

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



Figure 1: Lodging of wheat [1].

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).

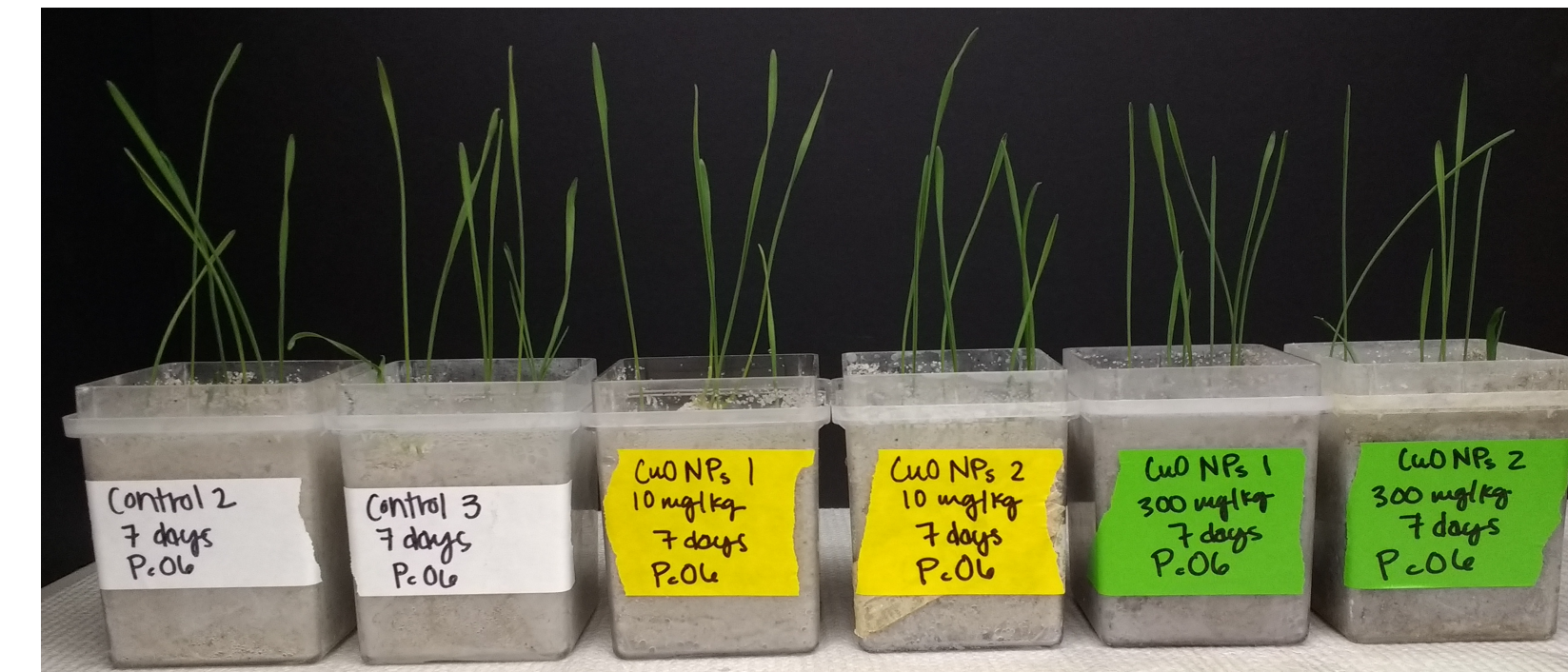


Figure 2: Growth of wheat plants (7 d) with root colonization by *PcO6* and amendments with CuO NPs. Images are typical of 3 replicates per treatment.

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.

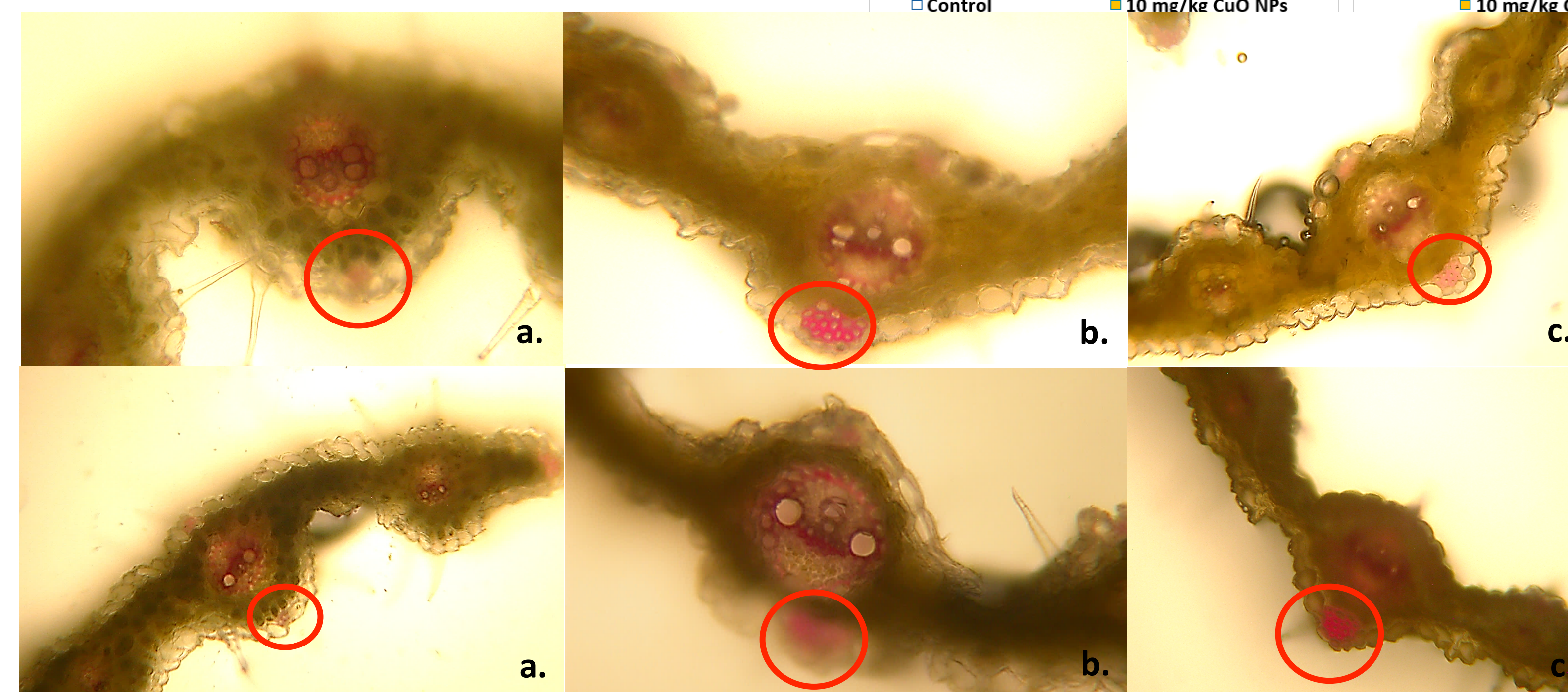


Figure 4: Lignification in transverse leaf sections. (a) Control treatment grown only with *PcO6*. (b) Treatment with 10 mg Cu/kg of CuO NPs grown with *PcO6*. (c) Treatment with 300 mg Cu/kg of CuO NPs grown with *PcO6*. Circles show bundles of sclerenchyma cells stained red with phloroglucinol in the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

IV. Conclusions

- CuO 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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