

# PhD School on Agriculture, Environment and Bioenergy

([http://sites.unimi.it/dottorato\\_aab/](http://sites.unimi.it/dottorato_aab/))

(XXXVII cycle, 2021-24)

## Project draft

### 1. Field of interest

BIO-03; BIO-01; AGR07

### 2. Project title

Breeding and evolution of plant architecture in rice and other wild *Poaceae*

### 3. Tutor prof. Simon Pierce

co-tutor/s Dr. Vittoria Brambilla

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

Rice is an herbaceous crop belonging to the *Poaceae* family that is the main staple food for humans worldwide and Northern Italy is the first European rice producer. Appreciated Italian varieties, adapted to its environments, has seen a stable trend of growing yields thanks to optimized agronomic practices and to cultivar breeding. Current efforts of rice breeders mainly concentrate on enduring this improvement, towards the production of new high yielding rice varieties that combine positive traits and can overtake the hybrids' performance.

Rice yield is predominately controlled by the plant's architecture: this includes the size of the plant, the number of culms, the shape of the panicle, the number of seeds and the efficiency of the root apparatus. Another important aspect related to rice yield is the capacity to cope with pathogens and abiotic stresses.

Developmental genetic studies over the past 20 year have characterized many genes controlling the growing of the rice plant (Gao et al., Trends in Plant Science 2017; Brambilla et al., The Plant Cell 2017; Gomez-Ariza et al., Nature Plants 2019) including those specifically affecting the plant's architecture and yield also related to different environmental conditions. Additionally, molecular plant pathology studies have uncovered important genes and mechanisms directing the plant's defense system.

Information on the genetic basis of plant traits gained by basic research studies can easily be transferred to breeding better yielding varieties by genome editing (Zhu et al., Nat Rev Mol Cell Biol 2020). Genome editing technologies, as CRISPR/Cas9, allow the insertion of small mutations in specific regions of the genome, thus eliminating or modifying a gene function. The mutations can be aimed at producing a frameshift or substituting by base editing a single base. Also, the CRISPR system has recently been improved into the prime editing system that allows re-writing of larger DNA fragments (Lin et al., Nature Biotechnology 2020). Modifying by CRISPR/Cas9 the loci controlling important breeding traits is a straightforward method to improve a variety for that trait.



Figure 1. High yielding varieties in the field have an optimal plant architecture

Rice belongs to the large *Poaceae* family of monocotyledonous herbaceous plants that, besides cereals, also include bamboos and lawn and grassland species. As many genes are known to control plant architecture in rice, the functional characterization of a group of them specifically involved in the definition of growth habits in a panel of diverse wild and cultivated *Poaceae* would elucidate the evolution and ecology of different plant morphologies and the possible conservation of their molecular bases (Araneda et al., Plos One 2013).

## 5. Layout of the project (draft)

The project focuses on the rice plant architecture, focusing on two aspects:

- 1) improving rice to achieve a better crop yield
- 2) studying the evolution and conservation within the *Poaceae* family in an ecological context

Plant architecture in the *Poaceae* family is largely determined by the activity of the meristematic cells that control cell and organ proliferation. These meristems are organized in a shoot apical meristem, that support the growth and differentiation of the top of the shoot, some axillary meristems that control lateral shoots development and intercalary meristems that support stem elongation.

In the lab two novel proteins controlling axillary and intercalary meristems proliferation have been characterized: these are respectively an F-BOX protein that we have not specifically named yet (Mineri et al., in preparation) and we will just refer to it as F-BOX and a C2H2 Zinc Finger transcription factor named PINE1 (Gomez-Ariza et al., Nature Plants 2019; Nagai et al., Nature 2020). CRISPR mutants in *F-BOX* have more proliferating **axillary meristems** that result in a higher number of culms per plant at maturity and higher yield. PINE1 controls internode elongation by regulating the activity of **intercalary meristems**. While *f-box* mutation in the temperate *japonica* model variety Nipponbare results in plants with an increased yield, *pine1* mutants in Nipponbare produce plants that are prostrated and are less productive but resemble.

Mutation in the *F-BOX* gene will be inserted by genome editing in elite Italian varieties to verify the conservation of its axillary meristem proliferation promoting function. Also *f-box* mutation could be pyramided with other known and already produced and studied in the lab mutations in genes that affect lateral stems growth like *OsCKX2* (Ashikari et al., Science 2005) to further increase the activity of the axillary meristems and rice productivity. In order to produce an optimal rice plant these mutations could be further combined with other two CRISPR mutation produced and verified in the lab: one that promotes root growth (in *ZOG* genes) and *pi21* (Fukuoka et al., Science 2009), *hma1* and *hma2* triple mutation that improves resistance to the fungus *Magnaporthe oryzae* that causes the deadly rice blast disease.

*PINE1* is an important gene controlling internode elongation in response to environmental stimuli as photoperiod (Gomez- Ariza et al., Nature Plants 2019) and also flooding (Nagai et al., Nature 2020). Knock out mutants in PINE1 show a prostrated habit, similarly to those in *PROG1* (Jin et al., Nature Genetics 2008) and *LAZY* (Yoshihikara et al., Plant Physiology 2017). These are currently the major characterized genes controlling growth habit in *Poaceae*. A panel of wild and cultivated *Poaceae* with different habits will be produced: then these three genes, if conserved, will be cloned, sequenced and will undergo comparative expression analyses. The panel will be constructed based on the phylogenetic relationship of the species with rice and the diversification of their growth habit. One species already identified that will be included is *Agrostis stolonifera*, that is a grass widely present in local meadows and has a

prostrated growth habit due to elongation of internodes similarly to that observed in *pine1* mutant.

### **5.1. Materials & Methods:**

CRISPR mutant rice will be genotypically and phenotypically analyzed by molecular biology tools (PCR and sequencing) and microscopy respectively.

New CRISPR mutants will be produced with the aim to achieve an improved plant ideotype- CRISPR frameshift mutants but also base edited and prime edited mutants could be produced. Appropriate constructs will be designed and produced by molecular biology tools (cloning), then rice calli will be transformed by rice in vitro culture and *Agrobacterium tumefaciens* infection.

A previously defined panel of *Poaceae* species will be collected, and their geographical and ecological distribution will be reported. DNA will be extracted and the plant habit genes sequenced.

Also, expression analyses by RNA extraction and qRT and eventually *in situ* hybridization will be performed on the species belonging to the *Poaceae* panel.

### **5.2. Schedule and major steps (3 years):**

#### YEAR 1:

CRISPR mutants genotyping, propagation and phenotypic analysis

New CRISPR mutants design, cloning and transformation – gene multiplexing and pyramiding

*Poaceae* panel establishment and collection in the field – geographical distribution and phylogenetic relationship

#### YEAR 2:

Propagation, genotypic and phenotypic characterization of single and multiple high yielding CRISPR mutants

Cloning of plant architecture genes in the *Poaceae* panel species – sequence analysis

Set up of expression analyses (qRT, *in situ* hybridization) in the *Poaceae* panel

#### YEAR 3:

Possible field trials (depending on evolution of European legislation) of CRISPR high yielding varieties

Data analysis from *Poaceae* panel genotypic and expression results

Writing and submitting manuscripts

### **6. Available funds (source and amount)**

SEED-DISENGAGE project total 30.000 euro

Funding from BASF- about 15.000 euro

## 6. Literature:

- Gao XQ, Wang N, Wang XL, Zhang XS. Architecture of Wheat Inflorescence: Insights from Rice. *Trends Plant Sci.* 2019 Sep;24(9):802-809. doi: 10.1016/j.tplants.2019.06.002. Epub 2019 Jun 27. PMID: 31257155.
- Brambilla V, Martignago D, Goretti D, Cerise M, Somssich M, de Rosa M, Galbiati F, Shrestha R, Lazzaro F, Simon R, Fornara F. Antagonistic Transcription Factor Complexes Modulate the Floral Transition in Rice. *Plant Cell.* 2017 Nov;29(11):2801-2816. doi: 10.1105/tpc.17.00645. Epub 2017 Oct 17. PMID: 29042404; PMCID: PMC5728136.
- Gómez-Ariza J, Brambilla V, Vicentini G, Landini M, Cerise M, Carrera E, Shrestha R, Chiozzotto R, Galbiati F, Caporali E, López Díaz I, Fornara F. A transcription factor coordinating internode elongation and photoperiodic signals in rice. *Nat Plants.* 2019 Apr;5(4):358-362. doi: 10.1038/s41477-019-0401-4. Epub 2019 Apr 1. PMID: 30936438.
- Zhu H, Li C, Gao C. Applications of CRISPR-Cas in agriculture and plant biotechnology. *Nat Rev Mol Cell Biol.* 2020 Nov;21(11):661-677. doi: 10.1038/s41580-020-00288-9. Epub 2020 Sep 24.
- Lin Q, Zong Y, Xue C, Wang S, Jin S, Zhu Z, Wang Y, Anzalone AV, Raguram A, Doman JL, Liu DR, Gao C. Prime genome editing in rice and wheat. *Nat Biotechnol.* 2020 May;38(5):582-585. doi: 10.1038/s41587-020-0455-x. Epub 2020 Mar 16. PMID: 32393904.
- Araneda L, Sim SC, Bae JJ, Chakraborty N, Curley J, Chang T, Inoue M, Warnke S, Jung G. Comparative genome analysis between *Agrostis stolonifera* and members of the Pooideae subfamily, including *Brachypodium distachyon*. *PLoS One.* 2013 Nov 11;8(11):e79425. doi: 10.1371/journal.pone.0079425. PMID: 24244501; PMCID: PMC3823605.
- Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M. Cytokinin oxidase regulates rice grain production. *Science.* 2005 Jul 29;309(5735):741-5. doi: 10.1126/science.1113373. Epub 2005 Jun 23. PMID: 15976269.
- Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M. Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science.* 2009 Aug 21;325(5943):998-1001. doi: 10.1126/science.1175550. PMID: 19696351.
- Nagai K, Mori Y, Ishikawa S, Furuta T, Gamuyao R, Niimi Y, Hobo T, Fukuda M, Kojima M, Takebayashi Y, Fukushima A, Himuro Y, Kobayashi M, Ackley W, Hisano H, Sato K, Yoshida A, Wu J, Sakakibara H, Sato Y, Tsuji H, Akagi T, Ashikari M. Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature.* 2020 Aug;584(7819):109-114. doi: 10.1038/s41586-020-2501-8. Epub 2020 Jul 15. PMID: 32669710.
- Jin J, Huang W, Gao JP, Yang J, Shi M, Zhu MZ, Luo D, Lin HX. Genetic control of rice plant architecture under domestication. *Nat Genet.* 2008 Nov;40(11):1365-9. doi: 10.1038/ng.247. Epub 2008 Sep 28. PMID: 18820696.
- Yoshihara T, Spalding EP. LAZY Genes Mediate the Effects of Gravity on Auxin Gradients and Plant Architecture. *Plant Physiol.* 2017 Oct;175(2):959-969. doi: 10.1104/pp.17.00942. Epub 2017 Aug 18. PMID: 28821594; PMCID: PMC5619908.

