Nanoparticles: Lignification of Wheat with Pseudomonas chlororaphis O6 (PcO6)

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I. Introduction
- Wheat production decreases due to many stresses. Drought stress and lodging (Fig. 1), caused by storms and overgrowth, both reduce yield [1]. My previous work showed induction of drought tolerance in wheat by root colonization of PcO6; drought tolerance was maintained when wheat seedlings were grown with CuO nanoparticles (NPs) (Doxey, Biology Undergraduate Research Symposium Dec 2016). The shoots of wheat grown with PcO6 and CuO NPs were more rigid than shoots grown without either treatment. This work examines whether the rigidity in the shoots was due to lignification as well as a higher water content.
- Toluidine Blue O (TBO) and phloroglucinol stain lignin, blue and red respectively [3,4,5]. Consequently, I used these stains to determine whether the lignin content in wheat was increased by growth of PcO6- colonized plants with CuO NPs.
- Increased lignification of shoots could reduce lodging of wheat and decrease pressure under field conditions.

II. Methods
- Surface-sterilized wheat seeds (12) were inoculated with PcO6, planted into sterile sand (300 g) wetted with sterile water (50 ml).
- Sand amended with 0, 10, and 300 mg Cu/kg from CuO NPs.
- Wheat seedlings grown for 7 d (Fig. 2).
- Growing of wheat plants (7 d) with root colonization by PcO6 and amendments with CuO NPs. Images are typical of 3 replicates per treatment.

III. Results

Question: What happens when wheat plants, grown with a beneficial microbe, PcO6, are exposed to CuO NPs?

Findings:
- Growth
  - Little variation in shoot growth between treatments (Fig. 3).
  - Plants with PcO6 colonization retained NP inhibition of root growth (Fig. 3).

Increased lignification in specific cells
- Control leaves show lignification in vascular bundles (Fig. 4). With addition of Cu from CuO NPs, lignification of sclerenchyma cells increased.

Figure 1: Lodging of wheat [1].

Figure 2: Growth of wheat plants (7 d) with root colonization by PcO6 and amendments with CuO NPs. Images are typical of 3 replicates per treatment.

Figure 3: Influence of NPs on plant root and shoot growth.

Figure 4: Lignification in transverse leaf sections. (a) Control treatment grown only with PcO6. (b) Treatment with 10 mg Cu/kg of CuO NPs grown with PcO6. (c) Treatment with 300 mg Cu/kg of CuO NPs grown with PcO6. Circles show bundles of sclerenchyma cells stained red with phloroglucinol in the presence of Cu.

IV. Conclusions
- Cu NPs increased lignification in sclerenchyma cells of the leaves of wheat colonized by PcO6.
- Sclerenchyma provides strength and support to the plant [6]. This induced lignification may explain increased stiffness of wheat shoots when grown with CuO NPs.

V. Future Work
- Improve consistency for tissue preparation for staining with repetition of CuO NP doses, with and without PcO6 colonization.
- Grow plants with Cu ions to look for sclerenchyma lignification.
- Examine differences in lignin composition by FTIR spectroscopy.
- Quantify lignification by acetyl bromide degradation and assessment.

VI. References

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