IOCAG
2025
Conference

# The 3rd International Online Conference on Agriculture



22-24 October 2025 | Online

## Enhancing Salinity Resistance in Zea mays through Biopriming with Pullulan from Aureobasidium pullulans and Chlorella vulgaris

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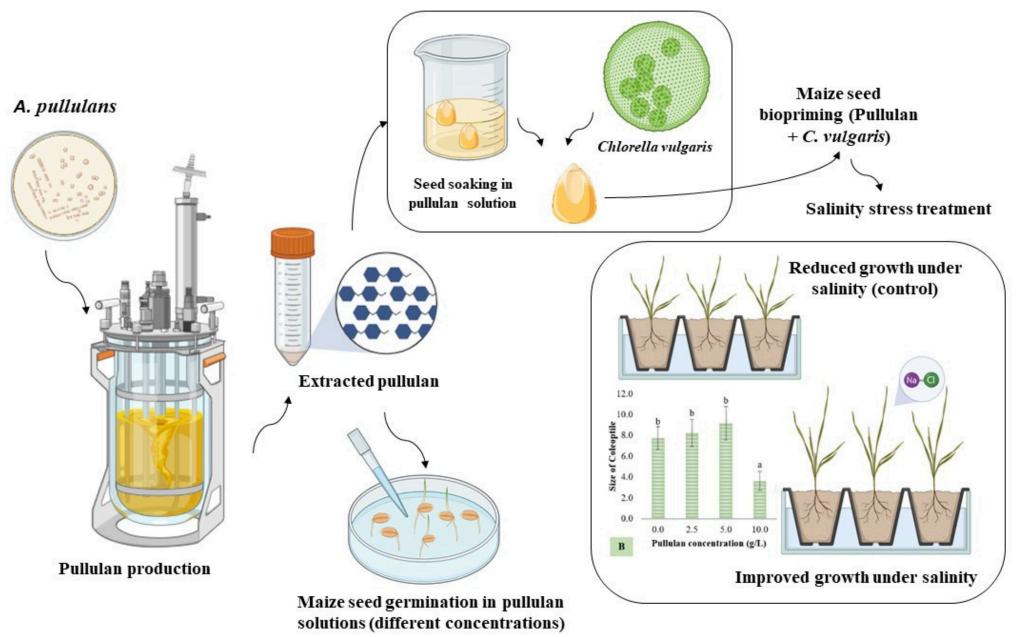
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#### Introduction & AIM

Soil salinity severely limits maize (Zea mays) growth by affecting germination and nutrient uptake. Biostimulants like pullulan, a biodegradable polysaccharide from Aureobasidium pullulans, and the microalga Chlorella vulgaris, rich in phytohormones and antioxidants, can improve stress tolerance. This study aimed to assess their synergistic effect on maize germination and early growth under saline conditions.

#### Method

Pullulan was produced by Aureobasidium pullulans ATCC 42023 in a 5 L stirred-tank bioreactor using glucose (60–100 g/L) as a carbon source. The polymer was recovered by ethanol precipitation and characterized by FTIR, confirming its  $\alpha$ -(1 $\rightarrow$ 6) and  $\alpha$ -(1 $\rightarrow$ 4) glycosidic linkages. Maize seeds were primed with pullulan solutions (0, 2.5, 5.0, and 10.0 g/L) and *Chlorella vulgaris* biomass (3.4–116.5 mg) for 24 h, then sown under 300 mM NaCl salinity stress.



A Central Composite Design (CCD) was applied to assess the combined effect of pullulan concentration and microalgae loading on plant height. Physiological parameters — chlorophyll content, malondialdehyde (MDA), and root activity (TTC assay) — were analyzed to evaluate oxidative stress and metabolic performance.

#### Results and Discussion

Pullulan production increased with higher glucose concentration, reaching the best yield at 100 g/L, where sugar consumption and biomass accumulation were highest (Figure 1).

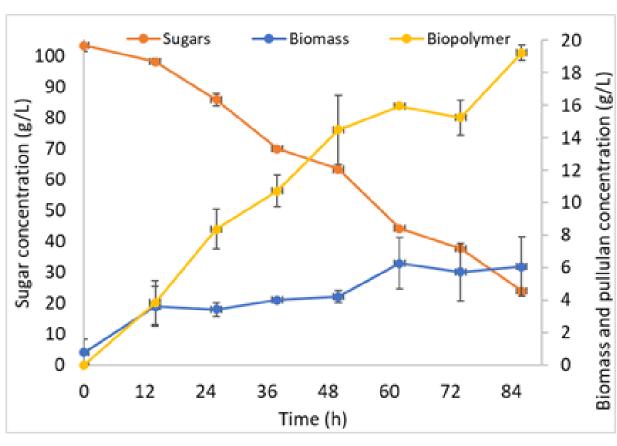
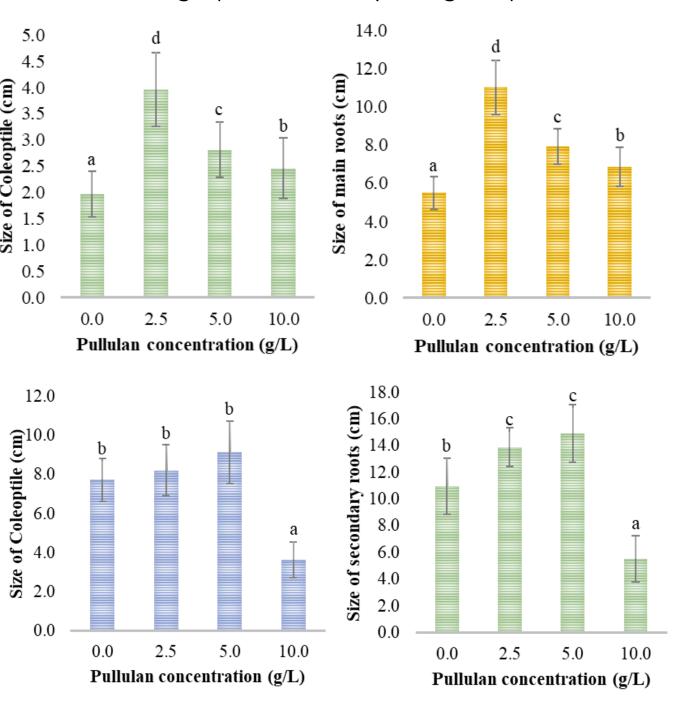


Figure 1. Profile of sugar consumption, biomass accumulation, and pullulan production by Aureobasidium pullulans ATCC 42023 during fermentation at 100 g/L glucose.

#### Results and Discussion

Moderate pullulan levels (2.5–5.0 g/L) promoted root and coleoptile growth, whereas 10 g/L inhibited it. Under 300 mM NaCl, salinity stress significantly reduced maize growth; however, biopriming with 5 g/L pullulan and 20 mg *Chlorella vulgaris* improved germination (91.7%), root activity, and chlorophyll content while reducing oxidative damage (MDA =  $0.04 \mu mol/g FW$ ).



**Figure 2**. Effect of pullulan concentration on coleoptile and primary root development of *Zea mays* during germination on plates.

Figure 3. Effect of pullulan concentration on coleoptile and root growth of Zea mays after 7 days of germination in soil trays (n = 24). Data are mean ± SD; different letters indicate significant differences (Tukey's test, p < 0.05).

The statistical model ( $R^2 = 0.97$ ; p < 0.001) confirmed a synergistic effect between pullulan and *C. vulgaris*, enhancing maize salt tolerance through improved metabolic balance and antioxidant protection.

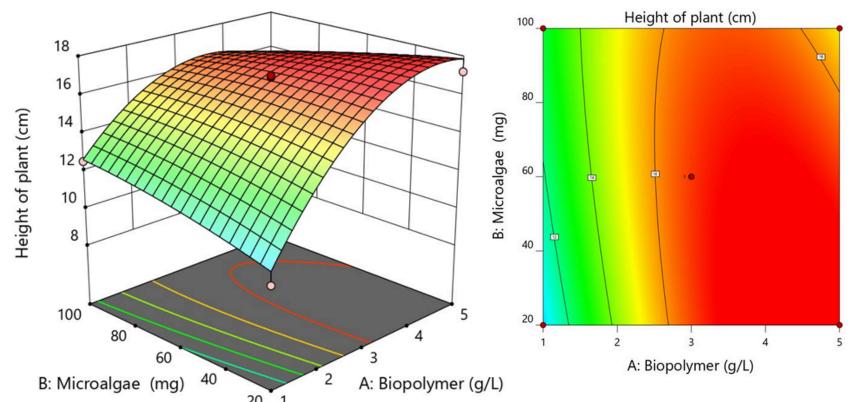


Figure 4. Contour and surface response plots showing the effect of pullulan concentration and microalgae loading on Zea mays seedling

height.

#### **Future Work / References**

Future research will focus on scaling up pullulan-based formulations, testing different microalgal species, and evaluating their long-term effects on field performance and soil microbiota.

#### **Key References:**

- [1] Li et al. (2024) Biosci Biotechnol Biochem, 88(8):923–931.
- [2] Usmanova et al. (2024) Polymers, 16(3):376.
- [3] Ortiz Silos et al. (2025) Biomass Convers Biorefin, 15(10):16059–16071.

### Acknowledgments

This research was supported by the Catholic University of Santa María (Grant no. 30493-R-2024). Special thanks to the Bioprocess Laboratory (F-405) and the Materials Science Laboratory (F-406).