Critical Review – Influence of resistance breeding in common bean on rhizosphere microbiome composition and function

Lucas William Mendes, Jos M Raaijmakers, Mattias de Hollander, Rodrigo Mendes and Siu Mui Tsai (2018)

Siu Mui Tsai is the director of CENA, the Centre for Nuclear Energy and Agriculture at the University of São Paulo, Brazil (Universidade de São Paulo, 2021). As a professor of Agronomy with a primary focus on plant-microbe interactions and symbioses, her lab group largely investigates environmental factors such as soil composition (Grossman *et al*., 2010; Rodrigues *et al*., 2012) and nitrogen availability (Rodrigues *et al*., 2012; Bossolani *et al*., 2020) in relation to plant-microbe interactions. The results of this research are used to inform agricultural research in Brazil and all over the world (e.g. Cordovez *et al*., 2019; Liu *et al*. 2019). In this essay, I will review Mendes, Raaijmakers, Hollander, Mendes and Tsai's 2018 paper "Influence of resistance breeding in common bean on rhizosphere microbiome composition and function", which experimentally investigated the impact fungal resistance has on the composition and metabolic potential of the rhizobacterial community of the different common bean (*Phaseolus vulgaris*).

In this paper, the authors propose that the plant's ability to defend against pathogens is increased by microorganisms in the rhizosphere, and that the physiochemical properties of this community are specific to biotic plant defence when the plant carries fungal resistance. The authors justify this hypothesis with recent research which showed that plants with fungal resistance expel exudates that are responsive to biotic and abiotic stress (Lebeis *et al*., 2015). Further research showed that exudates, in part, drive the selection of microbes with advantageous physiochemical properties (Jones *et al*., 2009). Therein, the authors suggest that in response to biotic stressors, the plant produces exudates that recruit a microbial community able to deal with this stressor. The authors argue that this research is of value to plant breeders. Plant breeders are continuously searching for ways to improve plant yield, growth and tolerance to biotic and abiotic stressors (Dias, 2019). This is usually achieved by modifying the genetic traits of plants (Pérez-Jaramillo *et al*., 2015). The authors seek to offer a new strategy to increase tolerance to stressors in the form of breeding cultivars with fungal resistance. In the experiment, bean cultivars with different levels of fungal resistance were grown in Amazonian bacteria-rich soil (ADE) and bacteria-poor soil used in industrial agricultural settings (AGR). Using 16s rRNA, metagenome and computational analyses, the authors provide an in-depth analysis of the resulting rhizobacterial community structure, abundance and network. These showed that the most-resistant cultivars' rhizosphere microbiome was enriched in bacterial families which form a network of biosynthetic pathways reinforcing the first line of defence. The authors suggest that breeding for fungal resistance in plants inadvertently selects for plant traits that recruit bacteria capable of defending against more than just fungal pathogens. Due to vast differences in bacterial abundance and network structure between the ADE and AGR soils, the results also showed that the rhizobacterial microbiome is dependent on the microbial diversity available to the plant outside of the rhizosphere. Overall, the results of this paper suggest that fungal resistance in the common bean assists in the plants' ability to recruit a bacterial network that is focused around defending the plant, and that this network is enhanced by being grown in bacteria-rich soil.

Agricultural literature has two perspectives, sustainability and industrial practicality. Historically, the authors have undertaken research that sought to balance these two (de Araújo *et al*., 2013). A similar intention becomes clear in this paper. The authors explicitly state in their introduction that optimising methods to grow crops is an ongoing interest in agriculture, and that they seek to inform these methods in their paper. The methods directly contrast industrial soil with bacteria-rich soil. Additionally, the soil and growth conditions – including photoperiods and day/night temperatures - are meticulously described to allow reconstruction of growth and environmental conditions. In their conclusion, the authors comment that, based on their results, plant breeding programs should consider the molecular interactions between plants and their rhizosphere microbiome to optimize plant breeding programs. As the authors seek to inform industrial agriculture in their research, I will elaborate a little more on the development of plant breeding research that is specific to industrial agriculture. Industrial plant breeding research has focused on improving plant growth by breeding for desirable genetic traits for several decades (Bradford *et al*., 2005). Originally, this manipulation was undertaken to breed specific parts of the plants (Friedt *et al*., 2007), a popular example of this is the variety within the *Brassica* crop species (Ghosh *et al*., 1970). The development of more eloquent methods led to breeding for larger fruit (Lin *et al*., 2014; Zhang *et al*., 2006) and higher yield (Gur & Zamir, 2004). Recently, the attention of plant breeding programs has turned towards a more pressing issue. Since the early 2000's, an increase of pathogenic, biotic stressors in agro-ecosystems are causing the destruction of yield (McDonald & Stukenbrock, 2016), or even the complete eradication of various crops and species (Gamliel & Fletcher, 2008; Sosnowski *et al*., 2009). One of the identified causes is a microbial imbalance in farming soil and has been termed "legume yield depression syndrome" (Fuchs *et al*., 2014). To alleviate this issue, plant breeders have turned their attention to resistance breeding.

Resistance breeding requires fine-tuning to the individual crops' pathogens (Wille *et al*., 2018), such as breeding for *Rhizoctonia solani* resistance in chickpeas (Hemissi *et al*., 2013), breeding for *Verticillium dahliae* and *Macrophomina phaseolina* resistance in strawberries (Lazcano *et al*., 2021), or breeding for *Rhizobium leguminosarum* resistance in peas and sugarbeets (Bardin *et al*., 2004). Through this fine-tuning method, meta-analyses and reviews have found that resistance breeding correlates with changes in rhizobial microbiomes (Lehmann *et al*., 2012; Tamiru *et al*., 2015). This revelation led to Mendes *et al*.'s paper, researching whether these changes may be relating to symbiotic bacteria-plant defence mechanisms. Findings similar to Mendes *et al*.'s have been replicated in other legumes, for instance Liu *et al*. (2019) found that bacteria-rich soil has a similar effect on rhizobacterial symbiotic defence in soybeans. Whilst Mendes *et al*. focused on genotypic changes in the plant, and want to use these changes to protect the plant from predators, other research investigated different ways of reducing crop spoilage. Bainard *et al*. (2017) found that, rather than modifying the plant, modifying the environment can help prevent the accumulation of fungal pathogens to a level that is threatening to the crop. Here, Bainard *et al*. showed that more frequent rotation of crops reduces fungal diversity in crop fields, resulting in less root rot and therefore, less crop spoilage. Whilst simply reducing the potential for pathogen exposure intuitively seems a good idea, further research (Fuchs *et al*., 2014; Mitter *et al*., 2019) showed that a reduction in pathogen exposure also results in reduction in beneficial microbe exposure, and therefore causes a reduction in yield and fruit size. Considering these findings, Mendes *et al*. offer a viable solution to legume yield

depression syndrome by revealing the relationship between fungal resistance and plant defence traits. Additionally, they highlight the importance of microbially-rich soil to strengthen this relationship, which is a connection uniquely made by the authors.

Mendes *et al*. established a main objective within their research which was broken down into two research questions. They sought to investigate the rhizosphere microbiome of a fungal-resistant common bean. The two research questions of this main objective were to determine the effect soil type and host genotype have on the composition of the rhizobacterial biome, and whether resistance breeding results in the plant recruiting microbial groups that confer an advantage to plant defence. The researchers achieved the objective as they used the research questions to guide the experimental design, data processing, and functional and network analyses. The results and discussion then largely focus on discussing the two main research questions. The authors postulate a hypothesis, for which they specifically outline their reasoning in the introduction. A description of observations made in previous research allowed me, as the reader, to follow the authors' justification for the research objective. However, upon conducting further research myself I believe that the case was not presented well. The introduction outlines that previous research has found that the plant genotype assists in the selection of the rhizobial microbiome. It also discusses that, in plant breeding, genetic traits are exploited to increase tolerance to biotic stress, and that specific microbial families found in disease suppressive soils have assisted in plant defence. They finally outline that further research revealed that changes in plant defence pathways change the rhizobacterial microbiome. I would argue that the authors only scrape the surface of the actual issue, which is that crops are wasted due to a microbial imbalance caused by artificial disruption of the agro-ecosystem (McDonald & Stukenbrock, 2016), for instance by excessive spraying of antifungal agents (Brauer *et al.*, 2019). This has been found to prevent crops from building their own fungal resistance (Brugman *et al*., 2018) and is a severe issue in modern agriculture (Qiu *et al*., 2019; Zhang *et al*., 2021), especially because this is very costly to agriculturists (Qiu *et al*., 2019). Despite their failure to accurately present this, the authors actually address this issue as the bacteria-poor soil used in the experiment is soil that is commonly used in agriculture and farming.

The methods in the main text are very detailed which allows for the study to be repeated easily. In the sampling method, the original location of the seedlings and soil before transferring into the green house are described, and the pot sizes of each cultivar. Additionally, the exact procedures for measurements of the physiochemical properties are described (i.e. soil pH measured in water suspension) as well as the photoperiod, moisture level and day/night temperatures were outlined. In the experimental procedure, details are provided about the exact primer sequences, number of PCR cycles and the brands of each analytical kit. The statistical methods provide a GitHub link to allow downloading of the code used. In addition to the methods described in the paper, the supplementary data also outlines the exact cultivars, physiochemical parameters of the soil, and further detail on the workflow and results of the metagenome and 16s rRNA analysis. Originally, I thought this approach was exemplary. This was because the authors chose to have 3 replicates of each conditions, resulting in 24 total samples (3 replicates x 4 resistance levels x 2 soil types). However, in my own research I noticed that many studies seeking to explore similar topics (Bardin *et al*., 2004; Hemissi *et al*., 2013; Bainard *et al*., 2017; Liu *et al*., 2019; Lazcano *et al*.,

2021) took place over several years, between 1 year and 4 years. The authors, however, only cultivated the 24 samples until R1 stage a single time. This severely impact the replicability of the results. A long-term experiment to observe the same phenomena would have been more useful and applicable in agricultural methods, as often the same cultivar is used over several years (Bainard *et al*., 2017). Additionally, the experiment took place in a greenhouse with extremely controlled conditions, however bean cultivars specifically are usually cultivated in the field (Rosales *et al*., 2012), naturally exposing the cultivars to stressors that could be impacting the formation of the rhizobial microbiome. However, the fact that the experiment took place in the greenhouse does pose the advantage that these stressors are removed and the authors could evaluate the exact impact resistance and soil type have on the rhizobial microbiome. Perhaps it could have been useful to complete another set of the same replicates and grow these in their natural environment or on an agricultural field. This would allow for comparison between the same parameters when looking at the setup inside the greenhouse, but would also allow to understand the impact the environment has, such as additional abiotic or biotic stressors, and to explore whether the natural environment allows for a larger accumulation of plant defence traits.

In the evaluation, the researchers also compared the bacteria in the rhizosphere of the most and least-resistant cultivars to determine whether the difference in composition and metagenome function is related to resistance to *Fox*. They argue that the selective breeding for *Fox* resistance inadvertently also selected for plants traits that allow for better, cultivar-specific rhizobacterial recruitment. The researchers investigated rhizobacterial species richness using Shannon's PD and diversity using Faith's PD, which are commonly used in to explore rhizobacterial richness (Trivedi et al., 2020). This showed that the most-resistant cultivar had largely increased their bacterial community diversity in both ADE and AGR soil when compared to the original soil samples, and that the proportional increase for both soil types was of similar magnitude. However, the actual bacterial abundance was much higher in the rhizosphere of the *Fox*-resistant cultivar grown in ADE soil. When looking at the corresponding data provided in the paper (Fig 2., Mendes *et al*., 2018) it seems odd that the authors disregard the results from the two other cultivars that have a moderate resistance to *Fox*. The data shows that the bacterial abundance in these cultivars is reduced when compared to the most and least-resistant cultivar, though only in the AGR soil. This poses the question why the combination of moderate resistance to *Fox* with bacteria-poor soil produces this result. One possibility may be that the moderate resistance prevents the plant from producing exudates that recruit microbes into its rhizobial microbiome. The other possibility is that the plant produces exudates to recruit microbes, but these microbes are unavailable in the AGR soil. To test this, one could expose the cultivars to various soils rich in microbes with different functional profiles. Here, a principal component analysis could help identify the functional profiles, and a low Shannon's PD would confirm that the species richness is restricted to microbes with the specific functional profile.

Notably, the analysis of microbial abundance and community structure was very well done. The additional network complexity analysis allowed the authors to understand the way the microbes interact and rely on each other in the cultivars. The authors identified a highly complex, modular network in the most-resistant cultivar's rhizosphere, and deduced that this indicates that the resistance to the fungal pathogen allowed the bacteria in the rhizosphere to produce a network that can react quickly to stressors. Arguably, the authors

did not test this and did not elaborate on this interpretation any further. Perhaps exposing the different cultivars to abiotic and biotic stressors and measuring their response time by measuring the exudates and time it takes to recover from the stressors could provide further insight into this interpretation. Another issue around the interpretation of the data is that the authors analysed the most-resistant cultivar in great detail by exploring the network structure and identifying key players in this network structure. Yet, not the same is done for the other cultivars. Thus, whilst the results may accurately reflect the rhizobial microbiome of the most resistant cultivar, it should not be concluded that this is unique to the most-resistant cultivar. Additionally, in the discussion the authors are not addressing the relevance to current issues in plant breeding and often draw comparisons between theirs and other papers (e.g. to Yao and Wu, 2010), despite their paper being short-term experiments whilst the compared papers are long-term experiments. This also makes me question whether these comparisons are appropriate as both, the experimental set-up, and the longevity of the experiment would likely impact the results.

Overall, Mendes *et al*. provide a well-thought-through experimental design that achieves what the authors seek out to investigate. The methods used to measure and interpret the data are used in other papers that investigate similar topics in plant breeding and are reported with a lot of detail to allow for replication of the experiment. Whilst it was generally interesting to read and the authors case seemed well-presented, further research into the topic shows some issues with the introduction, and the interpretation and discussion of the results. The introduction is lacking an explanation of the significance of and the context surrounding the authors' research, and there are some discrepancies in the interpretation of the data. Additionally, some of the conclusions drawn and comparisons made to other papers may not be appropriate as the authors compare the results of their short-term experiment to the results of other long-term experiments without highlighting this significant difference. To someone less familiar with plant breeding, this paper provides an interesting read and a great first insight into plant breeding. However, I suspect that for someone more familiar with plant breeding, it would become apparent quickly that this paper has significant flaws that cannot be overlooked.

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