PhD School on Agriculture, Environment and Bioenergy
(http://sites.unimi.it/dottorato_aab/)
(XXXV cycle, 2019-21)
Project draft

1.Field of interest: BIO-01

2.Project title: Improving rice plant and panicle architecture by molecular approaches

3.Tutors:, Dr. Simon Pierce; Dr. Vittoria Brambilla

4.Relevance of the topic and state of the art:

A rice plant is composed of a main shoot and several lateral branches (or tillers) that at maturity each bring an apical panicle. Modern rice is the result of thousands of years of **breeding** during which many favourable traits have been selected, including an **optimal plant and panicle architecture**[1]. These traits are crucial for grain quality and grain yield. In spite rice long breeding history, a large genetic variability is still present in modern rice plant and panicle architecture, that can be exploited with the knowledge gained from basic research. (Figure 1 A).

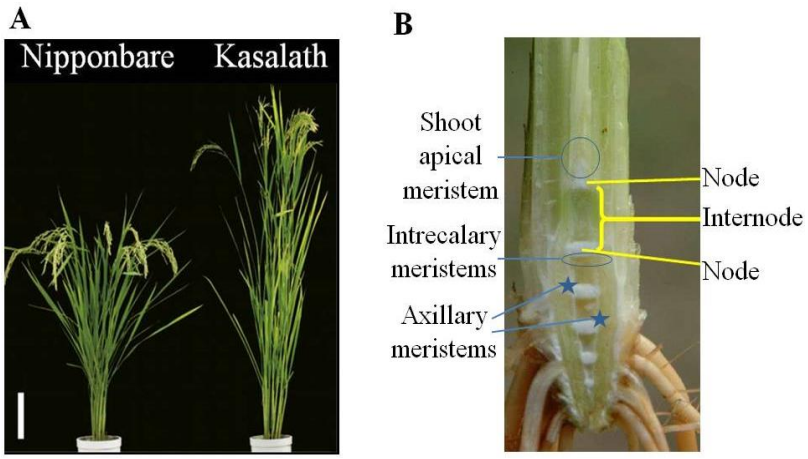

Figure 1. A: a large genetic variabilityfor plant architecture is still present in the rice germplasm; B: Plant growth is ensured by the activity of three meristems, the shoot apical meristem (SAM), the intercalary meristem(IM) and the axillary meristem (AXM).

Plants with reduced height and with an increased number of uniformly growing tillers are less prone to lodging, are more easily harvested and produce more panicles. Also, larger, more branched panicles produce more seeds.
The growth and development of the aerial rice plant occurs thanks to the activity of meristematic tissues (Figure 1B). These are:
- the **shoot apical meristem** (SAM) that ensures leaves and then panicles development;
- the **axillary meristems** (AXM) that originate the lateral tillers from the leaf axils;
-the **intercalary meristems** (IM) that support stem growth by internodes elongation.
Growth, development and synchronization of the activity ofthese meristems lead to the final shape of the plant and these processes are under a complex **hormonal and genetic control**.
HORMONES
It is well established that distribution of plant hormonesasAuxins (indole-3-acetic acid-IAA) and Gibberellins (GA) influences plant and panicle architecture.
Hormones signalling is also integrated into a complex genetic network where several components have been characterized. SAM and AXM development affect panicle development and tillering respectively, and these processes are synchronised with flowering.
GENES

Two major coordinators of flowering time with meristems differentiation are the two paralogous genesHd3a and RFT1. These are great targets to be modified to improve both plant and panicle architecture.Hd3a and RFT1 are produced in leaves in response to environmental conditions and act at the SAM where they can reprogram its development from vegetative to reproductive[2,3]. AlsoHd3a and RFT1 not only control the initial phases of floral transition at the SAM but they have also a role in subsequent panicle development. Preliminary data suggest that altering Hd3a or RFT1 function at the SAM might result in panicles that bear more seeds. Hd3a protein can similarly accumulate atthe AXM, where it promotesbranching [4].It is not yet known whether RFT1 can act redundantly with Hd3a in these processes.

It is also well demonstrated that AXM development is controlled by TB1 in many plant species.*TB1*genes repress axillary bud outgrowth in maize, Arabidopsis, bread wheat and rice[5]. Recent studies have shown that, in bread wheat, TB1 can also regulate panicle architecture, and that it carries out this function by interacting with a Hd3a wheat homologue[67]. OsTB1 function has also been linked to the hormone IAA.

BREEDING

The relationship between Hd3a/RFT1 and OsTB1 in rice has not yet been studied and it can provide a link for Hd3a (and RFT1) function in SAM and AXM development.Understanding the role of these genes and their correlation also with flowering and hormonal signalling might provide novel tools to fine tune SAM and AXM development and modify plant and panicle shape.

5.Layout of the project:

During the three-years PhD course, the candidate will focus both on hormones and genes that contribute to the definition of the shape of the rice plant and panicle. The objective is to gain knowledge on critical molecular pathways in order to exploit it for breeding rice plants with optimal plant and panicle architecture. As tillering and panicle branching are controlled by partially redundant gene-hormone modules, we will expect to be able to improve both traits by acting on these modules. All studies will be performed in the homogeneous genetic background of the model Nipponbare variety. Knowledge will then be transferred to other Italian agronomically relevant varieties.

The PhD project will start with studying the dynamics of IAA [8,9] and GA[10] distribution *in vivo*during SAM and AXM development thanks to fluorescent marker lined by **confocal imaging**. For IAAthe marker Nipponbare rice lines containing *DR5:VENUS3X* [9] or *35S:DII:VENUS,* already available in the lab, will be used to study the accumulation or the degradation of the fluorescent protein VENUS in the presence of IAA. The artificial promoter *DR5* is activated by IAA, while the fusion protein DII-VENUS is degraded. For bioactive GA visualization *in vivo*, we will optimize the optogenetic biosensor GIBBERELLIN PERCEPTION SENSOR 1 (GPS1) based on FRET for rice [10].The GA marker, developed for Arabidopsis and improved in the lab for rice, is under transformation and will need to be tested in the rice plant.These analyses will be first performed in the *wt* and subsequently, if validated, also inthe mutant lines.

Several **mutant CRISPR alleles** of *hd3a*and *rft1*are already available in the lab and could be studied and crossed with the marker lines. *Ostb1* mutant is not yet available in the cultivar Nipponbare[5], and it will be necessary to produce it by CRISPR/Cas9. The candidate will perform the cloning and the rice transformation for CRISPR mutant production.

The mutants will be morphologically characterized, with a focus on AXM and SAM development. **Expression analysis** of known SAM and AXM target genes will be performed in the mutant compared to the wt. *Hd3a*, *rft1* and *Ostb1* mutants will also be crossed to produce **multiple mutants** and study their **genetic interaction**.

Once a well expressing marker line will be selected, this will be crossed into the mutants. By testing IAA and GA distribution in *hd3a, rft1*and *Ostb1* mutants, we will understand if these hormones are perturbed in the mutants compared to the *wt*.

Similarly to what it has been proposed for bread wheat panicle, we will test if plant architecture is modulated by the interaction between OsTB1 and Hd3a/RFT1. This**protein-protein interaction**will be

then tested *in vitro* by yeast-two hybrid assay and in a FRET/FLIM experiment in tobacco leaves. Also transcriptional regulation of target genes will be studied.
We expect that elucidating the role of Hd3a, RFT1, OsTB1 and the hormones IAA and GA in rice development will open new possibilities for fine tune SAM and AXM development with the purpose of rice breeding.

5.1Materials&Methods:

CONFOCAL IMAGING

In vivo visualization of IAA accumulation/degradation in rice plants (DR5:VENUS3X; DII:VENUS transgenic rice plants, available in the lab).
Crossing of the *DR5:VENUS3X* and *DII:VENUS* marker lines in *hd3a* and *rft1*, genotyping and confocal observation.
In vivo visualization of GA accumulation in rice plants:
-rice*wt* and mutantstransformation with the optogenetic biosensor GIBBERELLIN PERCEPTION SENSOR 1 (GPS1).
-Transgenic plant selection and genotyping,
- transgene expression analysis by real time PCR,
-confocal analysis and validation of the GPS1 system in rice by tissue specifictranscriptional analyses of genes involved in GA biosynthesis, catabolism and signal transduction.

CRISPR MUTANTS
-CRISPR design, cloning and rice transformation.
-Selection and genotyping by Sanger and /or NGS
-Morphological characterization (see below)

MUTANTS MORPOLOGICALANALYSIS
- sampling, inclusion, sectioning and light microscopy observations.
- staining for different compounds for tissue characterization.

GENETIC INTERACTION
-crossing and F2 selection for different mutant combination – genotyping and phenotyping
-analysis of epistatic interactions

PROTEIN-PROTEIN INTERCTION STUDIES
-cloning and yeast two hybrid screens
-cloning and tobacco infiltration; FRET FLIM assays by confocal means of tobacco leaves

5.2Schedule and major steps (3 years):

YEAR 1
The project will start with confocal analysis of IAA distribution and parallelly with the cloning of the constructs for CRISPR *tb1* mutation. Subsequently rice will be transformed with CRISPR and the already available GA GPS1 sensor construct. During this time all constructs for protein-protein interaction studies between Hd3a, RFT1 and OsTB1 will be performed. Tests for protein-protein interaction will be performed. First test will be the yeast two hybrid and subsequently promising interactions will be validated with FRET/FLIM in tobacco.
YEAR 2
GPS1 GA marker plants transformed in year 1 will be analyzed with the confocal microscope.
Ostb1 mutant lines will be genotyped, propagated and characterized. Special attention will be paid to AXM and SAM development in *Ostb1* mutant compared to the *wt*.

Promising protein-protein interaction results could be eventually verified with further approaches during the second year.
IAA and GA sensors will be crossed into *hd3a*, *rft1* and *Ostb1* mutants. Also, double and triple mutants will be created to test genetic/epistatic interactions.
YEAR 3
In the last year IAA and GA distribution will be analyzed in *hd3a*, *rft1* and *Ostb1* mutants, compared to the *wt*. Also, morphological analysis of multiple mutants will be performed, together with comparative expression analyses at the SAM and AXM.Finallysome time will also be devoted to manuscript preparation.

See also diagram in Figure 2 below.

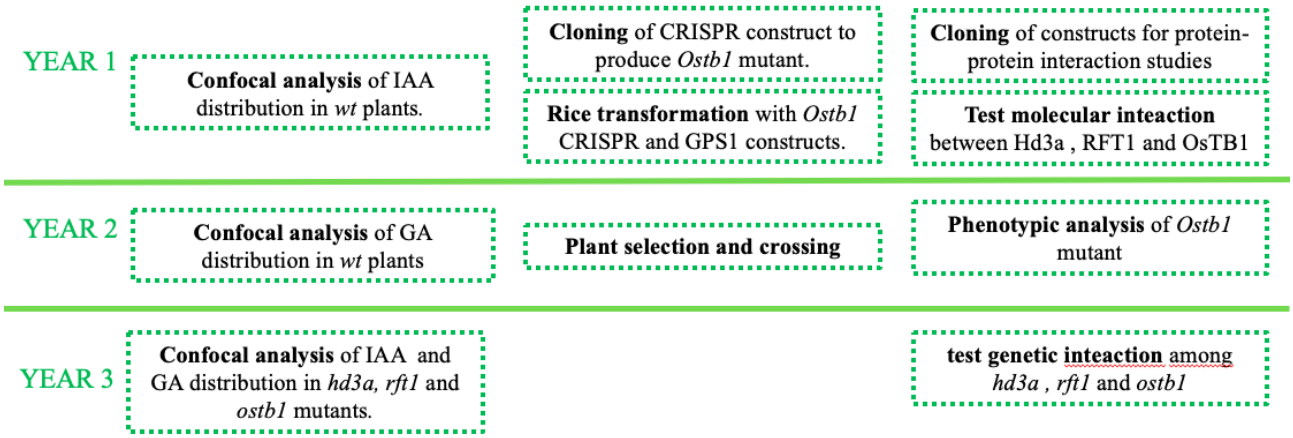

Figure 2. Schematic representation of the research development during the three years of PhD project.

6. Available funds (source and amount)

22.090 Euro – BASF project
13.000 Euro – PSR Ateneo 2018

7. Literature:

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