PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXVIII cycle, 2022-25)

Project draft

1.Field of interest

*Indicare **il/i** settore/i scientifico disciplinari:* BIO-01/BIO-03

2.Project title

Breeding of Lugano rice varieties to reduce water, fertilizers, and agrochemicals field inputs

3.Tutor prof**.** Simon Pierce

- Academic co-tutor dr.ssa Vittoria Brambilla

-Industrial co-tutor: dr.ssa Michela Martinotti

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

Rice is the main crop for human nutrition worldwide and it is predominantly cultivated in Southern Asia. Within Europe, the main rice producer is North Western Italy, where many varieties adapted to local environments and optimized for consumers requirements are grown. Decades long efforts of local breeders kept improving Italian rice varieties for many important breeding traits. Lugano Sementi s.r.l. is a rice seed company with a breeding department that developed many elite varieties that are currently among the most cultivated in Europe. Rice cultivation is currently facing unprecedent struggles for access to required inputs. In particular water scarcity, increased pricing for fertilizers and agrochemicals and uncontrolled disease infections are undermining rice production. For this reason, it is very important to further improve the elite varieties in the present context. This project intends to combine classical and biomolecular approaches, university technical expertese with that of an active company in the field to breed novel elite rice varieties optimized for three major agronomic traits: water use efficiency, fertilizer (nitrogen) use efficiency and resistance to a major rice disease (blast). For this project a tight collaboration between the proponent research group at Milan University and the rice breeding department of the company Lugano Sementi will be established.

5.Layout of the project (draft)

5.1.Materials & Methods:

The PhD candidate will follow two complementary approaches: the first based on classical breeding techniques driven by molecular markers and the second based on genome editing biomolecular technologies to produce novel elite rice varieties improved for three major agronomic traits. The targeted breeding traits are:

A. AN IMPROVED ROOT SYSTEM TO REDUCE WATER INPUT

A.1 Classical breeding approach.The Kinandang Patong (KP) variety provides the *dro1* allele that makes the root system deeper and the plant more drought resistant.

A.2. Targeted genome editing by CRISPR/Cas9 of *DRO1* gene

B. IMPROVE NITROGEN USE EFFICIENCY TO REDUCE NITROGEN INPUT

B.1. Classical breeding approach. The Tequing variety provides the allele *nrt1.1b* which improves the efficiency of nitrogen use. It will be introduced by crossing and followed by molecular markers.

B2. Targeted genome editing by CRISPR/Cas9 *prime editing* to insert the *nrt1.1b* allele.

To further enhance the performance of the root system *dro1* and *nrt1.1b* alleles could be combined

C. IMPROVE RESISTENCE TO RICE BLAST – REDUCTION OF AGROCHEMICAL INPUT

C.1. Classical breeding approach to stack three blast resistance loci. They will be introduced by crossing and followed by molecular markers.

C.2. Targeted genome editing of elite Lugano varieties by CRISPR/Cas9 of three known genes that confer resistance to blast (Pi21, HMA1 and HMA2)

5.2. Schedule and major steps (3 years):

Classical breeding techniques driven by molecular markers and genome editing targeted mutagenesis will be carried out simultaneously.

CLASSICAL MOLECULAR BREEDING

Year 1. Crosses of KP or Tequing X 2-3 Lugano varieties will be done in the field or in the phytotron. The variable to check is the flowering period. During winter, F1 individuals with *dro1* or *nrt1.1b* allele and cycle acceleration alleles will be selected by molecular markers and backcrossed with the corresponding parental (BC1F1). Crosses for blast resistance will be also performed.* *The Tequing variety also carries the PiQ1 allele of resistance to rice blast which can be selected in the progeny.*

Year 2. BC1F1 plants will be selected by molecular markers to keep only early flowering lines and plants carrying *dro1/ nrt1.1b* alleles. The selected lines will be again crossed in the field to produce the BC2F1 generation. During winter, BC2F1 individuals with *dro1/ nrt1.1b* and cycle acceleration alleles will be selected by molecular markers and backcrossed (BC3F1).

Year 3. BC3F1 will be again molecularly selected for *dro1/ nrt1.1b* and early flowering alleles to plant in the field only appropriated *dro1/ nrt1.1b* lines. BC4F1 will be produced by backcrosses in the field.

A similar approach will be used to stack blast resistant alleles.

During the II and III year it is also possible to start phenotypic tests

GENOME EDITING

Year 1 Production and selection of CRISPR/Cas9 mutagenized lines with the *dro1* allele and CRISPR prime editing *nrt1.1b* lines in 2-3 varieties of interest.

** The system used for nrt1.1b (prime editing) is more complex than that used for dro1 and less efficient to require the analysis of multiple plants.*

Year 2 Selection of homozygous non transgenic mutant lines and propagation.

Year 3 Phenotypic tests in the laboratory to evaluate the increase in depth of the root system and the increased resistance to drought, blast and evaluation of nitrogen use efficiency in the edited varieties *vs* the *wt*.

6. Available funds

(about 8000 euros overheads from previous projects)

7. Literature:

Hu B, Wang W, Ou S, Tang J, Li H, Che R, Zhang Z, Chai X, Wang H, Wang Y, Liang C, Liu L, Piao Z, Deng Q, Deng K, Xu C, Liang Y, Zhang L, Li L, Chu C. Variation in NRT1.1B contributes to nitrate-use divergence between rice subspecies. Nat Genet. 2015 Jul;47(7):834-8. doi: 10.1038/ng.3337. Epub 2015 Jun 8. PMID: 26053497.

Zhang J, Liu YX, Zhang N, Hu B, Jin T, Xu H, Qin Y, Yan P, Zhang X, Guo X, Hui J, Cao S, Wang X, Wang C, Wang H, Qu B, Fan G, Yuan L, Garrido-Oter R, Chu C, Bai Y. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol. 2019

Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet. 2013 Sep;45(9):1097-102. doi: 10.1038/ng.2725. Epub 2013 Aug 4. PMID: 23913002.

Brambilla V, Gomez-Ariza J, Cerise M, Fornara F. The Importance of Being on Time: Regulatory Networks Controlling Photoperiodic Flowering in Cereals. Front Plant Sci. 2017 Apr 26;8:665. doi: 10.3389/fpls.2017.00665. PMID: 28491078; PMCID: PMC5405123.

Borrelli VMG, Brambilla V, Rogowsky P, Marocco A, Lanubile A. The Enhancement of Plant Disease Resistance Using CRISPR/Cas9 Technology. Front Plant Sci. 2018 Aug 24;9:1245. doi: 10.3389/fpls.2018.01245. PMID: 30197654; PMCID: PMC6117396.

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.

Maidment JHR, Franceschetti M, Maqbool A, Saitoh H, Jantasuriyarat C, Kamoun S, Terauchi R, Banfield MJ. Multiple variants of the fungal effector AVR-Pik bind the HMA domain of the rice protein OsHIPP19, providing a foundation to engineer plant defense. J Biol Chem. 2021 Jan-Jun;296:100371. doi: 10.1016/j.jbc.2021.100371. Epub 2021 Feb 4. PMID: 33548226; PMCID: PMC7961100.