An investigation on callose presence in rhizoids of *Ceratopteris richardii* gametophytes grown in increasing concentrations of silver nanoparticles

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Introduction

Nanoscience is an emerging area of research that offers promising new technology and developments to the medical and environmental fields. The ability to transform metals such as silver and gold into their nanosize causes changes in the metal's chemical, physical and optical properties.[1] Due to their antimicrobial properties, silver nanoparticles (AgNPs) are used in a variety of applications such as: medical treatment for bacterial infections, burns, and wounds; household appliances like vacuums and washers; and household disinfectants.[2] The extensive use of silver nanoparticles in commercial products causes an increased risk of release into the environment which leads to unknown environmental concerns.[2] A prior study by Yanik and Vardar showed that 10 nm-sized AgNPs had a negative effect on the roots of wheat plants.[3] Callose is a beta-glucan polymer located in the cell walls of several higher plants.[4] Deposition of callose occurs rapidly and in large amounts in response to abiotic and mechanical stresses, wounding, and pathogen attack.[4, 5] In this study, we used the model fern *Ceratopteris richardii* (C-fern) to investigate the effects of AgNPs on rhizoids; threadlike structures which function to anchor the gametophyte to the substrate.[6]

Research Question

Does the exposure of silver nanoparticles in the growing media induce callose deposition in the rhizoid cell walls of *Ceratopteris richardii* gametophytes?

Hypothesis

If the concentration of silver nanoparticles is increased, then the amount of callose present in the rhizoids of *Ceratopteris richardii* gametophytes will increase.

Methodology

- Sowing: Pre-sterilized spores were soaked in autoclaved double distilled water overnight. Using the laminar flow hood, one drop of spore solution and two drops water were added to control (0ppm) and treatment (20ppm, 40ppm, 60ppm, 80ppm,100ppm) plates.
- Incubation: Spores were incubated at 27.83°C under constant light and 82% humidity.
- Histochemical Staining: Germinated spores from varying stages (4 days and 9 days from sowing) were placed in 1% aniline blue in 0.067 M Na2HPO4 (pH 8.5) solution to test for callose. Controls were made using the respective buffer without the presence of aniline blue. Both treatment and control specimens incubated for 30 minutes to an hour in darkness. Stained material was viewed using a Leica DM5000 B compound microscope using UV fluorescence. Digital images were collected using a Q-Imaging Retiga 2000R digital camera.

Results

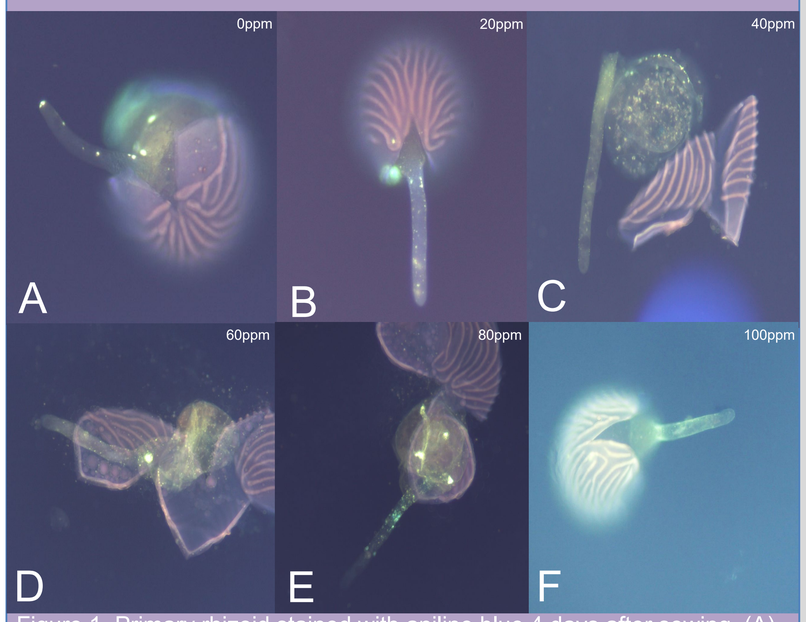

Figure 1. Primary rhizoid stained with aniline blue 4 days after sowing. (A) Small amount of callose deposition in cell wall in 0ppm. (B-F) Increased amounts of callose deposition in treatments compared to the control. (A-F, magnification 20x)

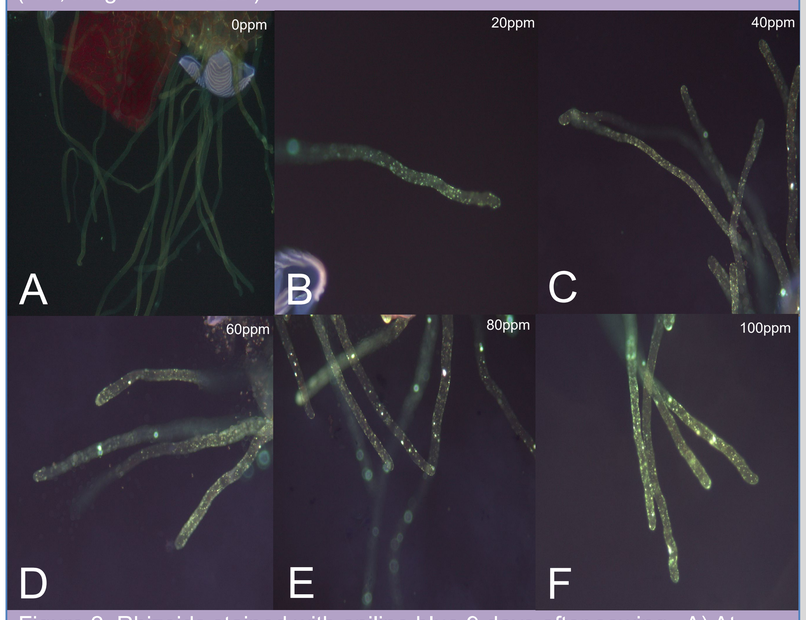

Figure 2. Rhizoids stained with aniline blue 9 days after sowing. A) At 0ppm rhizoid cell walls shows little to no callose deposition. (B-F) Notable amounts of callose in cell walls of the treatments. (A, magnification 10x; B-F, magnification 20x)

Conclusion

- We found that as the concentration of AgNPs increased, the presence of callose deposition in the cell walls of the rhizoids also increased.
- Silver nanoparticle presence in the growing media is an abiotic stress that induced callose deposition in the cell walls of rhizoids.
- Our findings are comparable to the 2019 Yanik and Vardar study where they found an increase in callose deposition in wheat roots exposed to varying concentrations of AgNPs.[3]
- It was observed that removing the gametophytes from the agar plates became easier as concentration increased. This indicates the possibility that the primary function of rhizoids to anchor the gametophyte to the substrate was hindered.
- For further research we would like to look at the effects of AgNPs as it relates to callose deposition throughout the gametophyte phase and the sporophyte phase where roots are produced.

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