

PhD School on Agriculture, Environment and Bioenergy

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(XXXVIII cycle, 2022-25)

Project draft

1. Field of interest

BIO-03; BIO-01

2. Project title

Genetic and molecular control of rice plant architecture and evolution of growth habit in the *Poaceae* family.

3. Tutor Prof. Simon Pierce

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

Rice is an herbaceous crop 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 a novel protein controlling intercalary meristems development have been characterized: these is a C2H2 Zinc Finger transcription factor that we named PREMATURE INTERNIDE ELONGATION 1 PINE1 (Gomez-Ariza et al., Nature Plants 2019; Nagai et al., Nature 2020) and controls internode elongation by regulating the activity of intercalary meristems 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. Species already identified that will be included are *Agrostis stolonifera*, *Cynodon dactylon*, *Dactyloctenium aegyptium* that are all grasses widely present in local meadows and has a prostrated growth habit due to elongation of internodes similarly to that observed in *pine1* mutant.



Figure 2. PINE1 represses internode elongation – these grow constitutively in *pine1* mutant plants

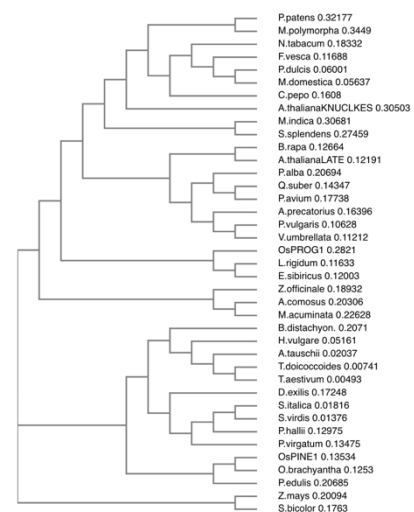


Figure 3. PINE1-like and PROG1-like genes are present across plant groups. Protein sequences were searched by tBLASTn using the PINE1 sequence.

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. For those for which there is no genomic sequence available, the DNA will be extracted and the plant habit genes sequenced. For few of them also transcription of the homologues genes will be assessed by RNA extraction and qRT-PCR.

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)

currently about 8.000 euro -waiting for grant applications results

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.

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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.

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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.

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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.

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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.

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