PhD School on Agriculture, Environment and Bioenergy (http://sites.unimi.it/dottorato_aab/)

(XXXIV cycle, 2018-20)

Project draft

1.Field of interest

BIO-01

2.Project title

Hormone signaling in defining plant architecture

3.Tutor Dr. Vittoria Brambilla, **co-tutor:** Prof. Alberto Spada

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

Rice is the most important crop for human nutrition worldwide. Modern rice is the result of thousands of years of breeding during which many favourable traits have been selected, including optimal plant architecture (Teichmann and Muhr, 2015). A rice plant is composed of a main shoot and several lateral branches (or tillers) that at maturity each bring an apical panicle (Figure 1). Plant architecture is crucial for grain quality and grain yield, as plants with reduced height and with an increased number of uniformly growing tillers produce more panicles (and seeds), are less prone to lodging and are more easily harvested. A large genetic variability is still present in modern rice plant architecture (Figure 2A).

The aerial rice plant growth and development occurs thanks to the activity of meristematic tissues (Figure 2B). During the vegetative growth, these are:

- the shoot apical meristem (SAM) that is at the top of the stem and ensures leaves and panicles development (Brambilla et al., 2017);
- the intercalary meristems (IM) that support stem growth by internodes elongation (Wang et al., 2018);
- the axillary meristems (AXM) that originate the lateral tillers from the leaf axils (Hussien et al., 2014; Wang et al., 2018).

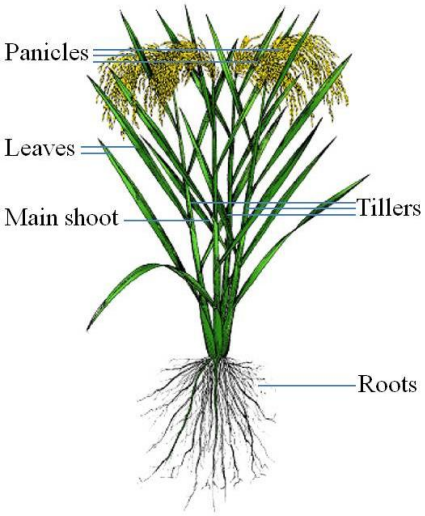

Figure 1. Schematic representation of the rice plant.

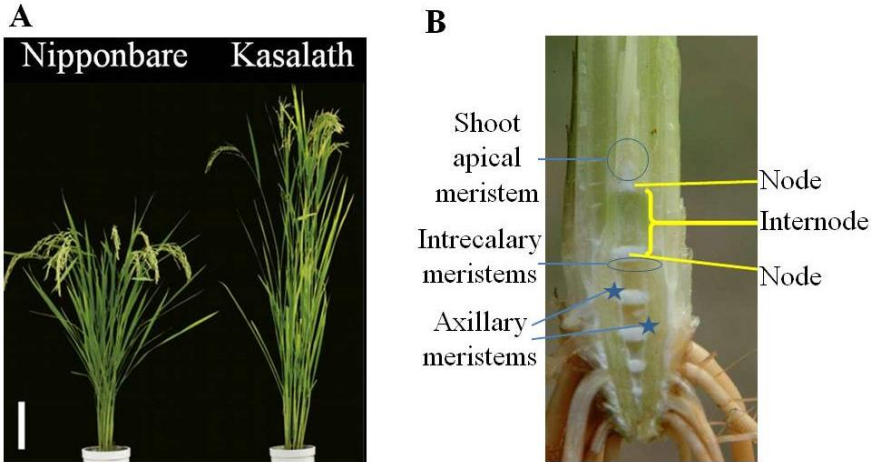

Figure 2. A:two rice varieties with different plant architecture; B: position on the SAM and the IM in the shoot.

It is well established that plant hormones like Auxins (indole-3-acetic acid, IAA) and Gibberellins (GA) are involved in plant growth and tillering and that their distribution influences plant architecture. Hormones signalling is also integrated into a complex genetic network where several components have been characterized. In the lab we have previously produced and characterized two mutants with defective meristems growth. One is *pine1* that constitutively grows IM,

resulting in a prostrate plant. The second one is an *hd3a rft1* double mutant that is defective in SAM and AXM development.

Layout of the project (draft)

During the three-years PhD course, the candidate will perform a detailed morphological analysis, using a light microscope, of *pine1* and *hd3a rft1* mutants. Subsequently she/he will study the dynamics of IAA (Lau et al., 2011; Yang et al., 2017) and GA (Dai and Xue, 2010) distribution *in vivo* by confocal imaging. For IAA the marker rice lines containing *DR5:VENUS3X* (Yang et al., 2017) 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 (Rizza et al., 2017). While IAA marker lines are already available in the lab, GA marker, already available for Arabidopsis, will need to be first introduced and tested in rice.
These analyses will be first performed in the *wt* and subsequently, if validated, also in *pine1* and *hd3a rft1* to study IAA and GA distribution perturbation in the mutants compared to the *wt*.
DR5:VENUS3X and *35S:DII:VENUS* will be introduced into the *pine1* and *hd3a rft1* mutants by crossing, while GPS1 will be transformed into the mutants. Double/triple crossings between *pine1*, *hd3a rft1* and various architecture/growth altered mutants and overexpressors (some of these are *oxSLR1*, *slr1-1*, *Sub1A*, *ostb1*...) (Dai and Xue, 2010; Schmitz et al., 2013; Guo et al., 2013) will be produced, to clarify their genetic interaction. Possibly the IAA marker will also be crossed into these multiple crossings (dashed lines in Figure 3).

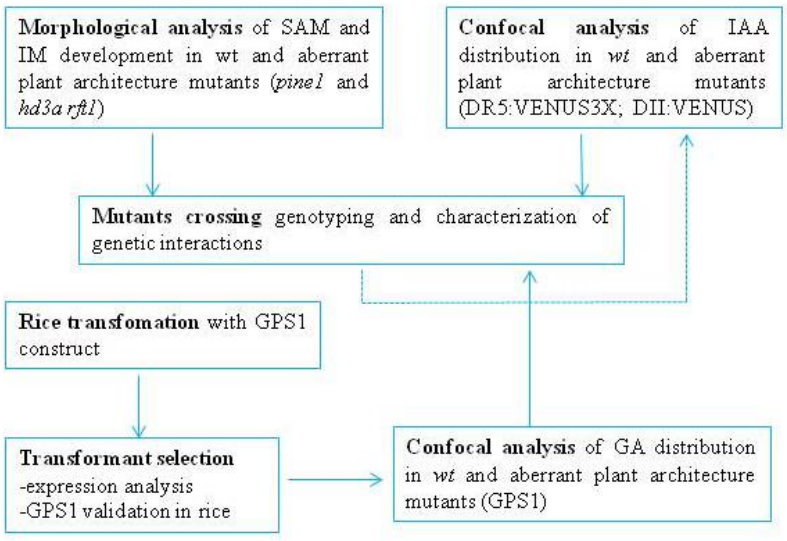

Figure 3. Schematic representation of the research development during the PhD course.

5.1.Materials & Methods

A) Morphological analysis of mutants available in the lab that present aberrant plant architecture:
- plant genotyping and sampling
- inclusion, sectioning and light microscopy observations.

- staining for different compounds for tissue characterization.
- quabtutative measure of internode elongation and statistical analyses.

B) *In vivo* visualization of IAA accumulation/degradation in rice plants (DR5:VENUS3X; DII:VENUS transgenic rice plants, available in the lab) by confocal microscopy.
- Crossing of the *DR5:VENUS3X* and *DII:VENUS* marker lines in *pine1* and *hd3a rft1* mutants and study of IAA accumulation in these, genotyping and confocal observation.

C) *In vivo* visualization of GA accumulation in rice plants:
- rice *wt* and developmental mutants *Agrobacterium tumefaciens* mediated transformation 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 specific transcriptional analyses of genes involved in GA biosynthesis, catabolism and signal transduction.

E) Crossing of *pine1* and *hd3a rft1* with characterized mutants/allelic variants/overexpressors with altered plant development, to verify their additive or epistatic effect.
-Rice crossing,
-genotyping by various molecular markers,
-Plant phenotypic analyses.

5.2.Schedule and major steps (3 years)

YEAR 1
The project will start with the detailed morphological characterization of the mutants already available in the lab that present aberrant plant architecture compared to the *wt*. Particular attention will need to be paid to the meristems development (SAM, IM, AXM). Different staining will allow the description of the cellular types and of the compounds accumulated in these tissues.
At the same time rice transformation will be set up for the GPS1 construct, already available. The transformation will be performed in *wt* and also in *pine1* and *hd3a rft1* backgrounds. Rice transformation will take around 1 year to have the first transgenic seeds to be propagated, therefore it is essential to start transformation as soon as possible during the project.
During year 1 all crossings will be performed to have homozygous F2 plants during year 2.

YEAR 2
IAA and GA accumulation will be studied at different developmental stages by confocal imaging in *wt,* and in *pine1* and *hd3a rft1* mutants.
The *GPS1* system will need to be validated by tissue specific expression analyses of genes involved in GA biosynthesis, catabolism and signal transduction. Rice tissues will be manually dissected under a stereo microscope.
Epistatic interactions will be studied between the *pine1* mutant, the *hd3a rft1* double mutant and known plant developmental regulators mutants/allelic variants/overexpressors.
Eventually the *GPS1* biosensor will also be introduced by crossing into the multiple mutants.

YEAR 3
Experiments performed during this time will largely depend on the results of the previous years. The analysis of hormone localization and quantification *in vivo* in rice *wt* and mutants will be refined, depending on the clues

obtained during the previously described experiments. IAA/GA accumulation will be also analyzed in multiple mutants produced during the previous years. Some time will also be devoted to manuscript preparation.

6. Available funds (source and amount)

22.090 Euro – BASF project

7. Literature:

Brambilla, V., Martignago, D., Goretti, D., Cerise, M., Somssich, M., de Rosa, M., Galbiati, F., Shrestha, R., Lazzaro, F., Simon, R., and Fornara, F. (2017). Antagonistic Transcription Factor Complexes Modulate the Floral Transition in Rice. Plant Cell: tpc.00645.2017.

Dai, C. and Xue, H.W. (2010). Rice early flowering1, a CKI, phosphorylates della protein SLR1 to negatively regulate gibberellin signalling. EMBO J. **29**: 1916–1927.

Guo, S., Xu, Y., Liu, H., Mao, Z., Zhang, C., Ma, Y., Zhang, Q., Meng, Z., and Chong, K. (2013). The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. Nat. Commun. **4**: 1566.

Hussien, A., Tavakol, E., Horner, D.S., Muñoz-Amatriaín, M., Muehlbauer, G.J., and Rossini, L. (2014). Genetics of Tillering in Rice and Barley. Plant Genome **7**: 0.

Lau, S., De Smet, I., Kolb, M., Meinhardt, H., and Jurgens, G. (2011). Auxin triggers a genetic switch. Nat. Cell Biol. **13**: 611-U249.

Rizza, A., Walia, A., Lanquar, V., Frommer, W.B., and Jones, A.M. (2017). In vivo gibberellin gradients visualized in rapidly elongating tissues. Nat. Plants **3**: 803–813.

Schmitz, A.J., Folsom, J.J., Jikamaru, Y., Ronald, P., and Walia, H. (2013). SUB1A-mediated submergence tolerance response in rice involves differential regulation of the brassinosteroid pathway. New Phytol. **198**: 1060–1070.

Teichmann, T. and Muhr, M. (2015). Shaping plant architecture. Front. Plant Sci. **6**: 1–18.

Wang, B., Smith, S.M., and Li, J. (2018). Genetic Regulation of Shoot Architecture. Annu. Rev. Plant Biol. **69**: annurev-arplant-042817-040422.

Yang, J., Yuan, Z., Meng, Q., Huang, G., Périn, C., Bureau, C., Meunier, A.-C., Ingouff, M., Bennett, M.J., Liang, W., and Zhang, D. (2017). Dynamic Regulation of Auxin Response during Rice Development Revealed by Newly Established Hormone Biosensor Markers. Front. Plant Sci. **8**: 1–17.