Phosporous in rice paddy field, balance between productivity and environment in view of new agronomic practices

BACKGROUND

Phosporous is an essential macroelement for plant development, indeed, it plays a major role in the energy metabolism and biosynthesis of nucleic acids and cell membranes. Moreover, P makes and important contribution to seed formation, root elongation, disease and cold tolerance. Deficiencies in this element can lead to growth inhibition and, as a consequence, yield losses.

P is abundantly present in the soil, although only a very small percentage (about 1%) is available for plant uptake, indeed plants absorb P as $H_2PO_4^-$ and HPO_4^-. P is usually found within the soil in mineral or organic forms. Mineral P, such as apatite, hydroxyapatite or oxyapatite, are poorly soluble in soil, on the other hand, organic P, such as phosphodiesters, phospholipids and nucleic acids, tend to precipate with a variety of metals (Aluminum, Calcium and Iron), becoming unavailable for plants. Many strategies have been developed by plant and microorganism for solubilizing mineral and organic forms of P.

Because of the scarce availability of P in soils, and the significant yield losses that a defiency of P can leat to, abundant phosphoric fertilization is often adopted. Nevertheless, P becomes quickly unavailable within the soil, precipitating with other elements and becoming insoluble. This practice, besides increasing the cost of agricultural production, has negative effects on the environment, indeed it palys a major role in the eutrophication of surface water that could lead to algal blooms and it causes a reduction of soil fertility .

Agronomic practices have a huge impact on the cycle and availability of nutrient elements in the soil. Other macroelements such ad N and K received many attentions, especially in the distinctive flooded water regime present in rice paddies. But there is a gap in the knowledge regarding how P is affected by agronomic practices.

METHODS

The microbiome of rice plants (Oryza sativa) is explored in light of different agronomic practices: presence (+CC) and absence (-CC) of a cover crop (i.e, hairy vetch, Vicia villosa) and supplement (+P) of P fertilizer compared to a lack of P addition (-P).

Rice plants are sampled from Italian rice paddies located in Castello D'agogna (PV) and Nicorvo (PV) at two different stateges: tillering, in June 2021 (Fig. A), and booting, in July 2020. The cover crop is also sampled before the ploughing in preparation of rice seeding. The schemes of experimental conditions are reported in fig C and D.

Each plant is processed as follow:

Rhizosphere soil, rhizoplane and endosphere are separated according to (Luo et al., 2011) and (Chen et al., 2012).

BACTERIA ARE ISOLATED on agar media from each fraction and classified into different homogeneous groups according to their colony morphology and BOX PCR DNA profile.

A variable number of bacteria isolates are screened for PGP activities (i.e, tricalcium phosphate TCP and phytate solubilization, siderophores production, nitrogen fixation, EPS production, Indole-3-acetic acid (IAA) and ACC deaminase production. Because of the considerable strains collection, the PGP tests, usually performed on agar media, require a scale up for screening the large number of strains. The tests are therefore performed on agar media, require a scale up for screening the large number of strains. The tests are therefore performed in microplates and analyzed by an automatic reader. The total bacteria DNA is isolated from rhizosphere soil, rhizoplane and root endosphere,

and subjected to metabarcoding analysis targeting the V3-V4 region of the 16S rRNA gene for exploring bacterial and archaeal communities and ITS for fungi.

RESULTS

Heterotrophic variable bacterial counts of bulk and rice rhizosphere soils were in the order of 10^7, those of rice rhizoplane 10^5-10^9 and those of rice endosphere 10^3-10^7 CFU/g dw; on the other hand, the bacterial communities associated with the vetch used as a cover crop appeared to be riches, as the rhizosphere soil harboured bacteria in the order of 10^7, those of rhizoplane 10^{10} and those of endosphere 10^7 (Table 1).

Table 1: Results of bacterial count on agar plates per each fraction of the root and condition and of the bulk soil.

Plant	Fraction	Condition	CFU/g dw
Rice (booting stage)	Rhizosphere soil	+CC	3.67×10^7
		-CC	6.49×10^7
		+P	2.42×10^7
		-P	3.96×10^7
	Rhizoplane	+CC	1.17×10^6
		-CC	1.37×10^6
		+P	9.15×10^9
		-P	4.57×10^9
	Endosphere	+CC	8.37×10^4
		-CC	3.25×10^3
		+P	7.44×10^3
		-P	3.90×10^3
Rice (tillering stage)	Rhizosphere soil	+CC +P	6.70×10^6
		+CC -P	2.62×10^8
		-CC + P	8.70×10^7
		-CC -P	1.25×10^8
	Rhizoplane	+CC +P	8.90×10^7
		+CC -P	3.40×10^7
		-CC + P	7.20×10^6
		-CC -P	5.90×10^5
	Endosphere	+CC +P	2.70×10^6
		+CC -P	1.89×10^7
		-CC + P	4.60×10^6
		-CC -P	1.32×10^6
Soil	Bulk soil	+P	7.13×10^7
		-P	4.94×10^7
Hairy vetch	Rhizosphere soil	/	1.17×10^9
	Rhizoplane	/	1.01×10^{10}
	Endosphere	/	6.23×10^7

A total of 641 strain were isolated from rice plants at the booting stage (559 strains) and from the cover crop (82 strains). In detail 254, 101 and 208 were isolated from the rhizosphere, rhizoplane and endosphere of rice plants, respectively. On the other hand 21,21,20 and 20 strains were isolated from rhizosphere soil, rhizoplane, endosphere and nodules from the cover crop, respectively. Regarding the rice plants, 30 morphological classes were identified from the rhizosphere from the rhizosphere soil, along with 23 strains that presented a unique morphology.

From the rhizoplane, 23 classes and 7 unique strains we found, while within the endosphere 35 morphological classes and 35 unique strains were observed within the strains recovered; regarding the unique strains, 3,5,2 and 4 were observed for rhizosphere soil, rhizoplane, endosphere and nodule.

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Regarding the PGP tests, the strains have been screened for their ability to solubilize TCP, a form of inorganic phosphorous for their ability to solubilize TCP, a form of inorganic phosphorous present within the soil. A total of 113, 69 and 118 strains belonging to rhizosphere soil, rhizoplane and endosphere of rice (bootiung stage) were screened, respectively.

Regarding the PGP tests, the strains have been screened for their ability to soluble TCP, a form of inorganic phosphorous present within the soil. A total of 113, 69 and 118 strains belonging to rhizosphere soil, rhizoplane and endosphere of rice (booting stage) were screened, respectively. As shown in figure 2, rhizoplane and endosphere presented the highest percentage of strains able to solubilize TCP: 29% and 28,8%, respectively. A few strains constituting the 8,8; 4,3 and 10.2 % of the strains screened for the rhizosphere soil, rhizoplane and endosphere, respectively, did not grow on the media used for the test.

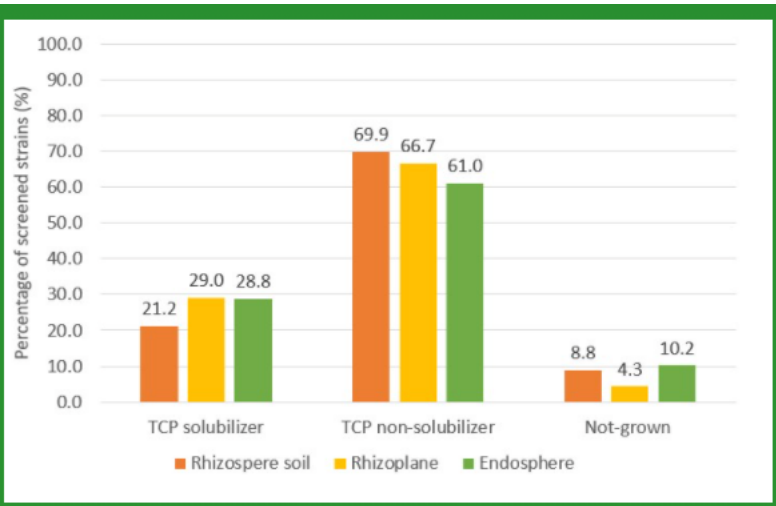

Figure 2: Bar-chart showing the percentage of strains isolated from rice (booting stage) able to solubilize TCP per rice root fraction.

The presence of the cover crop and the supply of P fertilizer revealed the presence of a higher percentage of strains with TCP-solubilizing ability (Figure 3).

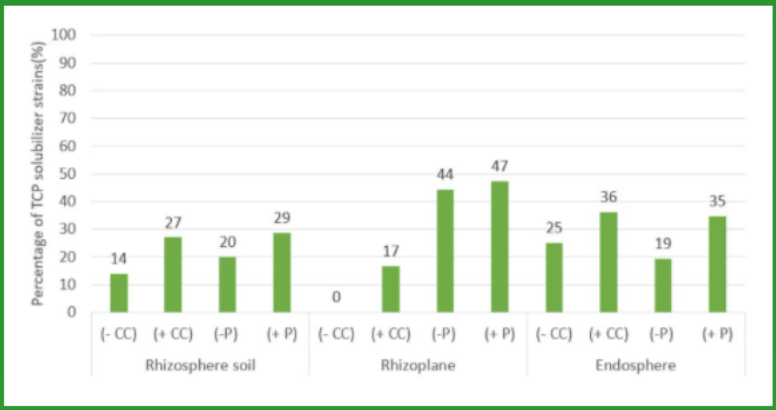

Figure 3: Bar-chart showing the percentage of strains isolated from rice (booting stage)

Figure 3: Bar-chart showing the percentage of strains isolated from rice (booting stage) able to solubilize TCP per condition. +CC: presence of cover crop; -CC: absence of cover crop; +P: addition of P fertilizer; -P: lack of P fertilizer.

In contrast with these results, none of the 23 strains recovered from the cover crop was able to solubilize this inorganic form of P. about the EPS production, 97 strains isolated from booting rice showed this activity; amongst them, 60 were recovered from the rhizosphere soil, 8 from the rhizoplane and 29 from the endosphere. Preliminary results of the IAA production test revealed that 9 out of 19 strains tested and isolated from the rhizosphere soil can produce IAA; interestingly, 88%

of these strains are also able to solubilize TCP. The total DNA from each fraction of rice (both tilling and booting stages9, COVER CROP AND bulk soil was successfully extracted metabarcoding analysis of the 16S rRNA gene (V3-V4 region) for bacterial and archaeal communities and ITS for fungal communities are ongoing.

DISCUSSION AND FUTURE WORK

Rice plants sampled in Northen Italy harbour a community of bacteria characterized by a high degree of diversity. Cover crop ploughing and phosphate fertilization positively affected endosphere bacterial abundance and promoted the establishment of bacterial strains with TCP solubilization ability.

Further analyses are needed in order to assess the effect of agronomic practices on PGP characteristics of the whole bacterial collection and on the P cycle in rice paddies. The project is still ongoing, indeed PGP tests (IAA and ACC deaminase production, nitrogen fixation, siderophores, EPS and phytate solubilization) on the strains are in due course. It is important to note that some strains are in due course. It is important to note that some strains were not able to grow on TCP solubilization and other PGP tests media, thus underlying the need to asses several recipes per each PGP activity of different strains. In addition, because of the large number of isolates, some strains may belong to the same taxonomic classification, therefore the strains will undergo a dereplication thought BOX-PCR and the profiles will be analysis will provide a fingerprint profile per each strain that will allow to carefully distinguish with a molecular approach the whole collection of bacteria strains. In this way, it will be possible to screen for PGP activities all the strains that morphologically looked like the same taxon. Per each profile, the strains will also be identified through 16S rRNA gene SANGER sequencing. A workflow for this part of the project is represented in figure 4.

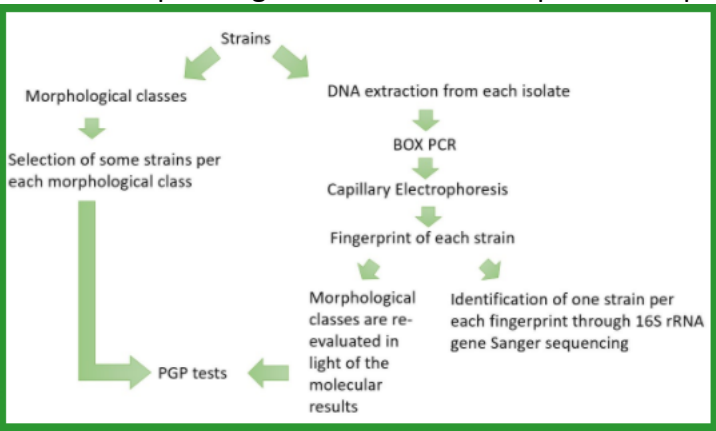

Figure 4: Workflow of the dereplication of the strains isolated.

Finally, the results of the metabarcoding analysis of environmental DNA will allow us to better explore the microbial ecology of rice rhizosphere communities as primed by the different agronomic practices, rice stages, cover crop and P ferilization. Along with the results collected from the other partners, Ente Nazionale Risi and the University of Turin, regarding P availability within the soil, the project will provide valuable information that will be helpful for filling the gap in knowledge regarding the P cycle in rice paddies in Northen Italy. This project provides useful data for farmers, for encouraging the reduction of P fertilization and the use of more sustainable practices such as cover crop in rice cultivation system in Northen Italy.

OVERVIEW AND AIMS OF THE PROJECT

The P-RICE project aims to increase the knowledge regarding P mobility and bioavailability in rice paddies in Northern Italy, whit the goal of optimizing phosphatic fertilization for enhancing P use efficiency, mercing productivity and environmental sustainability. The project started in June 2020, involving Ente Nazionale Risi and the University of Turin for the activities that regard agronomic management and P availability in soils, and the university of Milan for the aspects concerning the microbial communities associated with rice.

Regarding the work undertaken at the university of Milan, the objective of this part of the project is to characterize the microbial community associated with rice in Northern Italy, both with culture-dependent and independent techniques. Culture-based techniques. Culture- based techniques are focused on bacteria and they allow the isolation of bacterial strains from the rhizosphere soil, rhizoplane and endosphere of rice plants that are screened for different plant growth promoting (PGP(activities, particularly on the solubilization of organic and inorganic forms of P. One of the main goal of the culturome approach is to create a collection of PGP bacterial isolates belonging to the rice rhizosphere that will be delivered to public microbial culture collection. On the other hand, the culture – independent approach based on illumine analyses of environmental DNA will provide information on the complete bacterial, fungal and provide information on the complete bacterial, fungal archaeal communities associated with the rice root environment, with the aim to better understand the ecology and dynamics of microorganisms in light of different agronomic practices.