BELSPO Final Activity Report Post-Doc Research David Kothamasi

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Title: Effect of external gamma radiation and uranium on mycorrhiza in presence or absence of higher plants

Host Institute: Belgian Nuclear Research Centre (SCK•CEN)

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Purpose of proposed Research Plan

Symbioses between plants and soil microbes are almost universally present in natural plant communities. Mycorrhizal symbiotic associations with fungi are formed by around 90% of vascular plant species. As mycorrhizal associations are important in providing nutrient uptake, in offering tolerance to stress and in maintaining plant species diversity, they are considered key drivers of ecosystem functioning. Environmental stresses that may have an effect on mycorrhizas and mycorrhizal communities can consequently impact ecosystem functioning. Arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) are the most prevalent mycorrhizal associations and are important functional groups in the rhizosphere. Any environmental stress that can inhibit or affect their growth and functioning has ecological implications. For instance, exposure to UV radiation was found to reduce AMF root colonization by up to 20%. The numbers of arbuscules and vesicles were also reduced. While several studies have shown the effects of ionizing radiation on soil fungi, few studies have investigated the effects of ionizing radiation on AMF or EMF. We therefore proposed to study the effect of external gamma radiation and uranium on the functioning of EMF and AMFs.

Objectives

Three objectives were envisaged:

1) Analyses of the effects of ionizing radiation on ecto- and endo mycorrhizas
2) Analyses of functional traits of mycorrhizas exposed to ionizing radiation

3) Analyses of the effects of uranium on mycorrhizas and investigations into uptake and translocation of uranium by the mycorrhiza into the host

Following the execution of the work, the initially proposed activities to reach the objectives have been changed. The following experiments were carried out:

1) Experiment 1 - Effects of gamma radiation on the growth and metabolism of five strains of ectomycorrhizal fungi
2) Experiment 2 - Effects of gamma radiation on the functional traits of arbuscular mycorrhizal fungus *Rhizophagus irregularis*
3) Experiment 3 - Effects of gamma radiation on arbuscular mycorrhizal fungal community assembly inside host roots

Achievements:

Experiment 1 - Effects of gamma radiation on the growth and metabolism of five strains of ectomycorrhizal fungi

This experiment proposed to study the effect of gamma radiation on growth and metabolism of ectomycorrhizal fungi. Ectomycorrhizal cultures of *Suillus luteus* strains PD8, LMD10 and LMSL8; *S. bovinus* M7 and *Rhizopogon luteolus* HH4 were exposed to four dose rates (0, 55, 109 and 404 mGy h^{-1}) of gamma radiation from a ^{137}Cs source for 137 hours resulting in total doses of 0, 7.5, 15 and 55 Gy. Each treatment was replicated five times. Effects of radiation on the ectomycorrhizal fungi were analyzed by studying effects on biomass, radial growth, melanin production and enzyme activity.

The five strains of ectomycorrhizal fungi used in this study varied in tolerance to ionizing radiation. While mycelial biomass was lower in cultures of *S. luteus* strains PD8 and LMSL8 exposed to 404 mGy h^{-1} compared to the control, the fungal biomass of *S. luteus* LMD10 was not affected by radiation. With the exception of strain LMD10, radial growth of cultures exposed to 404 mGy h^{-1} of all strains was lower compared to the unexposed cultures. The fungi responded metabolically to radiation through secretion of radioprotective pigments like melanin and increased capacities of reactive oxygen species (ROS) scavenging enzymes.

Previous studies on fungal resistance to ionizing radiation have shown that tolerance in fungi can operate at two levels: non-enzymatic and enzymatic. The non-enzymatic response is mostly through melanin production. The enzymatic response is through production of enzymes such as catalase (CAT) and superoxide dismutase (SOD). Melanin can absorb radiation and the enzymes CAT and SOD will scavenge reactive oxygen species produced in the cell.

Unexposed cultures of *S. bovinus* M7 and *R. luteulus* HH4 produced significantly higher amounts of melanin compared to the *S. luteus* strains. In these strains (M7 and HH4) melanin production did not differ in the unexposed control and irradiated cultures. The differences in melanin production appeared to have a bearing on the enzymatic component of fungal

antioxidation mechanisms. *S. luteus* strains that produced lesser melanin compared to *R. luteolus* HH4 responded to radiation with higher SOD and CAT enzymatic capacities. However, CAT and SOD capacities of *S. bovinus* M7 which produced high amounts of melanin did not differ from enzyme capacities of the three strains of *S. luteus*. Our results nevertheless indicate that melanin and ROS scavenging enzymes such as CAT and SOD are the first line of defense against the damaging effects of ionizing radiation.

In this study, *S. luteus* strains PD8, LMD10 and LMSL8 showed a dose dependent increase in melanin production when exposed to ionizing radiation. In these strains melanin production was highest in cultures subjected to radiation dose rates of 404 mGy h^{-1}. This finding is consistent with previous studies which have found increased melanin production in fungi exposed to ionizing radiation.

Experiment 2 - Effects of gamma radiation on the functional traits of arbuscular mycorrhizal fungus *Rhizophagus irregularis*

This experiment investigated the effects of ionizing radiation on the functional traits of AMF. Approximately three month old root organ culture of *Rhizophagus irregularis* MUCL 41833 grown on *Agrobacterium* transformed carrot roots in modified Strullu Romand (MSR) medium was used for the experiment. Five square blocks, each measuring one inch were cut from the above culture and transferred to fresh Petri-plates. The Petri-plates were exposed in the dark at 21°C to four dose rates (0, 55, 109 and 404 mGy h^{-1}) of gamma radiation from a ^{137}Cs source for 280 hours resulting in total doses of 0, 15, 30 and 113 Gy. After 280 hours, AMF spores were extracted and transferred to 50 mL Falcon tubes containing 80 g autoclaved Rynz sand. Each tube received 100 spores. A single seedling (five-day old) of *Plantago lanceolata*, germinated from surface sterilised seeds, was placed in each tube. Falcon tubes that received filter sterilised extracts of MSR medium served as the non-mycorrhizal control. Plants were grown in a temperature controlled Green house at ~30°C and day-night cycle of 16–8 hours. The plants were maintained at 10–15% moisture and received fertilization once every 15 days by addition of 800 µL Hoagland solution. Pots were randomised weekly. Each treatment was replicated five times. After 14 weeks of growth, all the plants were harvested. Plant tissue was separated into roots and shoots. A second sub sample of the roots was stored in 50% ethanol for estimation of AMF colonization. Shoots and the remaining portions of the roots were dried at 70°C for five days and dry weights were measured. *P. lanceolata* seedlings inoculated with non-irradiated AMF spores and with spores irradiated with up to 30 Gy gamma radiation had similar levels of root colonization. Spores irradiated with 113 Gy gamma radiation failed to colonize roots of *P. lanceolata*. P content in the tissue of plants inoculated with non-irradiated spores and in plants inoculated with spores irradiated with up to 30 Gy gamma radiation was higher than in the tissues of non-mycorrhizal plants and plants inoculated with spores irradiated with 113 Gy gamma radiation. These results suggested that AMF *R. irregularis* spores are tolerant to high doses of ionizing radiation and exposed spores can colonize plant roots.

Experiment 3 - Effects of gamma radiation on arbuscular mycorrhizal fungal community assembly inside host roots

This experiment analyzed the effects of gamma radiation on AMF community assembly. Rhizosphere soil of herbaceous plant communities on the banks of the River Molse Nete in Mol (Belgium) was employed as a source of wild AMF inoculum. The rhizosphere soil was exposed for 280 hours to four dose rates of gamma radiation (0, 55, 109 and 404 mGy h^{-1}) from a ^{137}Cs source. Approximately 30 g radiation exposed rhizosphere soil was added to pots containing 900 g autoclaved Rynz sand. After addition of the rhizosphere soil, pots were overlain with autoclaved Rynz sand such that the final weight of each pot was 1000 g. Non-mycorrhizal controls received 30 g autoclaved radiation exposed rhizosphere soil. Each pot received five seedlings (2-3 cm) of *P. lanceolata* germinated from surface sterilised seeds. Plants were maintained at 10–15% moisture at 30°C with a day-night cycle of 16–8 hours in a Green house. Non-mycorrhizal pots received 10 mL microbial wash prepared by filtering a suspension (1:6) of rhizosphere soil in sterile deionised water through a series of sterile filters. The final filter had a pore size of 11 µm. Five replicates were maintained for each treatment. Pots were randomised weekly. Each pot received fertilization with 10 mL Hoagland solution once every 15 days. All the pots were harvested after 21 weeks of growth. Plant tissue was separated into roots and shoots. A sub sample of the roots was flash frozen in liquid nitrogen and stored at -80°C. These samples will be used for analysis of the AMF 18S small subunit (SSU) ribosomal DNA. A second sub sample of the roots was stored in 50% ethanol for estimation of AMF colonization. Shoots and the remaining portions of the roots were dried at 70°C for five days and dry weights were measured. The plant and AMF tissues from this experiment are still being analyzed for nutrient content, AMF colonization and AMF 18S SSU ribosomal DNA.

Highlights

Our results have shown that tolerance in EMF to ionizing gamma radiation is achieved through active metabolic processes that include protection through pigment production and enzyme activity. Our study on AMF community assemblage in roots following exposure to ionizing radiation will provide important insights into ecosystem dynamics of soil microorganisms when subject to radiation stress.

Publications

Kothamasi, D., Wannijn, J., Van Hees, M., Nauts, R., van Gompel, A., Vanhoudt, N., Cranenbrouck, S., Declerck, S., Vandenhove, H. The arbuscular mycorrhizal fungus Rhizophagus irregularis MUCL 41833 can colonize plants and improve their P uptake after exposure to ionizing gamma radiation. *Manuscript submitted*

Ectomycorrhizal fungi tolerate ionizing radiation through pigment production and release of enzymes that scavenge reactive oxygen species. *Manuscript in preparation*

Problems/issues (if any)

Because standardising for the above experiments took more time than originally envisaged, we could not include experiments on uranium.

Future work

Work proposed under Experiment 3 could not be completed within the 18 month tenure period of the BELSPO fellowship awarded. Following work will be undertaken for Experiment 3:

1. Plant and AMF tissues need to be analyzed for AMF colonization, gene expression and AMF community assembly
2. Data treatment and statistical analysis
3. Manuscript writing for publication of the results

In addition, results from Experiment 1 with ectomycorrhizal strains have thrown up questions on how radiation exposure would impact daughter generations of the exposed strains. Is radio-tolerance of the strains used in this study a phenotypic response or is it a genetic adaptation? In future works these questions are proposed to be addressed.