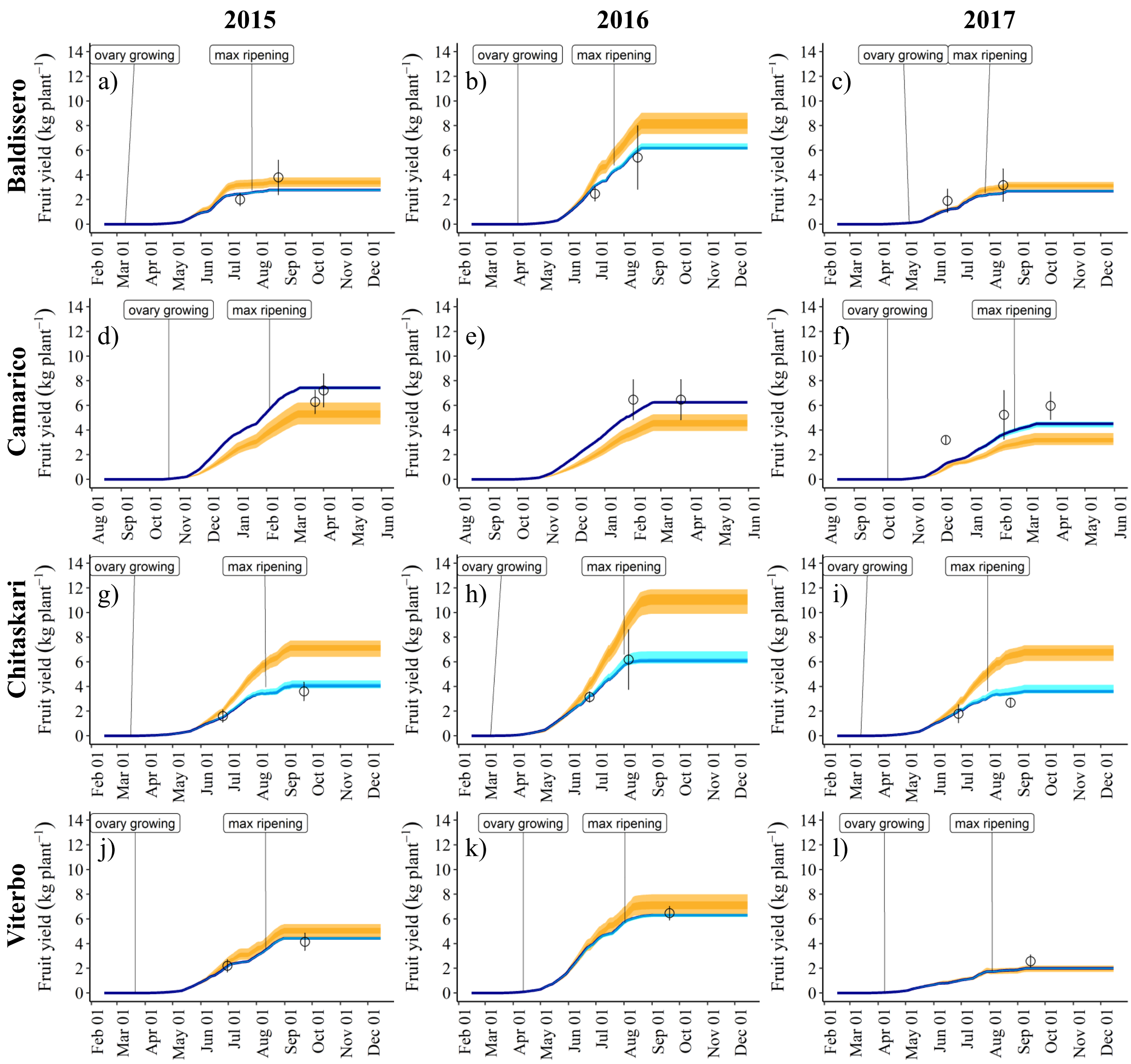


Figure 5.4 – Dynamic simulation of fruits yield (kg plant^{-1}) in screening sensitivity analysis runs (orange shades, dark orange corresponds to the 40th-60th percentile and light orange to the 25th-75th percentile) and in calibrated runs (blue shades, the line is the median, dark blue corresponds to the 40th-60th percentile and cyan to the 25th-75th percentile) in the four orchards of Baldissero (a, b, c), Camarico (d, e, f), Chitaskari (g, h, i) and Viterbo (j, k, l) in the three years of field trials. Unfilled points indicate the average fruits yield from field measurements, and the error bar corresponds to ± one standard deviation. Main reproductive phenological phases are labelled in the charts.

The measured dynamics of FY highlighted the marked alternate bearing of hazelnut trees in BAL (Figure 5.4a,b,c), CHI (Figure 5.4g,h,i) and VIT (Figure 5.4j,k,l). sSA and calibrated simulation runs correctly reproduced the trend with variable accuracy, whereas in CAM (Figure 5.3d,e,f) the hazelnut FY in the three years of experimental trials was quite constant, and ranged between 5.9 kg plant^{-1} in 2017 to 6.4 kg plant^{-1} in 2016. The model performances in sSA runs were better in BAL and VIT, according to LAI simulations (Figure 5.3). Both sites presented average lower production in 2015 (3.8 kg plant^{-1} in BAL, 4.1 kg plant^{-1} in VIT) and especially in 2017 (3.2 kg plant^{-1} in BAL, 2.6 kg plant^{-1} in VIT), the latter season also characterized by scarce precipitation amounts (Table 5.2) until maximum fruits ripening, which occurred in the last week of July in BAL and in the first half of August in VIT (Supplementary Table 5.1). The hazelnut model reproduced a very smooth increase of fruits dry weight from ovary growing stage, consistently to what has been recorded in similar periods in these two sites, i.e., March 10th (2015) to April 5th (2017) in BAL and March 21st (2015) to April 12th (2016) in VIT. Model runs in sSA showed a systematic overestimation in CHI (around 8 kg plant^{-1} in 2015 and 11 kg plant^{-1} in the on-year 2017) with respect to field measurements, coherently to LAI overestimation (Figure 5.3g,h,i), even if the alternate bearing was correctly reproduced, with 2016 as the only on-year in the field trial. The impact of automatic optimisation was evident here, with calibrated parameter sets leading to decreased fruits biomass accumulation rates from ovary growing (March 7th-16th) to maximum fruits ripening (July 30th-August 11th), coherently with observations. The model was able to reproduce the first samplings of fruits dry weight in R12 in the three cropping seasons (except in VIT-2017, missing data). Measured data of FY were higher in CAM in the three cropping seasons, where drip irrigation from ovary growing stage (October 6th -21st) was implemented. This site was the only one where sSA runs underestimated field measurements, with model calibrated parameter sets leading to an increase of simulated FY in all growing seasons (Figure 5.4d,e,f).

The simulated plant available water in sSA and calibrated runs, computed considering the ratio between SWC and the difference between field capacity and wilting point in the four hazelnut orchards is presented in Figure 5.5, along with field measurements and simulated water stress in calibrated runs.

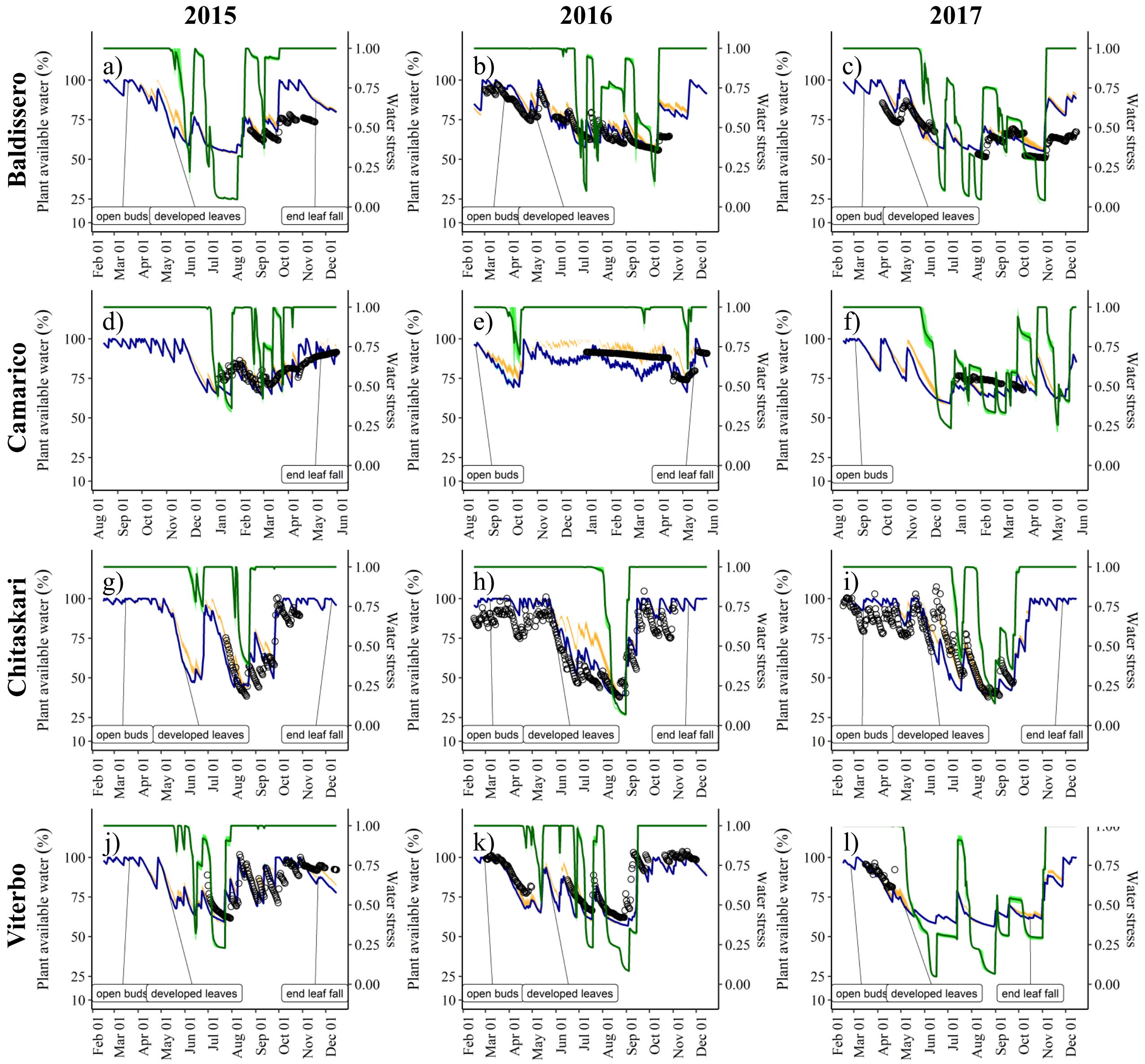


Figure 5.5 – Dynamic simulation of plant available water (primary y-axis, %, considering soil water content and the difference between field capacity and wilting point) in screening sensitivity analysis runs (orange shades, dark orange corresponds to the 40th-60th percentile and light orange to the 25th-75th percentile) and in calibrated runs (blue shades, the line is the median, dark blue corresponds to the 40th-60th percentile and cyan to the 25th-75th percentile) in the four orchards of Baldissero (a, b, c), Camarico (d, e, f), Chitaskari (g, h, i) and Viterbo (j, k, l) in the three years of field trials. Water stress, computed as the ratio of root water uptake to potential transpiration (0-1, with 1 corresponding to no stress) is plotted in the secondary y-axis. Unfilled points indicate the plant available water data according to soil water probes. The main vegetative phenological phases are labelled in the charts.

The simulation of plant available water during hazelnut growth denoted the model ability in reproducing measured dynamics of SWC both in sSA and calibrated runs. The simulations depicted similar trends in BAL (Figure 5.5a,b,c), CHI (Figure 5.5g,h,i) and VIT (Figure 5.5j,k,l), characterized by a progressive decrease of plant available water from open buds stage, being particularly marked in CHI-2016 and -2017 (minimum SWC = 0.15 m^3 m^{-3}). Coherently, simulated water stress in 2017 was more significant in the three sites of the northern hemisphere, with precipitations refilling the SWC and suddenly interrupting water stress conditions. In BAL (minimum SWC = 0.22 m^3 m^{-3}) and VIT (minimum SWC = 0.28 m^3 m^{-3}), the rainfall distribution in 2016 led to a higher soil water availability than in 2015, coherently with simulations. The model agreed with observations in simulating lower plant available water in 2017 in BAL and VIT, whereas in CAM the application of irrigation water led to higher SWC, especially in 2015 and 2016 (Figure 5.5d,e,f). Here, a steep decrease in soil water availability was simulated from September 2014 to January 2015, when precipitations and irrigation events started to refill the soil profile (no measured data). In the next cropping season, the simulated SWC decrease started in January 2016, with irrigation events gradually increasing soil water availability.

5.4.3 CORRELATIONS OF CALIBRATED PARAMETERS AND SIMULATED OUTPUTS DISTRIBUTIONS

The Spearman correlation coefficients computed between the model parameters and the simulated maximum value of LAI and FY in calibrated runs are reported in Figure 5.6, separately for each site.

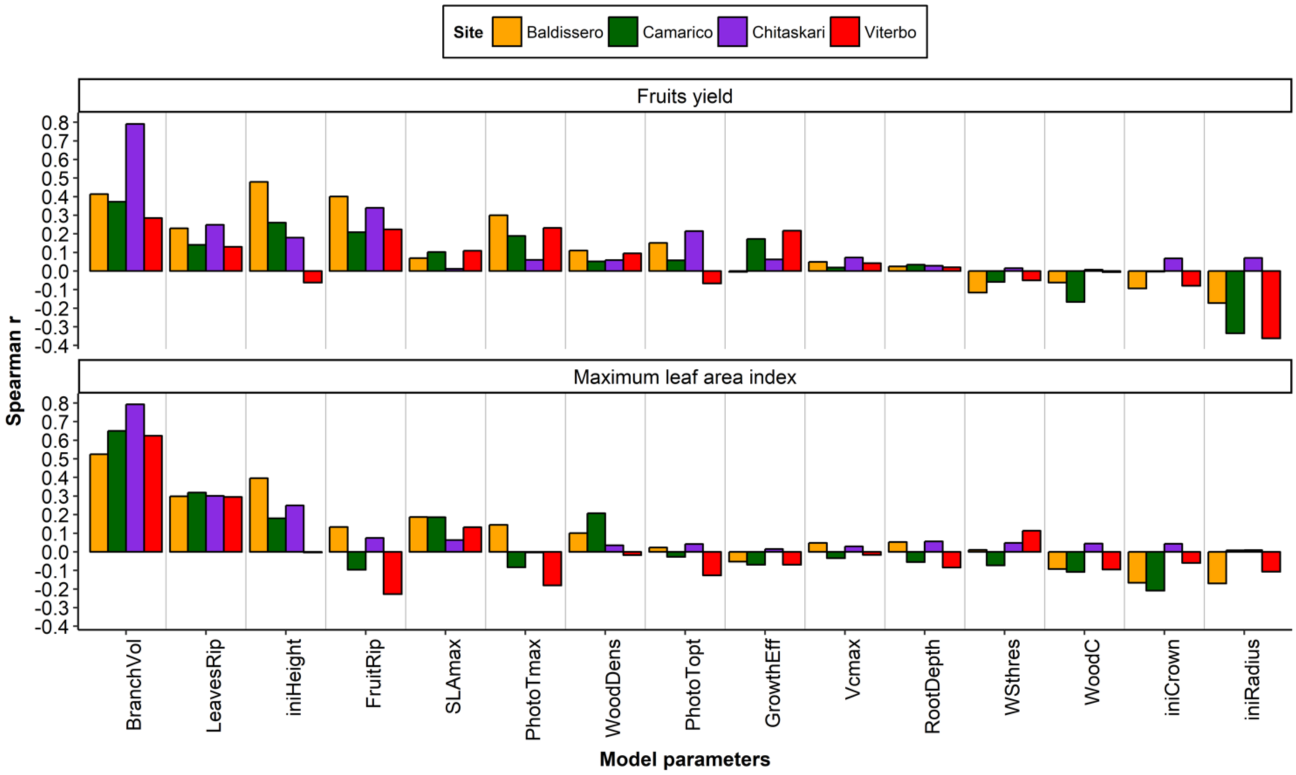


Figure 5.6 – Histograms presenting Spearman r computed between the distributions of each model parameter from screening sensitivity analysis and the corresponding simulated fruits yield (top chart) and maximum leaf area index (bottom chart) in the four sites after calibration runs.

The parameter BranchVol was the most positively correlated with LAI (Spearman r ranging from 0.525 in BAL to 0.793 in CHI) and FY (Spearman r ranging from 0.285 in VIT to 0.414 in BAL), with similar strength among sites. The second largest rank correlation with LAI was obtained by LeavesRip (Spearman r ranging from 0.295 in VIT to 0.318 in CAM), which was also positively correlated with FY, even if at a lesser extent (Spearman r ranging from 0.130 in VIT to 0.248 in CHI). FruitRip was positively correlated with FY in all sites (Spearman r ranging from 0.209 in CAM to 0.401 in BAL), whereas its impact on LAI was less clear, with weak positive correlations in BAL and CHI, and negative in CAM and VIT. The parameter SLAmax, ranked 1st in the qSA, showed slightly positive and quite constant relationships with LAI (Spearman r ranging from 0.06 in CHI to 0.187 in BAL), whereas the correlation with FY was not significant. PhotoTmax showed positive and significant correlations with FY (Spearman r ranging from 0.060 in CHI to 0.300 in BAL), whereas its impact on LAI was discordant, with weak relationships across sites. Among the model parameters connected to plant dimension, stronger correlations were obtained by iniHeight, which was positively correlated with LAI (Spearman r ranging from 0.179 in CHI to 0.479 in BAL) and FY (Spearman r ranging from 0.18 in CAM to 0.396 in BAL) in all sites but VIT, where the observed variance in the field was minimal (Table 5.1). We revealed negative correlations of the parameters iniCrown (Spearman r ranging from 0.18 in CAM to 0.396 in BAL) and iniRadius with LAI in VIT (Spearman r = -0.107) and BAL (Spearman r = -0.169). iniRadius was also negatively correlated with FY (Spearman r ranging from -0.172 in BAL to -0.362 in VIT, except in CHI). The other parameters obtained weaker correlations with LAI and FY, even if significant at p = 0.05 except for PhotoTopt, Vcmax, WoodDens and WSthresh.

5.5 DISCUSSION

The main objective of this study was the development of a reproducible methodology to extend the application of a hazelnut simulation model to new production environments, while testing it on four orchards in a three-year experimental trial. We formalized four steps i.e., two sensitivity analyses carried out for (i) screening the most relevant parameters and (ii) ranking their impact on the variability of model errors, followed by (iii) the automatic model calibration to match experimental data and (iv) the evaluation of the relationships between most relevant parameters and simulated LAI, FY and SWC. Each step consists of a standard analytic procedure commonly followed in the application of eco-physiological simulation models (Hamby, 1994; Pianosi et al., 2016) and provides pieces of information which could be used for various purposes, as discussed below. The main innovation here is represented by their integration in a unique workflow which is executed from start to finish. Available studies on ecological models usually focus on single aspects, e.g., the evaluation of the model sensitivity to parameter changes to deepen the knowledge on model (and system) behaviour (Ingalls, 2008), or the model calibration and validation on different experimental conditions (e.g., Kamali et al., 2018). The iterative adoption of sensitivity analysis assessments and automatic optimisation in the model building procedure are indeed considered as good practices in the development and evaluation of environmental models (Jakeman et al., 2006), either for determining how model structure and parameter values are to be found or to quantify the uncertainty in model estimates. Another peculiar element of this study is the use of an error evaluation metric (RRMSE) as the target variable of sensitivity analysis, computed on the different model outputs (FY, LAI and SWC). This is quite unusual in SA studies on ecological models, as most of them analyse the model sensitivity to parameter changes either focusing on single synthetic model outputs (e.g., aboveground biomass, Legaard et al., 2015; yield, Vanuytrecht et al., 2014) or on the variability of simulated variables, without any link with model error in reproducing reference data (e.g., Wang et al., 2013; He et al., 2016). Our choice was driven by the purpose of pointing out the most important sources of variability in model errors to individuate the processes deserving specific efforts in model development activities (Pappas et al., 2013) while gaining information on the typologies of data needed to improve model performances in reproducing measured data.

5.5.1 PRIORITIZING MODEL DEVELOPMENT ACTIVITIES AND FIELD DATA COLLATION

The main purposes of a sSA, involving fewer input requirements at less computational efforts (Gan et al., 2014), include the identification of research areas that should receive further evaluation for model improvement and simplification (Frey and Patil, 2002), the reduction of the number of model parameters considered for calibration (Makowski et al., 2011) and to prioritize field data collection (Chow et al., 2018). Clear indications emerge from the sensitivity analyses performed in our study, as the hazelnut model used here demonstrated to be most sensitive to parameters related to leaf area development (LeavesRip, SLAmax), plant architecture (iniHeight, iniRadius, iniCrown, BranchVol), photosynthetic process (PhotoTmax, PhotoTmin, GrowthEff, Vcmax), water stress (RootDepth, WSthresh), fruits ripening (FruitsRip) and carbon pool initialization (WoodDens and WoodC). Some of these parameters could be directly measured in field experiments with standard protocols at relatively low cost (e.g., SLAmax, Garnier et al., 2001), whereas others were already measured in our field experiments (iniHeight, iniRadius, iniCrown). Other relevant model parameters can be determined via destructive methods, e.g., wood density (Zobel and Jett, 1995), root depth (Crombie et al., 1988) and the ratio between branch and crown volume. The range of branch-volume ratio in model calibration was refined setting its minimum at 0.00025 m^3 m^{-3}, the default value defined in the seminal model of the Tree component (Donatelli et al., 2010) developed during SEAMLESS project (http://www.seamless-ip.org/) and used in our model. The field measurement of this parameter on species where branching is complex is particularly challenging and affected by large uncertainty (Ver Planck and MacFarlane, 2014), even if several approaches exist to predict dendrometric features as crown and branch volumes via allometric relationships with diameter and length (Akindele and LeMay, 2006), even if very few adapted to orchard fruit species (Fernandez-Puratich et al., 2013). The very high correlation obtained by BranchVol with FY and LAI was so clearly revealed likely due to the large range used in the settings of automatic optimisation. Despite the outcomes from the correlation step can be affected by the superimposed parameter ranges, as in every sensitivity analysis and calibration study (Shin et al., 2013; Jabloun et al., 2018), the reproducibility of the methodology would ensure a straightforward application with different settings and when new experimental data will be available. Nonetheless, the considerable importance gained by parameters related to plant dimension and carbon pool initialisation suggests a revision of the model representation of the plant architecture, rather than indicating the need of refining model calibration reducing the uncertainty in parameter values. The approach implemented here indeed relies on a simplified mono-dimensional representation of the plant, whereas new trends in tree modelling promote the realization of functional-structural models (Perttunen et al. 1996; Vos et al., 2009) with a spatially explicit definition of a three-dimensional plant architecture (e.g., L-PEACH, Allen, 2005). The upgrade of the current model formalisation would certainly improve the simulations of carbon source-sink interactions and the carbohydrate transport within the plant (Bidel et al. 2000), despite bringing additional complexity to the current model, which should be taken into account.

The remaining most relevant parameters from the sensitivity analyses (e.g., PhotoTmax, PhotoTmin, GrowthEff, Vcmax) highlighted the need of focusing on the related processes to collect new useful data for model improvement, especially for the photosynthetic process, which is currently simulated using a simplified Farquhar approach, with stomatal conductance decoupled from gross assimilation. The availability of photosynthetic flux data under different temperature conditions and soil water availability (Joseph et al., 2014) would allow comparing the current representation with alternative approaches (e.g., Tuzet et al., 2003; Yin and Struik, 2009), or using an ensemble of models in operational applications, as done in international initiatives in crop modelling (e.g., AgMIP, Rosenzweig et al., 2013).

5.5.2 MODEL PERFORMANCES IN REPRODUCING OBSERVED DATA

The model performances in reproducing experimental field data were evaluated twice in this study, using simulated outputs from sSA and after calibration of most relevant parameters. The evaluation allowed quantifying the model accuracy with original parameter sets (Bregaglio et al., 2016), thus performing a “blind” validation followed by the distinct evaluation of the impact of automatic optimisation on the explanatory power of the hazelnut model. According to Jamieson et al. (1991), who provided reference values to judge model accuracy according to RRMSE values, the average model performances across sites increased from “fair” (RRMSE 20-30%) to “good” (RRMSE 10-20%) for LAI (RRMSE = 21.35% and 15.45% in sSA and calibration, respectively), from “poor” (RRMSE > 30%) to “good” for FY (RRMSE = 37.65% and 13.04% in sSA and calibration, respectively), while remaining “good” for SWC (RRMSE = 10.74% and 10.01% in sSA runs and calibration, respectively). The simulation of phenological development, whose calibration preceded the sSA, led to “excellent” (RRMSE < 10%) model performances both for vegetative (RRMSE = 4.6%) and reproductive (RRMSE = 6.29%) phenological phases. The measured and simulated LAI values in the four sites (Figure 5.3, Supplementary Table 5.2) were in the range measured on cultivar ‘Tonda Giffoni’ in a mature hazelnut orchard in central Italy by Farinelli et al. (2005), who reported maximum values of 5.4-9.5 m^2 m^{-2} across pruning treatments, and agreed with Hampson et al. (1996), who measured LAI = 6.1 m^2 m^{-2} in 17- and 32-year old hazelnut orchards. Available data on FY are available in Farinelli et al. (2005), who reported 4.6-5.2 kg $plant^{-1}$ in low and dense canopies, whereas Rovira et al. (2017) and Baldwin et al. (2003) performed varietal trials in Spain and southeastern Australia, measuring average yields of 3.49 kg $plant^{-1}$ in 2005-2011, and ranging from 2.2 to 4.7 kg $plant^{-1}$ on ‘Tonda Giffoni’, respectively. Given the recent spread of hazelnut cultivation in Chile, available studies do not present yield data on orchards at full production, and the few studies report yields of 0.9 kg $plant^{-1}$ on cultivar ‘Tonda Gentile delle Langhe’ and 2 kg plant^{-1} on 'Tonda Giffoni' in a 4 year orchard placed very close to CAM (Grau et al., 2001; Grau and Bastias, 2005). The data collected in this work, which ranged between 2.7 kg plant^{-1} (sd = 0.5 kg plant^{-1}) in VIT-2017 and 6.4 kg plant^{-1} (sd = 1.6 kg plant^{-1}) in CAM-2016 agree with available information, while highlighting the marked alternate bearing of hazelnut tree and a similar productive potential in the four orchards tested (Supplementary Table 5.2). Soil water content was adequately reproduced by the model in all sites, with RRMSE ranging from 9.02% in CAM to 15.56% in BAL, which are in line with other studies where the performances of soil water model on agricultural soils were evaluated (Markewitz et al., 2010; Constantin et al., 2015).

5.5.3 LIMITATIONS AND PERSPECTIVES

It is a widely accepted concept across scientific disciplines that *the best model does not exist* (Schmidt et al., 2006; Carrizosa et al., 2014; Lauret et al., 2016), along with the claim of constantly improving (Antle et al., 2017) and overhauling (Rötter et al., 2011) current crop models, at least until the next paradigm shift (Kuhn, 1962) will occur, leading to other plausible and worthy model representation of the same phenomena (Feyerabend, 1975). In a smaller way, the main limitation of the hazelnut model considered here is the simplified simulation of the impact of pollen dispersal efficiency on FY, which currently relies on an empirical coefficient reducing fruits partitioning in off-years, despite the complexity of the biophysical processes involved (Ulger et al., 2004). The alternate bearing is a well-known habit in nut crops (e.g., almond, Valdebenito et al., 2018; pecan, Rohla et al., 2007; pistachio, Ak et al., 2016), and would certainly deserve specific experiments to reproduce the mechanisms as influenced by the variability in agro-meteorological conditions. Other limits of the hazelnut model emerged from the sensitivity analysis assessment, and were previously discussed; an additional consideration could be done for the processes which did not result as prominent in explaining model error (e.g., stomatal conductance, maintenance respiration): we leave the need to characterise the underlying processes further or to question current formalizations to another investigation.

The logical consequence of this study is the model application to realise dynamic monitoring of hazelnut growth and development, to gain timely information on the status of the orchard in order to e.g., anticipate the occurrence of water stress conditions, or to forecast a phenological stage associated to a high susceptibility of abiotic or biotic stresses. The implementation of such a decision supporting system would require the coupling of the model with a georeferenced source of information, to provide past and forecasted weather data as input at high-spatiotemporal resolution to consider the inner heterogeneity of the orchard (Perry et al., 2010), while allowing a user-friendly interaction (Mysiak et al., 2005) with the hazelnut grower. Another perspective concerns the model application to assess the suitability of hazelnut cultivation in new agro-environmental conditions, or to perform yield forecasting under alternative climatic scenarios, as extensively done for main food crops (Wheeler and von Braun, 2013). The crucial prerequisite for these studies is the reliability of the model in reproducing hazelnut growth patterns in current growing systems, which is one of the primary outcomes of this study.

5.6 CONCLUSIONS

The modelling work presented here aims at extending the application of a hazelnut model to different growing environments, via the combination of sensitivity analysis and calibration techniques. We organized the methodological workflow in four steps, which were progressively executed to screen most relevant model parameters and to rank their impact on the variability of model errors in reproducing field data. Then, the model accuracy has been improved by automatic optimisation of relevant model parameters, whose relationships with model outputs were investigated and quantified via Spearman rank correlation. The integration of these four steps led to the development of a reproducible method which provided useful information to prioritise new field data collection and to support further model improvement, while the results obtained in simulating hazelnut growth and development across sites and years encourage an operational model use for in-season farmer support and in scenario analyses.

5.7 ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

Supplementary Table 5.1 – Codes and description of the vegetative and reproductive phenological phases simulated by the hazelnut modelling solutions, according to the Italian Phenological Garden (IPG) scale. The corresponding Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) code is reported when available.

IPG code	BBCH code	Description
		Vegetative phases
V1	00	Bud dormancy
V2	03	End of bud swelling
V3	7-10	Bud breaking: swollen and opening buds with folded leaves
V4	11	Open buds and first young leaves with unfolded blade
V5	12-18	Young leaves unfolded, not yet full size
V6	19	Young leaves unfolded together with leaves fully expanded
V7	91	Leaves fully developed
V8	92	Beginning of leaf discoloring
V9	94	Leaves mostly discolored
V10	93	Beginning of leaf dryness
V11	95	Leaves mostly dried up
V12	93	Beginning of leaf fall
V13	95	Leaves mostly fallen
V14	97	End of leaf fall, plants dormant
		Reproductive phases
R7	71	Beginning of female flowering

R8	72-77	Full female flowering
R9	79-87	End of female flowering
R10	89	Beginning of ovary growing
R11	-	Beginning of fruit ripening
R12	-	Fruits visible but mostly unripe
R13		Fruits maximum ripening
R14		Beginning of fruits fall and seed dispersal

Supplementary Table 5.2 – Summary of the experimental data collected in the four hazelnut orchards in 2015-2017. Data concern the observations of the main vegetative (bud break, end of leaf fall) and reproductive (ovary growing, maximum fruit ripening) phenological phases, the measurements of the plant growth data (fruits dry weight and leaf area index) and the characterization of soil water content.

Measured data	Variable	Year	Experimental site			
			Baldissero	Camarico	Chitaskari	Viterbo
Vegetative phase	Open buds (date)	2015	Mar 19	Aug 15	Mar 16	Mar 21
		2016	Mar 28	na	Mar 7	Febr 29
		2017	Mar 14	Aug 16	Mar 13	Febr 25
	End leaf fall (date)	2015	Nov 18	May 22	Dec 9	Nov 21
		2016	na	May 12	Nov 21	na
		2017	na	May 16	Nov 27	Oct 16
Reproductive phase	Ovary growing (date)	2015	Mar 10	Oct 21	Mar 16	Mar 21
		2016	Apl 6	na	Mar 7	Apr 12
		2017	Apr 5	Oct 6	Mar 13	Apr 8
	Maximum ripening (date)	2015	Jul 27	Feb 4	Aug 11	Aug 11
		2016	Jul 21	na	Aug 1	Aug 2
		2017	Jul 27	Feb 16	Jul 30	Aug 4
Plant growth	Fruits dry weight, sd (kg plant^{-1})	2015	3.8 (1.4)	6.3 (0.9)	3.6 (0.8)	4.1 (0.7)
		2016	5.4 (2.6)	6.4 (1.6)	6.2 (2.4)	6.5 (0.6)
		2017	3.2 (1.3)	5.9 (1.1)	2.7 (0.4)	2.6 (0.5)
	Leaf area index maximum,	2015	5.2 (0.5)	4.4 (0.5)	5.4 (0.3)	5.4 (0.4)
		2016	5.2 (0.9)	4 (0.5)	5.2 (0.6)	4.5 (0.8)

	sd (m^2 m^{-2})	2017	4.7 (1)	5.4 (0.9)	4.7 (0.5)	4.1 (0.67)
Soil water availability	Average plant available water, sd (%)	2015	68	84	56	75
		2016	70	89	70	79
		2017	68	93	66	na

Supplementary Table 5.3 – Acronym, unit and description of the model parameters analized in this paper, with ranges used in sensitivity analysis assessments (Use, Y = yes, N = no) and calibrated values in the four orchards (CAM = Camarico, CHI = Chitaskari, VIT = Viterbo, BAL = Baldissero).

			Sensitivity analysis			**Calibrated values**			
Parameter	**Description**	**Unit**	**Use**	**min**	**max**	**CAM**	**CHI**	**VIT**	**BAL**
BaseTemperatureReproductive	Base temperature for reproductive phase development	°C	N			7			
BaseTemperatureVegetative	Base temperature for vegetative phase development	°C	N			5			
BranchVol	Branch to crown volume ratio	m^3 m^{-3}	Y	0.00045	0.00055	0.0003	0.0002	0.0003	0.0003
Chilling_GDD_Threshold	Chilling threshold to start thermal accumulation	h	N			415	690	510	410
Vcmax	Maximum carboxylation rate	micromoles m^{-2} s^{-1}	Y	31.5	38.5	34.45	32.75	34.12	35.13
FastSen	Daily amount of leaf senescence during leaf fall	kg C d^{-1}	Y	0.018	0.022	0.0			
GrowthEff	Cost of growth respiration	unitless	Y	0.612	0.748	0.71	0.69	0.74	0.66
ImbThr	Imbalance threshold	unitless	Y	0.63	0.77	0.7			
iniCabove	Tree initial aboveground carbon fraction	unitless	Y	0.45	0.55	0.5			
iniCrown	Tree initial crown base height	m	Y	0.36	0.44	0.90	0.50	0.99	0.39
iniRadius	Tree initial crown radius perpendicular to row	m	Y	1.62	1.92	1.31	1.25	1.73	1.75
iniHeight	Tree initial height	m	Y	1.8	2.2	4.06	3.34	4.10	3.89
LeafDens	Leaf area density	m^2 m^{-3}	Y	1.9305	2.3595	2.15			
LeafC	Leaf carbon content	kg C kg^{-1}	Y	0.45	0.55	0.5			
LeafMass	Leaf mass area	kg m^{-2}	Y	0.09	0.11	0.1			

Parameter	Description	Unit	Sensitivity analysis			Calibrated values			
			Use	min	max	CAM	CHI	VIT	BAL
LeafSen	Daily amount of leaf senescence	kg C d^{-1}	Y	0.00135	0.00165	0.0015			
LightCoeff	Coefficient for light response function	unitless	Y	0.009	0.011	0.01			
NSCfract	Maximum non structural carbon allocated to growth	%	Y	0.0252	0.0308	0.03			
FruitRip	Maximum partitioning to fruits	unitless	Y	0.234	0.286	0.28	0.20	0.26	0.22
RootDepth	Maximum root depth	m	Y	0.81	0.99	0.98	0.83	0.94	0.91
CondTmax	Maximum temperature for stomatal conductance	°C	Y	31.95	39.05	35.5			
PhotoTmax	Maximum temperature for photosynthesis	°C	Y	31.95	39.05	36.87	34.44	38.09	37.78
MaximumTemperatureReproductive	Maximum temperature for reproductive development	°C	N			35			
MaximumTemperatureVegetative	Maximum temperature for vegetative development	°C	N			35			
MaxDailyNSC	Maximum daily biomass from non structural carbon	%	Y	0.09	0.11	0.1			
CondTmin	Minimum temperature for stomatal conductance	°C	Y	3.6	4.4	4			
PhotoTmin	Minimum temperature for photosynthesis	°C	Y	3.6	4.4	4			
CondTopt	Optimum temperature for stomatal conductance	°C	Y	22.95	28.05	25.5			
PhotoTopt	Optimum temperature for photosynthesis	°C	Y	22.95	28.05	25.52	24.94	26.27	24.35
OptimumTemperatureReproductive	Optimum temperature for reproductive development	°C	N			22.00			
OptimumTemperatureVegetative	Optimum temperature for vegetative development	°C	N			18.00			
LeavesRip	Partitioning to leaves at bud burst	unitless	Y	0.4	0.48	0.40	0.46	0.43	0.47

Parameter	Description	Unit	Sensitivity analysis			Calibrated values			
			Use	min	max	CAM	CHI	VIT	BAL
PARtoGlobalSolarRadiationRatio	PAR to global solar radiation ratio	unitless	N			0.5			
PhotoperiodEffect	Effect of photoperiod on leaf fall	unitless	N			0			
PhotoperiodThresholdForLeafFall	Threshold of photoperiod to trigger leaf fall	hours	N			12.5			
R7_GDD_Threshold	Growing degree days to reach phenological phase R7	°C d^{-1}	N			6	23	2	37
R8_GDD_Threshold	Growing degree days to reach phenological phase R8	°C d^{-1}	N			43	55	13	59
R9_GDD_Threshold	Growing degree days to reach phenological phase R9	°C d^{-1}	N			73	120	59	85
R10_GDD_Threshold	Growing degree days to reach phase R10	°C d^{-1}	N			426	218	303	264
R11_GDD_Threshold	Growing degree days to reach phase R11	°C d^{-1}	N			630	676	640	561
R12_GDD_Threshold	Growing degree days to reach phase R12	°C d^{-1}	N			746	1153	916	864
R13_GDD_Threshold	Growing degree days to reach phase R13	°C d^{-1}	N			1251	1643	1505	1270
R14_GDD_Threshold	Growing degree days to reach phase R14	°C d^{-1}	N			1569	1909	1713	1383
R15_GDD_Threshold	Growing degree days to reach phase R15	°C d^{-1}	N			1788	2011	1828	1525
R16_GDD_Threshold	Growing degree days to reach phase R16	°C d^{-1}	N			2088	2154	1933	1765
Q10	Relative respiration rate per 10° temperature increase	unitless	Y	1.8	2.2	2			
BranchResp	Relative maintenance respiration of branches	g C g^{-1} d^{-1}	Y	0.0018	0.0022	0.002			
RootFResp	Relative maintenance respiration of fine roots	g C g^{-1} d^{-1}	Y	0.0018	0.0022	0.002			
LeavesResp	Relative maintenance respiration of foliage	g C g^{-1} d^{-1}	Y	0.009	0.011	0.01			

Parameter	Description	Unit	Sensitivity analysis			Calibrated values			
			Use	**min**	**max**	**CAM**	**CHI**	**VIT**	**BAL**
FruitsResp	Relative maintenance respiration of fruits	g C g^{-1} d^{-1}	Y	0.009	0.011	0.01			
StemsResp	Relative maintenance respiration of stems	g C g^{-1} d^{-1}	Y	0.00018	0.00022	0.0002			
RootHalfLife	Root half life	d	Y			250			
SLAmax	Maximum specific leaf area	m^{2} kg^{-1}	Y	18.9	23.1	21.45	19.59	20.64	20.71
SLAmin	Minimum specific leaf area	m^{2} kg^{-1}	Y	7.2	8.8	8			
SLAws	SLA water stress responsiveness	unitless	N	1.035	1.265	1.15			
CondTMax	Maximum stomatal conductance	°C	Y	0.873	1.067	0.97			
NSCtarget	Non structural carbon fraction target	%	Y	0.09	0.11	0.1			
TemperatureEffect	Effect of temperature on leaf fall	unitless	N			2			
TemperatureThresholdForLeafFall	Threshold of temperature to increase senescence	°C	N			25			
V1_GDD_Threshold	Growing degree days to reach phase V1	°C d^{-1}	N			9	50	17	42
V2_GDD_Threshold	Growing degree days to reach phase V2	°C d^{-1}	N			19	145	165	132
V3_GDD_Threshold	Growing degree days to reach phase V3	°C d^{-1}	N			50	210	188	187
V4_GDD_Threshold	Growing degree days to reach phase V4	°C d^{-1}	N			236	320	317	247
V5_GDD_Threshold	Growing degree days to reach phase V5	°C d^{-1}	N			351	440	397	332
V6_GDD_Threshold	Growing degree days to reach phase V6	°C d^{-1}	N			466	640	700	432
V7_GDD_Threshold	Growing degree days to reach phase V7	°C d^{-1}	N			496	970	846	565

Parameter	Description	Unit	Sensitivity analysis			Calibrated values			
			Use	min	max	CAM	CHI	VIT	BAL
V8_GDD_Threshold	Growing degree days to reach phase V8	°C d^{-1}	N			2090	2250	1830	1661
V9_GDD_Threshold	Growing degree days to reach phase V9	°C d^{-1}	N			3423	2758	1946	3014
V10_GDD_Threshold	Growing degree days to reach phase V10	°C d^{-1}	N			4082	5300	2055	4042
V11_GDD_Threshold	Growing degree days to reach phase V11	°C d^{-1}	N			4861	6000	2608	4820
V12_GDD_Threshold	Growing degree days to reach phase V12	°C d^{-1}	N			6426	6500	2974	5663
V13_GDD_Threshold	Growing degree days to reach phase V13	°C d^{-1}	N			7467	9000	6991	7899
V14_GDD_Threshold	Growing degree days to reach phase V14	°C d^{-1}	N			9016	10000	10494	9890
CondVPDmax	Vapor pressure deficit for maximum conductance	KPa	Y	0.18	0.22	0.2			
CondVPDmin	Vapor pressure deficit for minimum conductance	KPa	Y	3.33	4.07	3.7			
WSexp	exponential coefficient for water content response	unitless	Y	0.9	1.1	1			
WSmult	Multiplicative coefficient for water content response	unitless	Y	0.9	1.1	1			
WSthresh	Water stress threshold to impact on total daily growth	unitless	Y	0.45	0.55	0.47	0.53	0.51	0.46
WaterStressResponsiveness	Water stress responsiveness	unitless	Y	1.035	1.265	1.15			
WoodC	Wood carbon content	kg C kg^{-1}	Y	0.45	0.55	0.46	0.46	0.49	0.50
WoodDens	Wood volumetric mass density	kg m^{-3}	Y	666	814	777	755	753	702

CHAPTER 6 GENERAL DISCUSSION AND PERSPECTIVES

Specific objectives and achievements

The project aimed at reducing the gap of knowledge between plant breeding and crop modelling in rice. Specific experiments were carried out to characterise the phenological development and growth of the most important Italian rice varieties, and their morpho-physiological traits were translated into crop model parameter sets.

The specific objectives of this doctorate were:

- The release of resources aimed at extending the state of the art of rice cultivation in Italy, with emphasis on the aspects related to plant breeding and the biodiversity of the Italian rice varietal landscape, its evolutionary trends and the associations with published molecular data.
- Improving crop modelling capabilities in the area to support new integrated model-assisted breeding programs by providing ready-to-use data and context towards a detailed representation of cultivars in crop models.

The first objective involved the characterisation of the Italian rice varietal landscape, focused at exploring its phenotypic variability on few critical agronomic traits (phenology, culm and panicle length, grain biometrics), highlighting the evolutionary trends and associations with available molecular studies. A database containing quantitative information on seven yield-related traits (days to heading, days to maturity, stem and panicle length, caryopsis length and width, thousand seeds weight) for 351 cultivars representing the rice varieties released in Italy since 1900 was created, together with a graphic frontend used for performing database operations and querying. The purpose was twofold: a) to analyse the evolutionary trends and associations with phylogeny data present in literature; b) select a subsample of genotypes to be included in the field trial.

The analysis revealed the main historical trend in Italian rice breeding and allowed exploring traits variability and highlighting their associations. Evident temporal trends were found in Italian rice breeding history (Chapter 2) towards plant height reduction, earliness, and spindle-shaped caryopses. Previous evidence of maintained genetic diversity could not be confirmed as we found a considerable reduction of phenotypic variability in the cultivars released in the last 20 years. Two of the three phylogenetic groups considered in the study showed overlapping phenotypic characteristics with high variability in phenotypic expression. The study released quantitative information to assist future breeding programs and characterised the phenotypic space of variation of Italian genotypes, setting the basis for next applications in crop models to develop cultivar-specific parameterizations.

The developed database (cultivar data) was screened to derive a sample of 40 cultivars which were further characterised with a 2-year dedicated field experiment (2016-2017). The method used aimed at maximising the sample variance associated with the seven measured traits. The chosen accessions maximized the phenotypic variability, comprising both old historical and modern Italian rice cultivars. The field trial provided a valuable source of data of yield-related traits, including the duration of phenological stages, the leaf area development, the plant and grain biometrics, the biomass accumulation up to the final yield. Collected data were subjected to multivariate analysis to highlight correlations and evaluate traits variability. The analysis (Chapter 3) provided readymade insights for next crop modelling studies targeting the Italian rice area, particularly those involving the definition and evaluation of new ideotypes adapted for climate change conditions.

The second objective involved the development of a methodology to derive cultivar-specific model parameter sets with a minimum dataset of field observations (Chapter 4). Long-term experimental data collected in the Value and Cultivation of Use (VCU) trials on Italian rice varieties in 2004-2015 were used to perform a cultivar-specific calibration of the crop model WOFOST_GT. A minimum data set including phenological observations of heading and maturity, and canopy height and grain yield measurements were used as reference variables for model calibration. The WOFOST_GT model was chosen because of the availability of a dedicated sensitivity analysis performed on the same crop and in similar agro-environmental conditions. The results revealed a good ability of the crop model in reproducing reference observations of phenology, canopy height and yield across cultivars and years. The method was efficient in deriving robust cultivar parameterisations and released thirteen parameters sets representing the most cultivated Italian rice varieties of the last twenty years, fostering improvements of future modelling studies at different spatial and temporal resolutions.

Finally, the methodology outlined in Chapter 4 was evaluated in a different context, i.e. to improve the capabilities of a hazelnut simulation model (Chapter 5) to be applied in different growing environments. The methodological framework combining the use of sensitivity analyses techniques and automatic calibration fostered the model application in varying agro-pedo-meteorological conditions. The work offered insights about the effect of the model parameters in influencing simulated leaf area index, fruit biomass and soil water content, identifying areas for improvement and data collection to further investigate model performances. The hazelnut model sensitivity varied according to the model output considered, with parameters related to plant dimensions and photosynthesis resulting prominent in explaining leaf area index dynamics and fruit biomass accumulation.

6.1 FUTURE PERSPECTIVES

Further research originates from the present work. The integration of phenotypic and phylogenetic characterisations provides information that could support breeders in the selection of divergent genetic material for hybridisation programs. The synthetic representations of Italian cultivars developed in the study are preliminary work for further studies in different environments and management conditions. Large-scale modelling studies can exploit these resources to account for the heterogeneity of the genotypic component when studying G × E × M interactions in Italian rice area. Moreover, released data could drive new ideotyping studies that could account for the physiological limits and the compensatory effects that exist among crop traits. Thanks to the extended time-window covered by the data used in the study, our calibration could account for the increases in atmospheric CO_2 fostering its application in future climate change scenarios.

LIST OF PUBLICATIONS

Published

Mongiano, G., Titone, P., Tamborini, L., Pilu, R., & Bregaglio, S. (2018). Evolutionary trends and phylogenetic association of key morphological traits in the Italian rice varietal landscape. Scientific Reports, 8(1). http://doi.org/10.1038/s41598-018-31909-1

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