Microscopic Benefactors - More Than Nitrogen: Observations from field samples and trials showing increases in grain size, resistance to pathogens.

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Abstract

Soil quality indicators have often been limited to the chemical and physical components of soil, due to difficulties surrounding the analysis of microbiological diversity, which include the high diversity found in soil, as well as challenges in lab-based culture mechanisms (Bünemann et al., 2018). Novel techniques to allow for isolation of soil microorganisms were developed by Brooks, (2017) to detect rarer and slower growing microorganisms in the soil. Novel metabolites were characterised by Gurusinghe et al., (2019) from organisms isolated using this method, showing that the maintenance of overall diversity of the soil provides great potential in the realms of drug discovery (Piddock, 2015) and in the isolation of compounds that may provide plant growth promoting effects (Çakmakçi et al., 2006; Kumar et al., 2016), bioremediation possibilities (Ruiu, 2013; Luo et al., 2014), or increasing the general nutrient profile of the soil. The work detailed in this article is an overview of some promising results in this sphere – the increase in grain size through bacterial inoculation during the growth of wheat plants at a test site in Lake Cargelligo, NSW, as well as the isolation of a bacterial species from a wheat crop in Wagga Wagga, NSW producing an exudate which experimentally acts as an oomycete filament forming inhibitor. The identification of bacterial diversity can also highlight and remediate other potential issues, such as the influence of an Alternaria species on the root development of a wheat crop noted (also in Lake Cargelligo, NSW). Whilst these are all promising results, they indicate a pathway for further research and the synthesis of microbiology and agronomy to enhance crop performance.

Keywords

Allelopathic compounds, Bacteria, Nitrogen, Pathogens, Growth-Promotion, Metabolites.

Introduction

“I cannot conceive of the time when knowledge of soils will be complete” (Smith, 1928). As Smith predicted, our knowledge of soils is still far from complete. Completing the complex and challenging study of the interactions between soil, its inhabitants and the plants that grow in its matrix is still a faroff goal. From a microbiological perspective, the communities of bacteria, fungi and oomycetes live in a constant state of conflict and natural selection, mediated by soil nutrients (Bertin, Yang and Weston, 2003; Netthisinghe et al., 2013), the presence of chemical compounds such as pesticides (Lupwayi et al., 2009), heavy metals (Zhuang et al., 2007), and the presence of crop exudates within the soil (Lupwayi et al., 2009). Microbiota in the soil serve as pathogens and benefactors (van Elsas and Boersma, 2011), with noted connections between the community of microbiota and plant-plant interactions (Bonkowski and Roy, 2005), pathogenic invasion of soils (Wieland, Neumann and Backhaus, 2001; Sabuquillo, Cal and Melgarejo, 2006), and the tolerance of host plants to abiotic stress whether that be environmental (Yang, Kloepper and Ryu, 2009; Cheema, Farooq and Wahid, 2012) or chemical (Dell’Amico, Cavalca and Andreoni, 2008; Ruiu, 2013). Completing this picture is most important with the need for innovation in food security.

Methods

Bacterial isolation

Soil samples were obtained from fields (GI, S) in Lake Cargelligo (LC) and Wagga Wagga (U - WW): A notably well-performing oat crop (GIO - LC), a poorly performing wheat crop (SW - LC) and two wheat crops (GIW - LC and UW - WW) of no particular notable characteristics. Each of these was cultured using the water suspension and separated streak methods outlined by Brooks, (2017). The media used for these techniques was 1:10 diluted nutrient broth using the agar-replacing gelling agents of gellan and gelatin.

Bacterial Inoculation

After two dominant species were identified in the cultured microbial community from the GIO-LC sample using the aforementioned bacterial isolation procedure, these bacteria were then isolated further and purified before being grown in nutrient broth. Cultures from other sites were performed, but these were identified as of interest due to the well performing oat crop. After the broth had an equivalent turbidity of 1 MacFarland standard (3x10^8 equivalent cell density), samples of the inoculated broth were transferred into 3ml sterile syringes. Four sets of syringes were prepared – Bacteria A (a non-motile bacillus - 4), Bacteria B (a filamentous bacteria - 2), BacMix (a combination of Bacteria A and B - 3) and a placebo containing sterile nutrient broth (1). These syringes were transported to the wheat field testing site in Lake Cargelligo (GIW-LC) and inoculated directly into the rhizosphere zone of the test plants. A selection of 4 plants, located in the central area of the cropping field was chosen, and inoculation was performed 8 weeks after planting.

Identification of microbiological organisms

The oomycete and fungal species noted in the SW-LC and UW-WW were identified morphologically using a light microscope after their isolation. Characterization of the effect of all species noted in this paper was done experimentally, inoculating 4 replicates of 3 grass seeds planted in sterile soil with cultures of each isolated microorganism, and measuring height and root development of these seedlings in the early stages of their development.

Assessment of wheat grain development

Post harvest (GIW-LC), the inoculated plants were removed from the ground with soil and roots attached. The grains were hulled, counted and weighed, taking an average weight of the grains collected, as well as an average of number of grains present on the plant per head. Each plant was measured for height and this was also averaged. The soil from the roots was assessed once more using the bacterial inoculation method outlined above.

Results

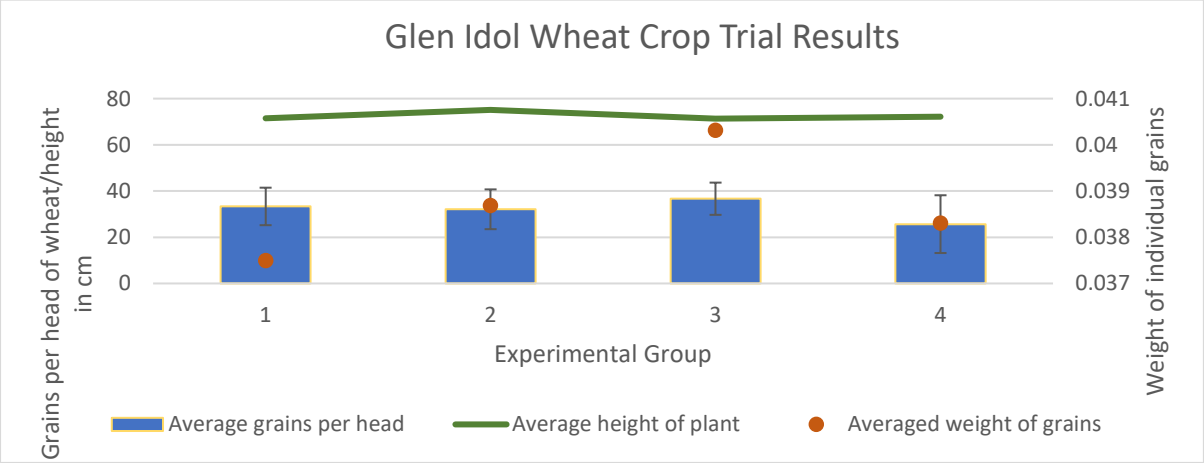

Figure 1: Summarised results from bacterial inoculation trial, assessing averaged weight of grains, height of plant and grains per head.

The most significant result from this study can be most clearly seen in Table 1 and Figure 1, where the inoculated bacteria in combination showed an increase in grain weight. When assessing presence of retained microorganisms, the distinctive Bacteria A was present as expected, though Bacteria B did not appear to successfully re-culture from the second round of root assessment.

Table 1: Summarised results from bacterial inoculation trial, assessing averaged weight of grains, height of plant and grains per head.

Treatment	Total Seeds	Weight of Seeds	Av. Grains/Head	Std. Dev. Grains/Head
1: Placebo	200	7.5	33.3	8.1
2: Bacteria B	321	12.42	32.1	8.6
3: Bacmix	220	8.87	36.7	6.9
4: Bacteria A	154	5.9	25.7	12.5

The wheat crops that were not part of the inoculation trial showed the presence of Oomycetes in the UW-WW sample and Alternaria spores in the SW-LC sample. The Oomycetes grew more effectively in gelatin based media, though this resulted in breakdown of the gelling matrix. A bacterial species was found within this same sample to be exuding a brown pigmented compound into the media, and oomycete filament formation stopped at the borders of the presence of this diffused compound. The plants in this field reported no signs of root rot or other negative implications from the high presence of oomycetes in the soil, however the experimental characterisation test grasses inoculated with this microorganism did not germinate. In the SW-LC sample however, almost no bacterial diversity was noted, and root formation of the crop was extremely poor. The assessed plants showed presence of only primary roots, with no significant secondary root structures appearing, and this effect was noted in the experimental characterisation plants as well. These plants reached maturity, however this early stunting of growth impacted on the final crop yield being less than expected.

Conclusion

The presence of high bacterial diversity within the soil has been found previously to be correlated with positive cropping outcomes such as disease resistance (Selim, Gomaa and Essa, 2017) and higher crop yields (Wu et al., 2008), and this was noted within the well performing GIO-LC oat crop sample. The two dominant species isolated were isolated and inoculated into a wheat crop, where the plants inoculated showed an increase in grain size. This correlated increase may be from the introduction of plant growth promoting factors, or the conversion of nitrogen within the soil. The protective influence of the microbial diversity in the soil was evident in the comparison of the health and root development of the UW-WW sample and the SW-LC crops, where in the former, which had high bacterial diversity, the high number of oomycetes isolated did not influence the crop health, though the genus Pythium is known to cause root rot (and experimentally impacted the germination of grass seeds exposed to the pure culture of this isolated microorganism). In the SW-LC crop, very little microbial diversity was noted, and this correlated with the lack of development of root structures. The overall picture of how microbial communities can be maintained, developed and how interactions can be quantified show strong potential for exciting developments to improve crop yields.

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