Nanoparticles: Lignification of Wheat with *Pseudomonas chlororaphis* O6 (*Pc*O6)

Stephanie Doxey
Biology Department Utah State University
Astrid Jacobson (PSC) and Anne Anderson (BIOL)

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 *Pc*O6; 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 *Pc*O6 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 *Pc*O6- 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 *Pc*O6, 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).

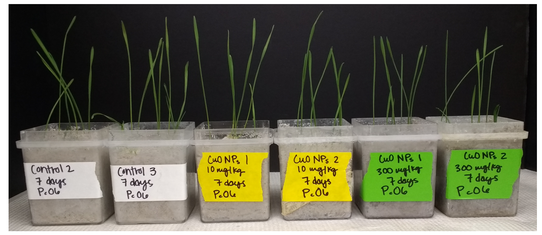


Figure 2: Growth of wheat plants (7 d) with root colonization by *Pc*O6 and amendments with CuO NPs. Images are typical of 3 replicates per treatment.

III. Results

Question: What happens when wheat plants, grown with a beneficial microbe, *Pc*O6, are exposed to CuO NPs?

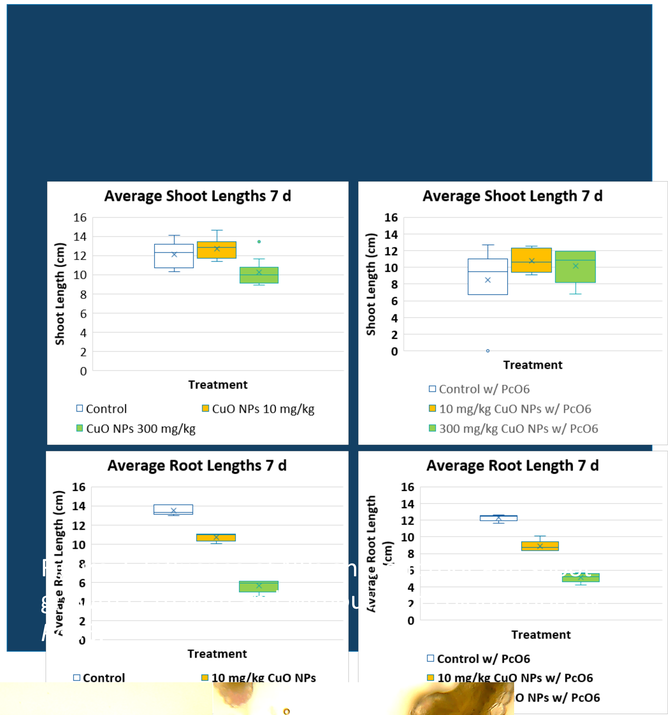
Findings:

Growth

- Little variation in shoot growth between treatments (Fig. 3).
- Plants with *Pc*O6 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.



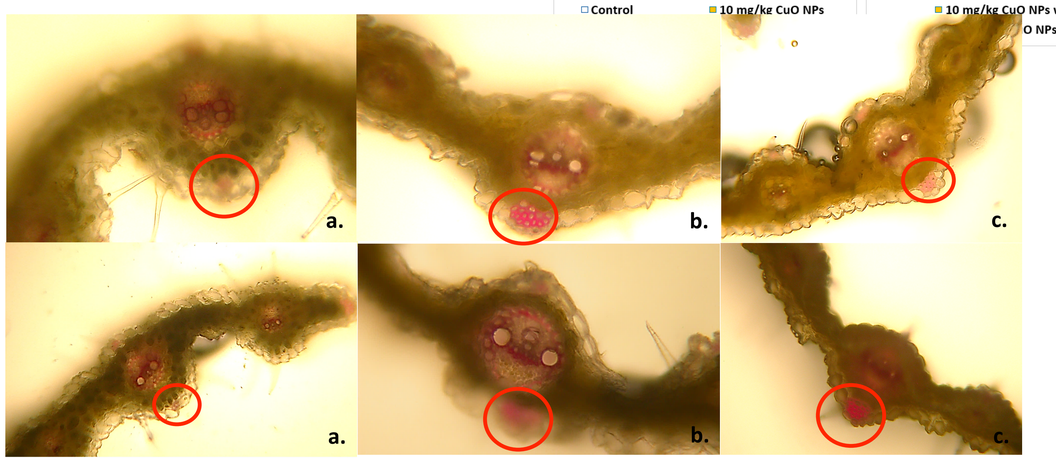


Figure 4: Lignification in transverse leaf sections. (a) Control treatment grown only with *Pc*O6. (b) Treatment with 10 mg Cu/kg of CuO NPs grown with *Pc*O6. (c) Treatment with 300 mg Cu/kg of CuO NPs grown with *Pc*O6. Circles show bundles of sclerenchyma cells stained red with phloroglucinol in the presence of Cu.

IV. Conclusions

- CuO NPs increased lignification in sclerenchyma cells of the leaves of wheat colonized by *Pc*O6.
- 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 *Pc*O6 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

[1] Marx, Gary. Three Best Practices to Minimize Lodged Crops. Decisive Farming, 31, Aug. 2015, www.decisivefarming.com. Accessed 22 Feb. 2017.

[2] *Lignoworks*. Western University Institute for Chemicals and Fuels From Alternative Resources, www.icfar.ca/lignoworks. Accessed 21 Feb. 2017.

[3] O'Brien, T.P., Feder, N., McCully, M.E. (1964) Polychromatic staining of plant cells walls by toluidine blue O. Protoplasma 59(2):368-373. doi:10.1007/BF01248568.

[4] Mitra, P. P., Loqué, D. (2014) Histochemical staining of *Arabidopsis thaliana* secondary cell wall elements. J. Vis. Exp. (87), e51381, doi:10.3791/51381

[5] Speer, E.O. (1987) A method of retaining phloroglucinol proof of lignin. Stain Technol. 62(4):279-280.

[6] Bouzizi, H., Jouili, H., Geitmann, A., El Ferjani E. (2011) Cell wall accumulation of Cu ions and modulation of lignifying enzymes in primary leaves of bean seedlings exposed to excess copper. Biol Trace Elem Res. 139(1):97-107.

This work was supported by an URCO Grant Spring 2017.

UtahStateUniversity
DEPARTMENT OF BIOLOGY

Stephanie Doxey
Biology Department
steph.wallentine1@aggiemail.usu.edu