Proceedings of the International Workshop:
“Crop Improvement in a Changing Environment: the RISINNOVA Project for sustainable rice production in Italy”
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“Crop Improvement in a Changing Environment: the RISINNOVA Project for sustainable rice production in Italy”  
Venezia, Italy - 29/30 October 2012

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BAKANAE DISEASE OF RICE IN ITALY: SURVEY OF INCIDENCE, IDENTIFICATION OF *FUSARIUM FUJIKUROI* AS THE MAJOR CAUSAL AGENT AND CHARACTERIZATION OF PATHOGEN POPULATION STRUCTURE USING MICROSATELLITE MARKERS.


*) CRA – Plant Pathology Research Centre, Via C. G. Bertero 22, 00156 Rome, Italy; **) AGROINNOVA – University of Turin, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy

*Fusarium fujikuroi*, population genetics, SSR.

*Fusarium fujikuroi* Niremberg, belonging to *Gibberella fujikuroi* complex, is the causal agent of Bakanae disease of rice, an increasing problem for rice cultivation in Italy. The objective of this study is to determine the genetic structure within and among populations of *F. fujikuroi* coming from the major Italian rice areas by using efficient Simple Sequence Repeats (SSRs). Though *F. fujikuroi* is the most abundant *Fusarium* species found on rice, other species can also be isolated from rice. Multiple alignment of translation elongation factor (TEF) gene sequences of different *Fusarium* spp. showed a deletion of six nucleotides in *F. fujikuroi* sequence. This element of variability was used to develop a conventional PCR assay for diagnosis. Primer specificity was confirmed by analysing the DNA of several strains of *Fusarium* spp. isolated from rice plants and seeds in Italy. 221 fungal isolates derived from symptomatic culms, previously selected by morphological analysis, were examined by *F. fujikuroi*-specific PCR and 215 isolates were confirmed as *F. fujikuroi*. Despite the detection of SSRs in fungi is harder than in other organisms, they are considered the most robust and efficient markers in population genetics. Twenty-eight SSRs, all derived from expressed sequence tags (ESTs), were found and tested by PCR on a sub-sample of *F. fujikuroi* isolates. The products were detected by polyacrylamide gel electrophoresis (PAGE): 27 SSRs gave the expected amplicon but only 3 resulted polymorphic, with an average number of alleles per locus ranging from 2 to 5. A preliminary statistical analysis on the distribution of polymorphisms in 135 isolates belonging to 5 pathogen populations showed 8% of molecular variance among populations and 92% within populations. Taking into account the low number of polymorphic SSRs found in *F. fujikuroi* published sequences, and the need of a larger number of markers for population structure analysis, we are planning to perform a shotgun genome sequencing of *F. fujikuroi*.

Supported by Progetto AGER, grant n° 2010-2369.
TRANSCRIPTOME PROFILING OF THE EARLY RESPONSE TO MAGNAPORTHE ORYZAE IN DURABLE RESISTANT VS SUSCEPTIBLE RICE GENOTYPES


*) CRA-GPG, Genomics Research Centre, Via S Protaso 302, 29017 Fiorenzuola d’Arda (Piacenza) (Italy)
**) CRA-RIS, Rice Research Unit, S.S. 11 to Torino, Km 2,5, 13100, Vercelli (Italy)
***) FPTP, Parco Tecnologico Padano, Via Einstein, 26900 Lodi (Italy)
****) CRA-DPV, Vegetal Biology and Production Department, Via Nazionale 82, 00184 Roma (Italy)

Rice accessions, blast, durable resistance, RNA-Seq

Gigante Vercelli (GV) and Vialone Nano (VN) are two temperate japonica old Italian rice cultivars identified after screening of a large germplasm collection as presenting contrasting response to blast infection. GV displays durable and wide resistance to several isolates of the blast fungus M. oryzae while VN is highly susceptible to blast. To gain insights with respect to the early molecular processes deployed during the resistance response of GV, an RNA-seq experiment was conducted with Illumina GAIIx on three biological replicates of four tested conditions, namely GV and VIA in control (mock inoculated) leaves and leaves at 24 h after blast inoculation. About 40 million paired-end reads (37 bp) for each biological replicate were obtained. Reads were aligned to rice japonica (cv Nipponbare) genome and, as estimated by DESeq R package, 1,070 and 1,484 genes, of which 726 and 699 up regulated, were found to be modulated as a consequence of infection (FDR 0.05) in GV and VN, respectively. GO enrichment analyses revealed a set of GO terms enriched in both varieties including “defence response to fungus, incompatible interaction”. Despite this commonality, however, the gene set contributing to common GO enriched terms were dissimilar. In order to investigate GV resistance mechanism, expression patterns of genes grouped in GV-specific enriched GO terms where examined in detail down to the transcript isoform level. At 24 hours post infection, GV exhibited a dramatic upregulation of diterpene phytoalexin biosynthesis (DPB) genes and wall associated kinases (WAKs). Further GV-specific upregulated genes included flavin-containing monooxygenase and discrete members of class I chitinase (GH19) and glycosyl hydrolase 17 (GH17) gene families. Exploitation of the sensitivity and high dynamic range of RNA-Seq technique allowed the identification of genes which appear to be critically involved in conferring resistance to GV through acting in the early steps of defence circuitry. These included chitin oligosaccharides sensing, MAPK cascades and WRKY transcription factors. Since triggering of the early defence response in GV is likely to stem from genotype-specific resistance (R) gene(s), candidate genes with expression patterns consistent with a potential role for GV-specific functional R gene(s) were identified.

The research was supported by Progetto AGER, grant n° 2010-2369.
DIFFERENTIAL RESPONSE TO OSMOTIC STRESS IN RICE: SELECTION OF TWO ITALIAN CULTIVARS FOR RNA-SEQ ANALYSIS

BALDONI E., LOCATELLI F., MATTANA M., GENGA A.

CNR – Institute of Agricultural Biology and Biotechnology, via Bassini 15, 20133 Milano (Italy)

Oryza sativa, Italian cultivars, osmotic stress, RNA-seq

Abiotic stresses are the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50 %. Rice is particularly susceptible to dehydration, and cultivars may considerably differ in their vegetative response to water deficit. The identification of key genes regulated by water shortage is a major objective for the development of drought-tolerant varieties by molecular breeding. Here, we provide a physiological analysis of 13 Italian rice cultivars (Oryza sativa L. ssp japonica) in response to osmotic stress: Arborio, Augusto, Baldo, Balilla, Carnaroli, Eurosis, Koral, Loto, SISR215, Thaibonnet, Venere, Vialone Nano, Volano. Rice plants were grown in hydroponic culture and then subjected to PEG treatment (20% PEG 6000). The measurement of physiological parameters (leaf Relative Water Content and Electrolyte Leakage) showed that the cultivars exhibited differential responses to osmotic stress. On the basis of these data, the cultivars Eurosis and Augusto were classified as high tolerant and Loto and Vialone Nano as high sensitive. Total RNAs separately extracted from leaves and roots of Eurosis and Loto plants were used for RNA sequencing. RNA-seq data are currently under analysis. The expression profile of genes of interest, found to be differentially expressed between these two cultivars, will be further investigated in Augusto and Vialone Nano varieties.

These gene expression data will be used to identify genes involved in the response to water shortage, with the aim to identify associated polymorphisms correlated with the stress response phenotype (tolerance/susceptibility).

This work was supported by Progetto AGER, grant n° 2010-2369.
BACTERIAL FOOT ROT: ISOLATION AND CHARACTERIZATION OF Dickeya zeae FROM ITALIAN INFECTED PLANTS

BERTANI I. AND VENTURI V.

Bacteriology Lab, AREA Science Park, Padriciano 99, 34049 Trieste, Italy

Dickeya zeae, Oryza sativa, bacterial foot rot

The reports on rice fields presenting bacterial foot rot symptoms increased recently not only in Asian countries but also in Italy. The causal agent of the disease is Dickeya zeae (previously known as Erwinia chrysanthemi pv zeae) a Gram-negative phytopathogen belonging to the Enterobacteriaceae group and able to infect important crops such as maize, rice, potato, banana, sugar cane and ornamentals plants causing relevant economic loss. At the molecular and genetic level little is known about this bacterium. Starting from Italian infected rice plants, through phenotypic and molecular tests, we were able to isolate 27 bacterial strains identified as D. zeae by sequencing the recA and expDz genes together with the 16s rDNA. Virulence of the new isolates was tested on seeds and young plants revealing different levels of resistance in different rice varieties. The genome of one of the Italian isolates strain is now sequenced: the draft version shows a genome size of 4.7Mb and the presence of 4649 putative ORFs; comparative analysis will now be performed with other similar plant pathogens (for example the D. zeae isolated from onion and other cereal pathogens). This work will then lead to the study of the virulence mechanisms and possible control strategies that can be used against this emerging pathogen.

This work was supported by Progetto AGER, grant n° 2010-2369.
NATURAL VARIABILITY IN SALT TOLERANCE AMONG ITALIAN RICE GENOTYPES

BERTAZZINI M., FORLANI G.
Department of Life Science and Biotechnology, University of Ferrara, via L. Borsari 46, I-44121 Ferrara, Italy.

Oryza sativa, salt stress, natural variability, Italian rice germplasm, damage threshold

Salt accumulation in the soil represents an increasing threat to agricultural productivity. According to the FAO, more than 800 million ha are affected by either salinity or sodicity worldwide, corresponding to over 6% of arable lands. Because of climate change, this amount is expected to grow steadily. This is the case of Northern Italy, where rice is grown as an irrigated crop in Po delta, playing an important role in soil desalinization. This notwithstanding, no information at all has been made available to date with respect to the susceptibility of Italian rice cultivars to excess salt in the soil. Here we report on the characterization of a group of 17 rice genotypes with respect to salt susceptibility during germination and early growth. A rapid plate bioassay was developed to assess growth inhibition rates. Results were validated on growth-chamber-grown seedlings at the 3-leaf stage. Little difference was evident at high salt concentrations, whereas a significant variability was found concerning the threshold at which growth inhibition becomes evident. The use of a complex salt solution mimicking ion composition of the soil liquid phase yielded different patterns than NaCl alone, indicating that a NaCl-based screening could lead to misleading inferences. Data are consistent with the current trend of rice cultivar use in salt-affected regions of Northern Italy. Results will provide the basis for a future search of molecular determinants for increased tolerance to saline environments.
IN SILICO PHENOTYPING: EVALUATION OF BIOTIC AND ABIOTIC STRESSES ON TEMPERATE RICE

CONFALONIERI R.*, BREGAGLIO S.*, BOSCHETTI M.**, ACUTIS M.*

*) University of Milan, Department of Agricultural and Environmental Sciences, via Celoria 2, 20133 Milano, Italy, roberto.confalonieri@unimi.it
**) National Research Council, IREA, via Bassini 2, Milano, Italy

WARM model, simulation, spikelet sterility, blast disease, climate change

One of the main drivers in plant model development is studying genotype × environment interactions to provide support to variety selection within a given species. This concept has been recently extended - via advanced simulation techniques - towards the identification of ideotypes able to maximize productions under specific conditions. However the few studies where in silico phenotyping techniques were applied are mainly related to plant features involved with photosynthesis and leaf area expansion, thus with traits involved in crop growth under potential conditions for biotic and abiotic damages. In this study, we developed a simulation platform for the evaluation of the possible benefits attainable via the use of cultivars improved for their resistance/tolerance to biotic (blast and brown spot diseases) and abiotic (e.g., cold shocks, salinity) injuries. The platform is based on the WARM model and is developed using advanced software architectures, allowing further extensions and multi-approach simulations. The platform, capable to perform evaluations under current and climate change conditions, is provided with high resolution data layers covering all the Italian rice districts. The complexity of the simulation engine behind the platform is handled with the aim of providing users with a friendly interface, specifically designed for the RISINNOVA community.
GENETIC DIVERSITY AND POPULATION STRUCTURE OF A LARGE COLLECTIONE OF \textit{Magnaporthe oryzae} ISOLATES FROM ITALIAN RICE FIELD

CRISPINO L.*, RODOLFI M.**, PICCO A.**, ODILE FAIVRE-RAMPANT****THARREAU D.***, LUPOTTO E.****, PIFFANELLI P. *, ABRUSCATO P.*

*) FPTP, Centro di Ricerche e Studi Agroalimentari, Lodi (Italy)
**) UNIPV, Dipartimento di Scienze della Terra e dell’Ambiente, Pavia (Italy)
***) CIRAD, UMR BGPI, Montpellier (France)
****) CRA- Dipartimento di Biologia e Produzione Vegetale, Roma (Italy)

\textit{Magnaporthe oryzae}, biodiversity, SSR, Italian isolates

Blast, caused by the filamentous ascomycete fungus \textit{Magnaporthe oryzae}, is the most severe disease of rice worldwide, causing yield loss to rice cultivation up to 50-70%. To investigate rice-\textit{Magnaporthe} interaction is crucial to understand the molecular mechanisms underlying durable blast resistance and establish improved rice protection strategies. However, durable resistance is a difficult task to achieve due to the high degree of pathogenic variability of host populations and large number of fungal races co-existing. The study of the genetic diversity and definition of the structure of existing populations are necessary to overcome this hurdle and identify new virulent genotypes. Therefore, the development of robust and reliable molecular markers allowing to monitor the dynamics of \textit{Magnaporthe} populations is a crucial goal to design strategies for rice blast control. During the last 20 years, the diversity and structure of \textit{M. oryzae} populations on rice were described using different molecular techniques such as RFLP, rep-PCR markers, RAPD or AFLP. Simple Sequence Repeat (SSR) has become the most popular marker system used in genetic mapping, diversity studies and pedigree analysis, since are highly informative and highly reproducible. SSR have been only recently developed to analyze \textit{Magnaporthe} genetic diversity, but studies have been carried out only at European or worldwide scale. The main goal of this study was to investigate the genetic diversity of Italian \textit{Magnaporthe oryzae} strains to implement national rice breeding program for durable resistance towards blast population inhabiting Italian ricegrowing areas. To this aim, in the framework of the RISINNOVA project we created a large \textit{Magnaporthe} collection constituted of 293 Italian strains isolated in the period 1998-2011, different locations in Italy and plant organs. To classify the biodiversity of RISINNOVA \textit{Magnaporthe} collection a molecular characterization was carried out by a set of selected informative SSR and preliminary results of the phylogenetic analyses will be presented.

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Rice spikelet fertility is negatively affected either by low and high temperature stress conditions. In both the cases, the male germ line is the most sensitive tissue. Heat stress mostly impairs pollen grain germination and pollen tube elongation along the ovary. In collaboration with IRRI, we have characterized transcriptomic variations occurring under heat stress conditions in pollinated ovaries of an highly heat stress tolerant rice variety, N22, by means of RNA-Seq. Several genes which belong to the most important heat shock protein families (HSPs, putative molecular chaperones) are strongly induced after only 30 minutes of heat stress treatment at 38°C. Among transcription factor encoding genes, an AP2 domain and a Heat Shock Transcription Factor (HSF, which are putative positive regulators of HSPs) encoding gene were the most induced, about 500- and 21-fold, respectively. Our efforts are focused on the isolation of genetic markers useful for the selection of heat tolerant varieties.

In the frame of RISINNOVA, we are also characterizing the molecular responses of rice spikelets to cold stress events, which also mainly affect the development of pollen. During cold stress (temperatures below 12°C), microspore meiosis and tetrad formation are strongly impaired in most of the rice accessions. We will give an overview of our morphological and genetic comparative analysis between sensitive, intermediate and strongly tolerant rice varieties.

In Italy, cold stress is harmful for rice also at early vegetative stages. A panel of 13 rice accessions was selected to be tested in controlled conditions for tolerance to cold (chilling) at early vegetative stages, as well as to evaluate their behavior in conditions of multiple stresses (cold and submergence on hypocotyl elongation). A set of measurements were chosen to evaluate the different responses to stress (i.e. electrolyte leakage, visual score and survival test). Significant differences among cultivars were observed for all the parameters used. Cold stress (10h at 10°C in the dark) damaged 2-leaves stage plants, as seen in terms of EL%, after 2, 7 and 14 days of stress, although differences among varieties were more evident after 7 and better after 14 days of treatment. The combination of cold and submergence stress on hypocotyl elongation identified also genotype x stress interactions. Ranking of accessions allowed us to select ‘Volano’ as the most resistant cv. and ‘Thaibonnet’ as the most susceptible one for future transcriptome analysis experiments. Quantitative RT-PCR experiments with stress-marker genes are being performed for optimizing the timing of our stress experiments.
1.9

SYSTEMIC ALLOCATION OF TRACE ELEMENTS IN RICE PLANTS.

FONTANILI L., LUCCHINI G., SACCHI G.A., NOCITO F.F., LANCILLI C.

DiSAA - Università degli Studi di Milano, Via Celoria 2, 20133 Milano

Oryza sativa, trace elements, HMA, aerobic rice

Plants have a complex network of homeostatic mechanisms to control the uptake, accumulation, translocation and detoxification of a range of transition metals; these processes involve a range of metal transporters, whose activity results decisive for the nutritional value and quality of food, for the aspects related to the content in essential and/or toxic elements. Concerning rice, these aspects have become particularly relevant with the development of irrigated rice systems that require less water than traditional flooded rice, since the aerobic cultivation affects metal accumulation in the caryopses. Here we present a research aimed at studying the general mechanisms involved in the uptake and systemic distribution of some trace elements in rice and under different stress conditions. Rice plants (Oryza sativa L. spp. Japonica cv. Roma) were grown in hydroponic solution with different oxygen availabilities at the root level: hypoxia (stagnant solution) or normoxia (oxygenated solution). The effect determined by the different oxygen availability on the expression patterns of some members of the P_{1B}-type ATPase (HMA) family of metal transporters in rice roots was evaluated. The expression of the more affected genes will be also tested in 300 varieties grown in aerobic or anaerobic condition in open field. Moreover, the functional characterization of some HMAs genes is ongoing by means of heterologous expression in Saccharomyces cerevisiae.

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ROS SCAVENGING SYSTEMS IN ITALIAN RICE GENOTYPES SHOWING DIFFERENTIAL TOLERANCE TO SALT STRESS

FORLANI G., BERTAZZINI M., GIBERTI S.
Department of Life Science and Biotechnology, University of Ferrara, via L. Borsari 46, I-44121 Ferrara, Italy.

Oryza sativa, salt stress, proline accumulation, antioxidant enzymes, Italian rice germplasm

Salinity and drought are well known to induce oxidative stress at the cellular level. To overcome damages of salt-mediated reactive oxygen species (ROS), plants up-regulate antioxidative systems. Their role is essential to maintain a balance between ROS production and their scavenging to keep them at signaling level for reinstating metabolic homeostasis. On the other hand, to avoid salt-driven water withdrawal from the symplast, cells synthesize some small molecules, collectively known as compatible osmolytes, among which the amino acid proline has the widest occurrence. Their accumulation maintains equal water potential with the environment, and under mild stress conditions represents another strategy to achieve resistance against salinity. Since it also acts as a singlet oxygen quencher and as a scavenger of OH· radicals, proline is the only solute that can play a role in both mechanisms. In order to understand the biochemical basis of their response to excess salt in the soil, we measured proline content in tissues of salt-stressed seedlings of 17 rice varieties. A statistically highly significant relationship was found between their relative tolerance to salt stress and the increase of free proline concentration in shoots. The expression of selected antioxidative enzymes was also investigated in 5 rice cultivars with a contrasting capability to cope with salt stress. Catalase, Cu/Zn-SOD and Mn-SOD levels increased with the severity of the stress, the latter being the most responsive enzyme.
MECHANISMS OF PERCEPTION AND ADAPTATION TO SALT STRESS: AN INITIAL ANALYSIS IN ITALIAN RICE CULTIVARS

FORMENTIN E., BARIZZA E., ZOTTINI M., LO SCHIAVO F.

Department of Biology, University of Padova, via U. Bassi 58/B 35131 Padova

Oryza sativa, salinity tolerance, compatible solutes, K+ channels

The salinization of the soil is a problem for crop production in the coastal areas surrounding the Po Delta. During low flow periods, between July and August, sea water intrudes for many kilometers inland from the river mouth and salt enters into the ground by capillarity. In saline environments, the difference in the water potential between soil and root cells is reduced or even inverted, leading to a reduction in water uptake or loss of water. Plant growth responds to salinity in two phases: a rapid, “osmotic phase” that inhibits growth of young leaves; a slower, “ionic phase” that accelerates senescence of mature leaves. Plant adaptations to salinity are of three types: i) osmotic stress tolerance, ii) Na$^+$ exclusion, iii) tolerance of tissue to accumulated Na$^+$.

In the frame of the RISINNOVA research project for integrated genetic and genomic approaches for new Italian rice breeding strategies, we investigated the response of selected Italian cultivars to the salt stress. On the basis of ionomic and physiological data, six Italian rice cultivars, with different capability to cope with salt stress conditions, have been selected for further analyses. Their salt sensitivity was assessed, both in hydroponic and agar medium, containing a salt mixture mimicking the composition of the soil solution. Baldo and Vialone nano have been chosen as the more contrasting genotypes to be compared by the RNA sequencing techniques to find out useful markers for the assisted breeding.

Salt tolerance relies on the coordinated action of many genes that perform a variety of functions such as ion sequestration, osmotic adjustment and antioxidative defense. Thus, the selected cultivars have been characterized biochemically by measuring both the content of proline, an osmoprotectant widely used by plants during osmotic stress, and the amount of enzymes involved in ROS scavenging. Moreover, the role of nitric oxide as a signal molecule in the salt stress response has been investigated both in vitro and in planta. In regard of ionic stress, five potassium channels, putatively accountable for K$^+$ release from intracellular compartments, have been cloned and studied.

In addition, cultured cell lines have been generated and used to investigate on the cellular processes that modulate the tolerance/sensitivity at plant level. Preliminary results suggest that different mechanisms of stress response exist among the selected cultivars, that are worthy of additional investigation.
PHENYLALANINE-AMMONIA LYASE EXPRESSION IN RICE GENOTYPES SHOWING DIFFERENTIAL TOLERANCE TO MAGNAPORTHE ORYZAE

GIBERTI S., FORLANI G.
Department of Life Science and Biotechnology, University of Ferrara, via L. Borsari 46, I-44121 Ferrara, Italy.

**Oryza sativa**, blast infection, aromatic biosynthesis, plant response to pathogens, candidate gene to select for increased blast tolerance

Phenylalanine ammonia lyase (PAL) specific activity levels were measured in suspension cultured cells of some rice cultivars following the treatment with cell wall hydrolysates prepared from various *Magnaporthe oryzae* strains. Early after elicitation, even low hydrolysate concentrations were able to induce a significant increase of enzyme levels. However, neither rice genotypes showing differential sensitivity to blast reacted differently, nor elicitors obtained from various pathotypes induced different reactions. At a later stage, higher hydrolysate concentrations were required to trigger maximal enzyme induction. In this case, highly significant differences were disclosed among plant genotypes. A remarkable relationship was evident between the mean increase in PAL activity and the overall resistance to blast at the plant level. A complex pattern of PAL time course specific activity levels was evident in cells of a blast-resistant genotype (cv Gigante Vercelli). Ion-exchange chromatographic fractionation of crude extracts suggested that the early and the late increase of activity rely upon the sequential induction of two different isoenzymes. Two out of eleven PAL genes were indeed found to be induced by treatments with the hydrolysates. Conversely, only the early-responsive enzyme form was observed following elicitation in a blast-sensitive genotype (cv Vialone nano). Therefore, the late-responsive isoform may represent a candidate gene to select for decreased sensitivity to blast.
PROTEOMIC AND ULTRA-STRUCTURAL ANALYSIS OF RICE GRAIN

GULLÌ M., IMPERIALE D., CAMPOLI D., MARMIROLI M., MARMIROLI N.

Department of Life Sciences, viale delle Scienze 11a, 43124 Parma

Oryza sativa, seed storage proteins, proteomic.

Italian rice cultivars with a divergent capability to cope with drought and salt stress have been analyzed to obtain seed proteomic maps. Two approaches have been utilized at this purpose: SDS- PAGE and 2D-LC (2D-Liquid Chromatography). The existing protocols have been adapted and optimized for rice seed samples. The analysis of the storage protein fraction was achieved using a SDS-UREA buffer for protein isolation and an high throughput proteomic apparatus such as Protean® i12™ IEF System for separation. Analysis by 2D-LC HT was performed with the Proteome Lab™ PF2D (Beckman Coulter) on the total protein extract, and the proteomic maps obtained for the varieties of interest have been compared. Protein fractions obtained were chosen for peptide mass fingerprinting analysis, after trypsin digestion. Samples were analyzed with a MALDI-TOF-TOF mass spectrometer. The online program Mascot (Matrix Science) was used for data analysis. The rice cultivars studied at proteomic level were also characterized at structural level using a Scanning Electron Microscope (SEM) equipped with X-ray microanalysis. The analysis performed will be repeated on the same cultivars grown in stressed condition to identify useful markers.
TRACE ELEMENTS AND NUTRACEUTICAL COMPOUNDS IN FLOODED AND AEROBIC RICE GRAINS.


DiSAA - Università degli Studi di Milano, Via Celoria 2, 20133 Milano

Oryza sativa, ionomics, metabolomics

A different field water management could influence rice grain quality concerning the content of trace elements and nutraceutical compounds. Here we present a ionomic and metabolomic survey conducted on the grains of six rice varieties grown as flooded or irrigated (aerobic) rice. ICP-MS analyses show that in all the varieties the contents of Cd, Fe, Cu and Se were higher following turnated irrigation with respect to continuous flooding; differently, As and Mn contents were higher in flooded rice than in the aerobic one. Interestingly, a strong variability in the strength of the variation observed was present between rice cultivars, showing that different genetic backgrounds have different sensitiveness to changes in field water management and that the changes in the trace element content do not depend only by a different bioavailability of the trace elements themselves. Metabolic analyses, performed by GC-MS techniques showed that glutaric acid, glycolic acid and phenylalanine content are positively affected by the aerobic growing condition, whilst the levels of adenosine, alanine, allantoin, GABA, F6P, histidine, proline and succinic acid are higher in flooded rice. Nitrogen assimilative metabolism seems the most affected one by the redox state of soils. HPLC analyses showed that water management deeply affects the content of phytic acid, tocotrienols, tocopherol and oryzanol. In the framework of the RISINNOVA project the same approach is going on concerning 300 rice accessions.

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1.15

INCREASING BLAST RESISTANCE IN ITALIAN RICE VARIETIES: MAPPING OF NEW DISEASE RESISTANCE LOCI AND MARKER-ASSISTED SELECTION

URSO S.*, TACCONI G. ***, DESIDERIO F.*, CRISPINO L.****, PIFFANELLI P.***, LUPOTTO E.****, CATTIVELLI L.*, VALTE G. ***/*

*) CRA-Genomic Research Centre, Via S.Protaso 302, Fiorenzuola D’Arda 29017, Italy
**) CRA-RIS, Rice Research Unit, S.S. 11 to Torino, Km 2,5, 13100, Vercelli, Italy,
****) CRA-Department of Plant Biology and Crop production, Via Nazionale 82, 00184 Roma, Italy

Gigante Vercelli (GV) is an old Italian rice (Oryza sativa L. sp. japonica) cv exhibiting wide and durable resistance to many isolates of the blast fungus Magnaporthe oryzae. To find out more about the genetic basis of GV blast resistance, an SSR-based linkage map was developed from an F2 population segregating for blast resistance derived from a cross between GV and the susceptible variety Vialone Nano (VN). About 140 SSR markers were mapped to the 12 rice chromosomes. The 120 lines of F3 families were inoculated with a mix of three M. oryzae Italian isolates, avirulent towards GV, and phenotypic data were used to perform QTLs analyses. Two QTLs responsible for GV blast resistance were identified to chromosome 1 (LOD=13.58; R² 25.9%) and to chromosome 4 (LOD =16.19; R² 32.7%) respectively. These results therefore indicate that the durable resistance of GV is derived from an unconscious accumulation of two resistance loci during the past century.

To date, introgression of single or multiple resistance genes is considered the best strategy for increasing blast resistance. In order to obtain a more durable blast resistance, advanced lines (F4 lines) were obtained from breeding procedures allowing introgression of known blast resistance genes. These lines were used as donors for pyramiding of multiple resistances (Pi-5, Pi-1, Piz, Pik, Pib) in susceptible traditional rice varieties. To identify homozygous lines containing more resistance genes, marker assisted selection (MAS) was used.

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2.1

DYNAMICS OF ROOTS AND AM COLONIZATION IN RICE PLANTS GROWING IN CHANGING ENVIRONMENTAL CONDITIONS

Fiorilli V.*, Vallino M*, Marziano E**, Bonfante P***.

*) Institute for Plant Protection – Consiglio Nazionale delle Ricerche, Viale P.A Mattioli 25, 10125, Torino
**) Department of Life Sciences and System Biology, University of Turin, Viale P.A Mattioli 25, 10125, Torino

Oryza sativa, root morphology, AM symbiosis

As one of the most important food source for humans, rice has a relevant social and economic role worldwide. Understanding rice responses to the interaction with arbuscular mycorrizal (AM) fungi, has gained increasing importance in order to enhance plant yield. While it is known that AM fungi do not preferentially colonize flooded roots, the mechanisms which control such events are fully unknown. The goal of our work is to elucidate, through morphological and molecular approaches, the factors which control colonization dynamics of rice roots by AM fungi in different water regimes such as flooding and dry conditions.

Different sets of mycorrhizal and control rice plants were grown in both water regimes, also including the transfer from water to dry and viceversa. Roots from all biological conditions were sampled at different time points, and some parameters (root branching, mycorrhizal colonization and expression of functional marker genes) were evaluated. As expected, mycorrhizal colonization decreased with flooding, while differences on root anatomy between dry and flooding conditions started from 7 dpi. In mycorrhizal plants, root branching was significantly higher as a consequence of the fungal presence added to the effect of the dry soil. Root branching changed after shifting the plants from flooding to dry, revealing a high plasticity of the root system. Molecular analysis showed that symbiosis functionality was directly linked to the root anatomy and the success of mycorrhizal colonization.

In conclusion, our experiments demonstrate that the water regime influences the fungal colonization, and therefore the expression of symbiosis marker genes, first modifying root morphology.

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MULTIPLE MOLECULAR APPROACHES REVEAL HOW BACTERIAL AND FUNGAL COMMUNITIES THRIVE IN THE RICE RHIZOSPHERE


*) CNR, Istituto per la Protezione delle Piante, UOS, Torino (Italy)
**) FPTP, Centro di Ricerche e Studi Agroalimentari, Lodi (Italy)
***) CNR, Istituto di Virologia, Torino (Italy)
****) CRA-Dipartimento di Biologia e Produzione Vegetale, Roma (Italy)

Metabarcoding, pyrosequencing, biodiversity, rice microbiome

Recent development of molecular tools and Next-Generation Sequencing techniques have revolutionized the study of microbial ecology, providing insights into complex systems, such as those created by rice roots. These below-ground plant organs identify, in fact, specific niches for various microorganisms which are affected by agricultural management systems (i.e. aerobic versus anaerobic growth conditions). The first aim of our project was the investigation of microbiome associated to both soil and root under these management conditions. Rice roots and surrounding rhizospheric soil were sampled from plants grown in upland and lowland conditions at three plant development stages (tillering, panicle formation and milky stage maturation) including triplicates, which were kept separate during the molecular analyses. The complexity of whole microbial community was estimated by ARISA analysis in the four different experimental setting (roots, rhizosphere in aerobic and anaerobic growth conditions). A metabarcoding approach based on tag-encoded 454 pyrosequencing spanning hyper-variable regions of the rRNA genes from both fungal and bacterial communities was applied. Eight multiplex amplicon pools were created and sequenced producing 559K reads (220.7Mb). A preliminary metagenetic study was conducted on Archaea and Bacteria 16S and Fungal 18S datasets. For each sample, reads were clustered and searched by BLAST against the RDP (Ribosomal Database Project) database. Fungal ITS reads were clustered and the taxonomic assignment was performed using BLAST against a customized reference repository (FunITSinnova), specially developed to overcome the limitations and biases of currently used ITS databases.

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3.1

GENOME WIDE ASSOCIATION STUDIES FOR ROOT TRAITS IN A TEMPERATE RICE COLLECTION


*) Rice Genomics Group, Parco Tecnologico Padano, Lodi (Italy)
**) CRA-Centro di Ricerca per la Genomica e la Postgenomica Animale e Vegetale, Fiorenzuola d’Arda, PC (Italy)
****) Plant Breeding and Genetics Dept., Cornell University, Ithaca (NY, USA)
***** ) CRA-Unità di Ricerca per la Risicoltura, Vercelli (Italy)
******) CRA-Dipartimento di Biologia e Produzione Vegetale, Roma (Italy)

Rice, drought, GBS genotyping, root system architecture, GWAS

During the last two centuries rice in Italy developed essentially as a water demanding crop, completing the growth cycle under submersion. Reduced water availability due to climate changes, especially if occurring during critical phases of the plant growing cycle, dramatically affects crop yield and quality. The root-system architecture is known to play a crucial role in conferring tolerance to water scarcity. Indeed a deep root system able to absorb water at depth is the most relevant trait contributing to the successful adaptation of the plant to temporary conditions of drought stress.

To explore the existing biodiversity of temperate rice in terms of drought tolerance, a collection of 96 rice accessions was selected and characterized through whole-genome genotyping coupled to root phenotypic evaluations in controlled growth conditions. The collection included traditional and modern accessions representing the genetic diversity of the Italian rice germplasm and a set of foreign varieties from temperate areas adapted to Italian climatic conditions. Genome-wide association analyses performed on a first set of phenotypic traits allowed the identification of SNPs co-localizing with QTLs previously shown to be associated with root traits related to drought avoidance strategies. These preliminary results confirmed the suitability of GBS genotyping for GWAS studies in rice. As a final goal, we aim at developing relevant know-how immediately available to devise marker-assisted breeding programs for a more sustainable rice cultivation in Italy.

Research activities were supported by Progetto AGER, grant n° 2010-2369.
3.2

RELEASE OF THE CEREALAB DATABASE V 2.0 FOR RICE MARKER-ASSISTED SELECTION

DANNAOUI A.R.*,**, TUMINO G.*, BENEVENTANO D.*,**, MILC J.*, CAFFAGNI A.*, PECCHIONI N.*

*). BIOGEST-SITEIA Interdepartmental Research Centre, Department of Life Sciences, University of Modena and Reggio Emilia, Reggio Emilia, IT
***) Department of Information Engineering, University of Modena and Reggio Emilia, Modena, IT

MAS, Cereals, Oryza sativa, Data integration, Database

The CEREALAB database (Milc et al., 2011) is a web-based tool realized for rice (wheat and barley) MAS (Marker-Assisted Selection), to help the breeders in choosing molecular markers associated to the most important economically phenotypic traits. It contains phenotypic and genotypic data obtained from the integration of available open source databases with the data obtained by the CEREALAB project.

In this paper we describe several significant extensions to the CEREALAB database, derived from real needs of the end-user, the modern breeder that is using molecular tools. Firstly, to offer to the breeders new significant data, the CEREALAB database was extended. As a second aim, to improve and simplify the access to the database, a new user-friendly Graphic User Interface (GUI) was developed. Third, to maximize and optimize the accessibility of the available information, new functionalities and additional tools were realized. In particular, to offer to the breeders an effective tool for the analysis of data, the possibility to obtain structured reports was introduced. Finally, to insert new data in the database, a new data entry module was implemented in the interface.

Database URL: http://www.cerealab.org
3.3

REGULATION OF FLOWERING TIME IN RICE AT NORTHERN LATITUDES

FORNARA F.

Università degli Studi di Milano, Dipartimento di Bioscienze, Via Celoria 26, 20133 Milano (Italy)

Oryza sativa, flowering, photoperiod, natural genetic variation

Flowering is a crucial developmental phase for most plant species and heavily depends on correct perception of environmental parameters such as light and temperature, in order to take place at the correct time of year. Many plants flower in response to a certain duration of the light phase, called photoperiod. Rice is a typical short day plant that flowers as the photoperiod falls below a critical threshold, but many varieties are known that can also flower under longer day lengths. These include also varieties typically cultivated in temperate areas of Europe, such as Italy, Spain and France where summer days are long and warm. Flowering occurs as a mobile signal generated in leaves, called florigen, moves to the shoot apical meristem to induce formation of inflorescences and flowers. We are investigating the molecular basis of adaptation of rice to northern latitudes using germplasm currently cultivated in Europe. Italian varieties undergo floral transition as days become longer and show low sensitivity to photoperiod. By sequencing known flowering time regulators and assessing their expression under different day lengths we aim at understanding how allelic composition at critical loci allowed rice to adapt to extreme areas.
3.4

EVALUATION OF A LARGE COLLECTION OF RICE ACCESSIONS UNDER AEROBIC AND FLOODING WATER MANAGEMENT CONDITIONS


*) CRA-RIS, Unità di Ricerca per la Risicoltura, Strada Statale 11 per Torino km 2,5, 13100, Vercelli, Italy
**) CRA-GPG, Centro per la Genomica e Post-Genomica animale e vegetale, v. S. Protaso 302, 29017, Fiorenzuola d’Arda (PC), Italy
***) Rice Genomics Unit, Parco Tecnologico Padano, Via Einstein, 26900, Lodi, Italy
****) CRA, Dipartimento di Produzioni Vegetali, Via Nazionale 82, 00184, Roma, Italy

Rice, aerobic soil, phenotyping, genotyping

Three hundred temperate japonica rice accessions were grown in field under two water management conditions (flooded and aerobic soil) to evaluate their agronomic performances under water-limited conditions. Three replicates for each accession and condition were tested in a randomised block design. An extensive list of agronomically relevant traits was recorded, including flowering and maturation time, plant height, anthocyanic pigmentation, chlorophyll and flavonols content, attitude of blade of the flag leaf, type and attitude of panicle in relation to stem and several others. Preliminary data analysis highlighted a large variability of the recorded traits among the accessions with a large effect of the aerobic conditions on the agronomic traits. A parallel genotyping of the rice accessions was carried out with a genotyping by sequencing approach. These data will allow the identification of robust and accurate associations between genetic haplotypes and the target aerobic adaptation traits.

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Durum wheat, stress tolerance response, quality, .

The expected climatic changes will led to an environment characterized by elevated atmospheric CO₂ increased temperatures and increased risks for drought due to an increased atmospheric evaporative demand. To understand the impact of these changes on durum wheat yield and quality a range of experimental approaches has been undertaken.

The presentation will summarize the main results achieved in terms of:

i) response of durum wheat germplasm to elevated CO₂ assessed in a Free Air CO₂ Experiments (FACE);

ii) molecular description of drought response in durum wheat cultivars contrasting for water use efficiency;

iii) functional characterization of some key genes involved in the response to drought;

Overall the date highlight an extent of genetic variability for adaptation to expected climatic conditions that will allow the selection of new cultivars with a specific adaptation to the new climatic conditions.
Invited lecture

HARNESSING DIVERSITY OF TEMPERATE RICES: OPPORTUNITIES OFFERED BY SEQUENCING DEVELOPMENTS

COURTOIS B.*, AHMADI N.*

*) CIRAD, UMR AGAP, Montpellier F34398, France.

Diversity, DArT, SNP, genotyping by sequencing, association mapping.

Breeders from Europe have established a collection of 425 temperate rice accessions by pooling their working collections. These accessions have first been characterised with 25 SSR markers, enabling to differentiate a tropical and a temperate japonica group that further split into two sub-groups. The three groups corresponded to specific maturity classes and grain types. A core collection of about 200 accessions was then defined and phenotyped for various traits (salinity tolerance, blast resistance, and water use efficiency). SNPs in candidate genes supposed to control these traits were genotyped on the core collection. Targeted association mapping was conducted for salinity tolerance. It showed that some European accessions were carrying specific alleles which, cumulated, could improve salinity tolerance above present levels. DArT markers were then genotyped on the core collection. The pattern of diversity revealed by DArT markers was similar to that shown by SNPs but only 300 markers were polymorphic. To run whole genome association studies (WGAM), a larger number of markers is necessary since linkage disequilibrium in temperate rices is expected to span about 400 kb in average. Genotyping by sequencing that associates DArT and next-generation sequencing technologies offers the possibility to go further. Preliminary results on a set of japonica rices enabled us to estimate the polymorphism to be expected in the core collection. The marker density seems satisfactory for WGAM although the results have to be confirmed at a larger scale. Ways to better exploit existing data and links with genomic selection are presented.
Rice agriculture represents a major source of the greenhouse gas methane which is produced in the anoxic bulk soil. However, methanotrophic microorganisms catalyze the oxidation of methane and thereby significantly reduce the amount released to the atmosphere. Aerobic methanotrophs are located at oxic-anoxic interfaces where methane and oxygen are present, such as the upper paddy soil layer and the rice rhizosphere. In addition, they have been found as part of the edaphic community within rice roots. Here, they potentially promote plant growth, and might be involved in nitrogen fixation. Thus, methanotrophic bacteria are important players in the paddy field ecosystem and understanding their functional diversity is essential to predict responses to a changing environment. We investigated the spatial distribution of aerobic methanotrophs within individual paddy fields located in Northern Italy (Vercelli), showing that different communities are present on the rice roots and the bulk soil. We furthermore exposed an effect of the rice genotype on the root-associated methanotrophic community. A comparison of various terrestrial and marine habitats revealed that paddy fields harbor a very distinct and selective methanotrophic community, very different even from the meadow located just next to the field. However, paddy field distributed world-wide harbor a very similar community. Nevertheless, we found a correlation between the geographical distance and community similarity. Such a distance-decay relationship is commonly found in plant and animal ecology, however, has only been rarely studied for microbes yet.
Invited lecture

GENOME STRUCTURE AND PLANT EVOLUTION: FROM ONE TO THE MANY GENOMES OF A SPECIES

MORGANTE M.*, **

*) Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, Via delle Scienze 208, 33100 Udine, Italy.
**) Istituto di Genomica Applicata, Parco Scientifico di Udine, Via J. Linussio 51, 33100 Udine, Italy

Pan-genome, structural variation, transposable elements, cooption

The analysis of variation in plants has revealed that their genomes are characterised by high levels of structural variation, consisting of both smaller insertion/deletions, mostly due to recent insertions of transposable elements, and of larger insertion/deletion similar to those termed in humans Copy Number Variants (CNVs). These observations indicate that a single genome sequence might not reflect the entire genomic complement of a species, and prompted us to introduce the concept of the plant pan-genome, including core genomic features common to all individuals and a Dispensable Genome (DG) composed of partially shared and/or non shared DNA sequence elements. The very active transposable element systems present in many plant genomes may account for a large fraction of the DG. The mechanisms by which the CNV-like variants are generated and the direction of the mutational events are still unknown. Uncovering the intriguing nature of the DG, i.e. its composition, origin and function, represents a step forward towards an understanding of the processes generating genetic diversity and phenotypic variation. Additionally, since the DG clearly appears to be for the most part the youngest and most dynamic component of the pan genome, it is of great interest to understand whether it is a major contributor to the creation of new genetic variation in plant evolution as well as in the artificial selection processes of plant breeding. We will discuss the extent and composition of the pan genome in different plant species.
Invited lecture

GENETICS AND GENOMIC PERSPECTIVE RELATED TO WATER USE IN RICE FROM THE NORTH OF SCOTLAND

PRICE A.

Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK

Oryza sativa, genotyping, stress tolerance, water, QTLs

For the past 20 years, research has been undertaken to map QTLs for stress resistance related traits in rice. The stresses include biotic (nematodes, parasitic plants, blasts) and abiotic (drought, heat and arsenic). There has been an emphasis on the role of roots in many of these traits and more recently on quality issues related to major and minor element in the grain. The Bala x Azucena mapping population has been the mainstay of this work, but now we are working as well on the Rice Diversity Panel (developed in a project lead by Susan McCouch) which allows genome wide association studies (GWAS) to be conducted. This presentation will highlight some of the genetic insight that has been gained that demonstrate the complexity of some traits (e.g. roots and drought) and the tractability of others (Striga-rice interactions). It will include a look towards the near and medium-term future of genetic mapping and candidate gene identification of traits related to the sustainability of water use in rice.
INVESTIGATING THE ROLE OF microRNAs IN THE RICE RESPONSE TO PATHOGEN INFECTION


* Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UB. Edifici CRAG, Campus UAB. Bellaterra (Cerdanyola del Vallés). 08193 Barcelona, Spain;
** Department of Environmental Biology, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Ramiro de Maeztu 9, 28040 Madrid, Spain;
*** Bioinformatics and Genomics Program, Center for Genomic Regulation, UPF-Barcelona, Catalonia, 08003, Spain.

Alternative splicing, evolution, miRNA, metal transporter

MicroRNAs (miRNAs) are small non-coding RNAs that regulate eukaryotic gene expression. They negatively regulate gene expression by triggering mRNA degradation or translational repression of the cognate mRNA. To identify miRNAs potentially involved in rice immunity, we performed deep sequencing of small RNA populations from rice tissues treated, or not, with elicitors prepared from the fungal pathogen Magnaporthe oryzae. Our data revealed dynamic alterations in the accumulation of miRNAs representing up to 58 known miRNA families, including novel members of previously annotated miRNA families. Members of the same miRNA family exhibited a differential expression pattern. Many of these elicitor-responsive miRNAs are known to target genes involved in stress responses, hormone regulation and development, or miRNA functioning. Genome-wide transcriptome analysis confirmed the expected anticorrelation between miRNA and target gene expression for known miRNAs that are conserved in plants. These findings revealed the complexity of microRNA-guided gene regulations operating during the rice response to fungal elicitors.

Novel miRNAs from rice are proposed. Among them, a novel evolutionarily young miRNA that is processed in a dcl4-dependent manner was characterized in more detail. This novel miRNA experienced natural and domestication selection events during rice evolution. Overexpression of miR-118 in rice results in down-regulation of an alternatively spliced transcript of the OsNramp6 (Natural resistance-associated macrophage protein 6) metal transporter gene. Altogether, these results provide a foundation for evaluating the relevance of alternative splicing events as a mechanism to specifically attenuate miRNA-based regulation of target gene expression.
COMMUNICATION IN RICE-ASSOCIATED MICROBIAL COMMUNITIES

VENTURI V.

International Centre for Genetic Engineering and Biotechnology, 34149, Trieste, Italy

Multispecies communities, Intra-/inter-species signalling, interkingdom communication

An emerging research field is the study of how bacteria communicate and form multispecies communities; it is also interest if and how these communities undergo chemical signaling with the eukaryotic host. Plants are a good working model to study microbial communities since many bacterial species are associated with them at different locations. We are using the rice (Oryza sativa) model to study intra- and inter-species bacterial communication as well as interkingdom signaling. Emerging rice pathogens like Dickeya zeae and Pseudomonas fuscovaginae are of concern as they are aggressive and quick colonizers; we have shown that they form communities via N-acyl homoserine lactone (AHL) LuxI/R quorum sensing (QS) signaling. In pathogenic Xanthomoa oryzae no AHL signaling is taking place however we determined that a new family of bacterial regulators (closely related to the QS LuxR-family proteins) bind low molecular compound(s) produced by plants and regulate gene expression. This is a new interkingdom signaling circuit between bacteria and plants. A similar system has been identified in rhizosphere beneficial Pseudomonas meaning that this system plays important roles in plant-bacteria interactions in both beneficial and pathogenic bacteria. Finally, bacterial communication and multispecies communities are also formed by rice endophytic bacteria. Understanding these communication networks will possibly lead to better strategies for disease resistance, tailored biological control and the resourceful use of biofertilizers.

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