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Understanding the role of SABP2-interacting proteins (SIP) 428: an NAD+-Dependent Deacetylase Enzyme in Abiotic Stress Signaling of Nicotiana tabacum

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Understanding the roles of SIP428, a NAD+ dependent deacetylase enzyme in mediating abiotic stress signaling in *Nicotiana tabacum*

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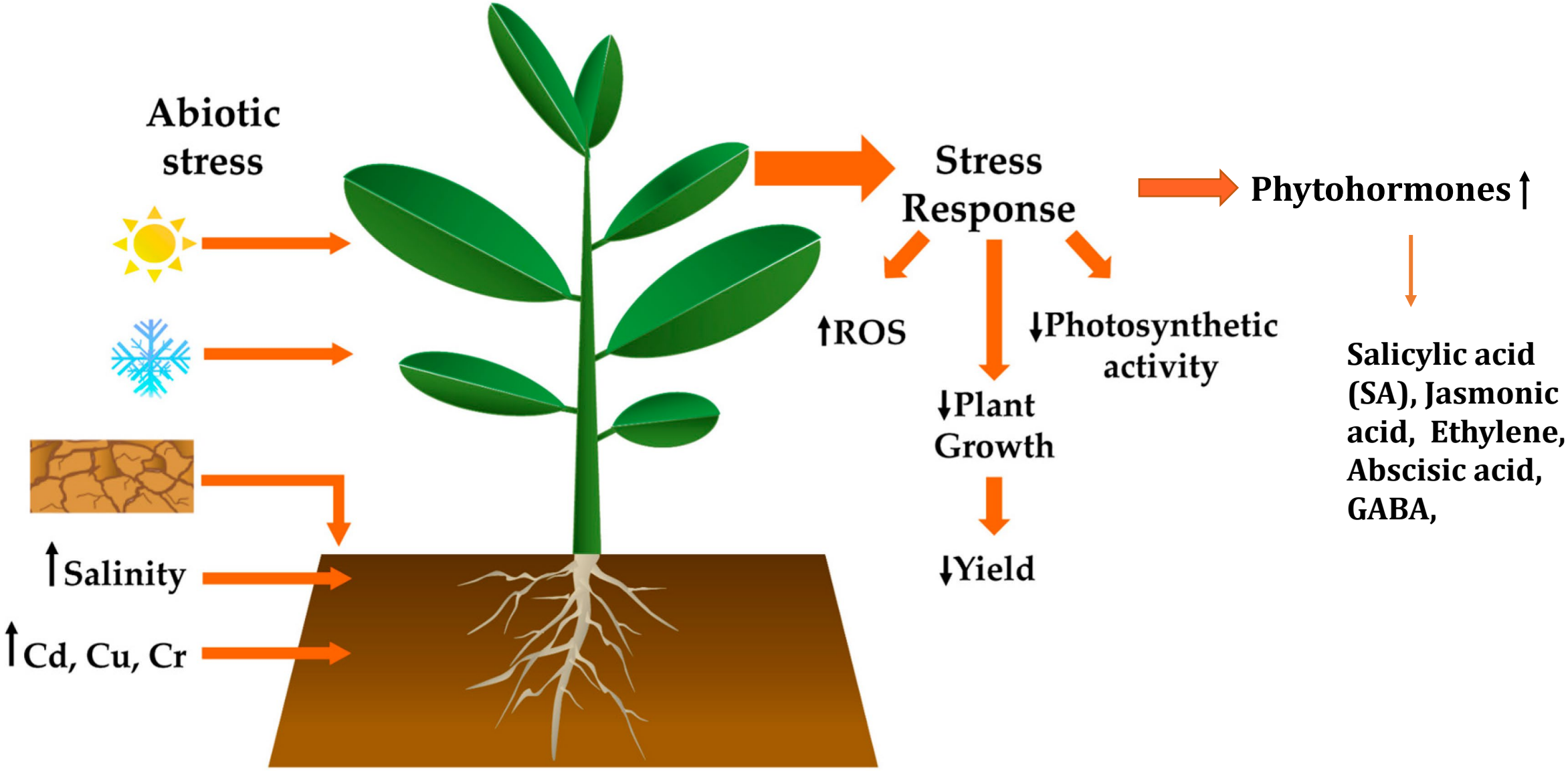
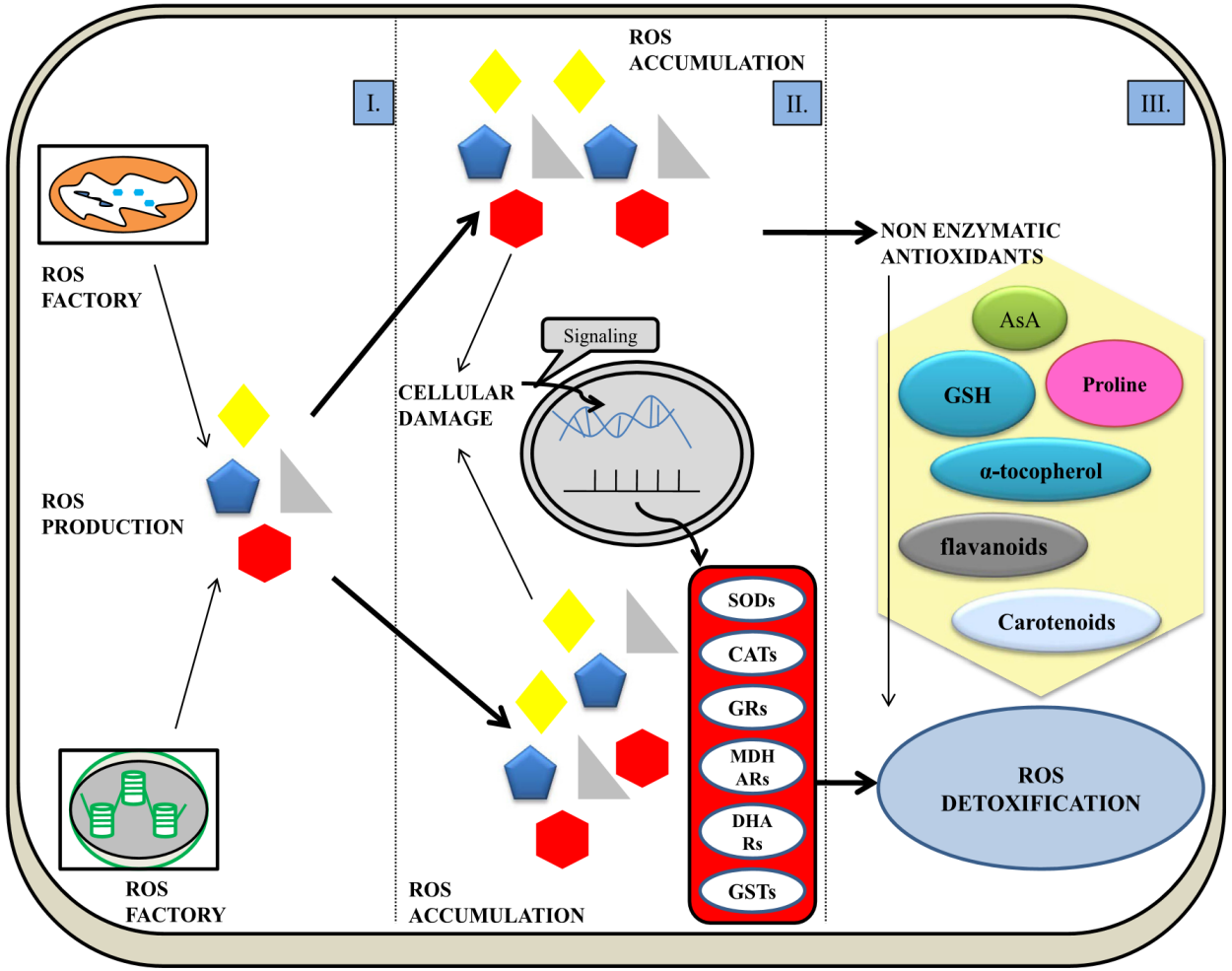
Plants and Abiotic Stress

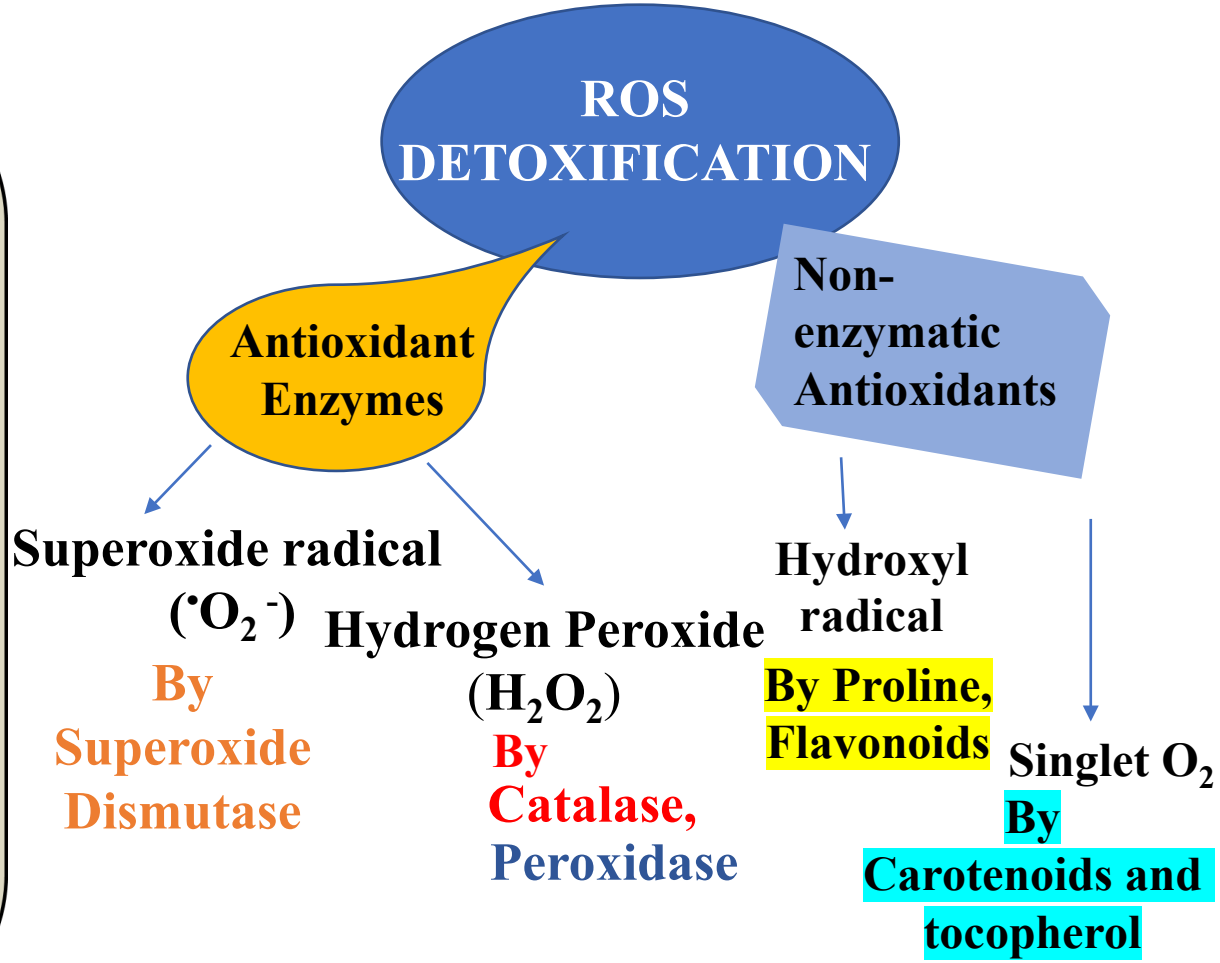


Reactive Oxygen Species (ROS) Signaling

ROS are reactive O_2 species produced from incomplete reduction of O_2.

Superoxide radical ($^{\bullet}O_2^-$), **Hydroxyl radical** ($OH^{\bullet}$), **Hydrogen Peroxide** (H_2O_2), **Singlet oxygen** ($^1O_2$),



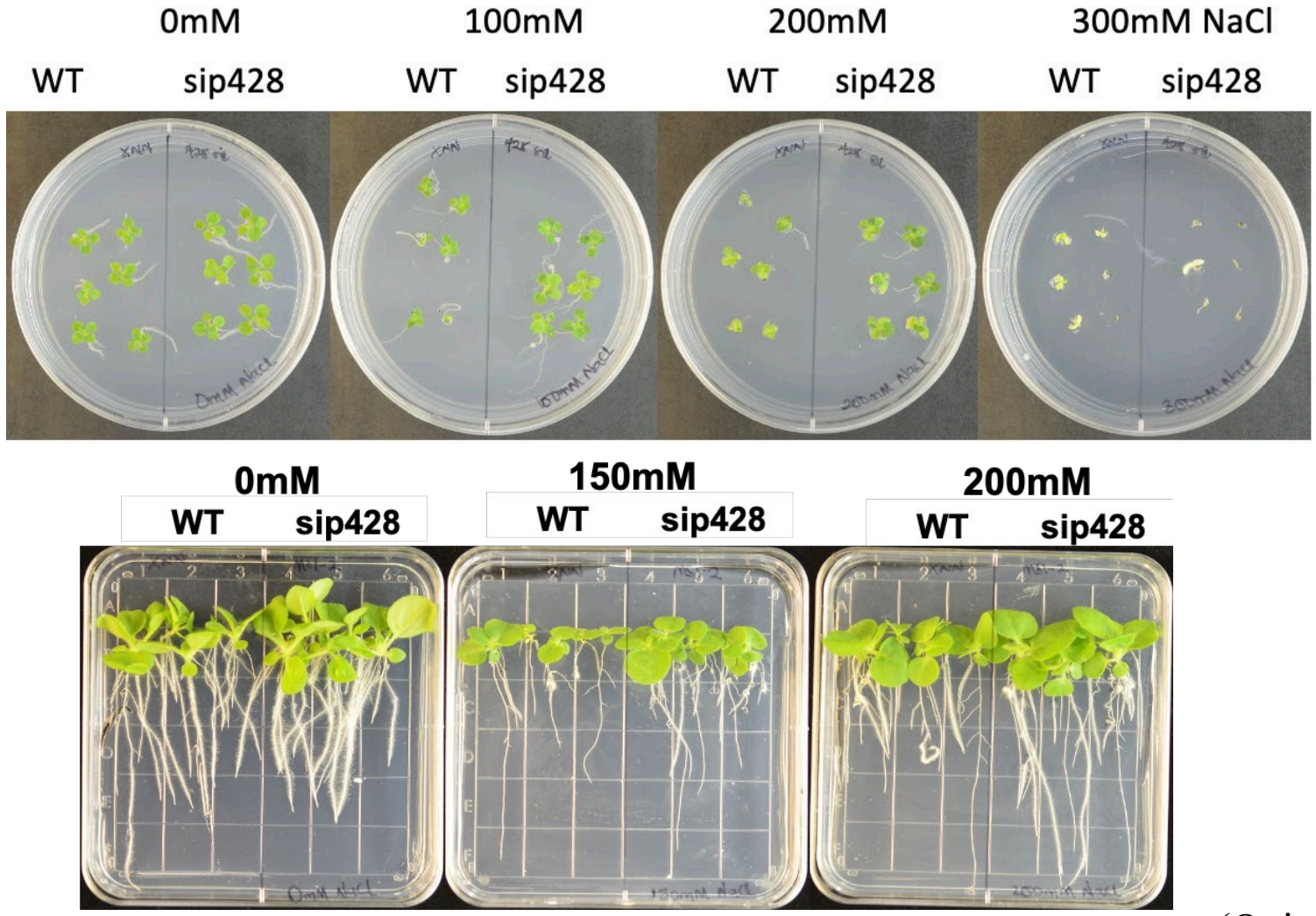


SIP428 in *Nicotiana tabacum*

- SA, a plant hormone has important role in mediating plant stress.
- SABP2- Salicylic Acid Protein 2 converts MeSA to SA that is required for plant immunity. (PNAS 2003; PNAS 2005; Science 2007)
- **SIP428** was identified as one of the interacting proteins in the SABP2 signaling pathway; a NAD^+ dependent deacetylase enzyme. (Haq, 2020).
- SIP428-silenced plants showed **higher resistance** to Tobacco Mosaic Virus infection (Thakuri, 2018)
- SIP428 is a **Negative Regulator** of plant growth under Abiotic Stress (Oviavo, 2021)

The Role of SIP428 in Abiotic Stress

- SIP428 plays a negative regulatory role in Salt stress

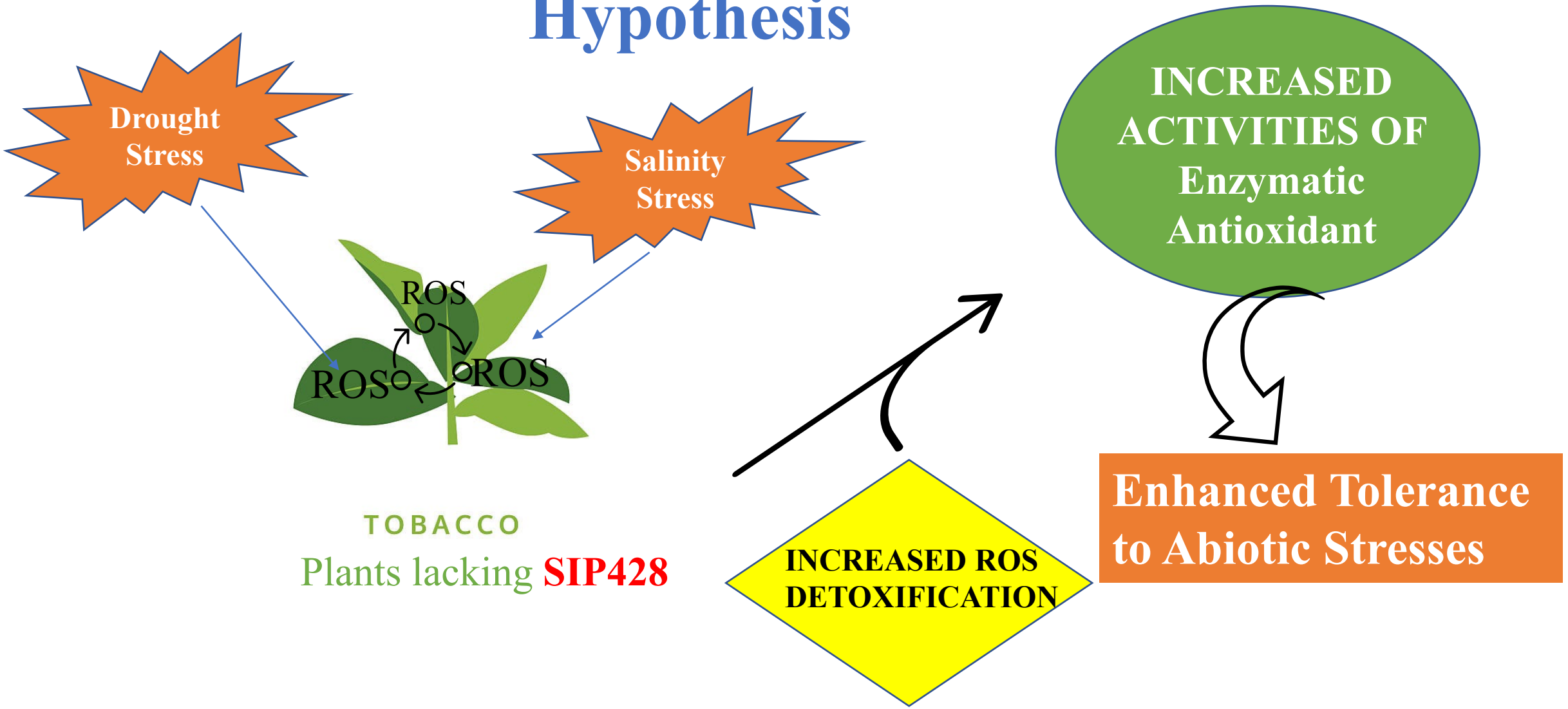


SIP428-silenced Plant and root growth under Salt Stress

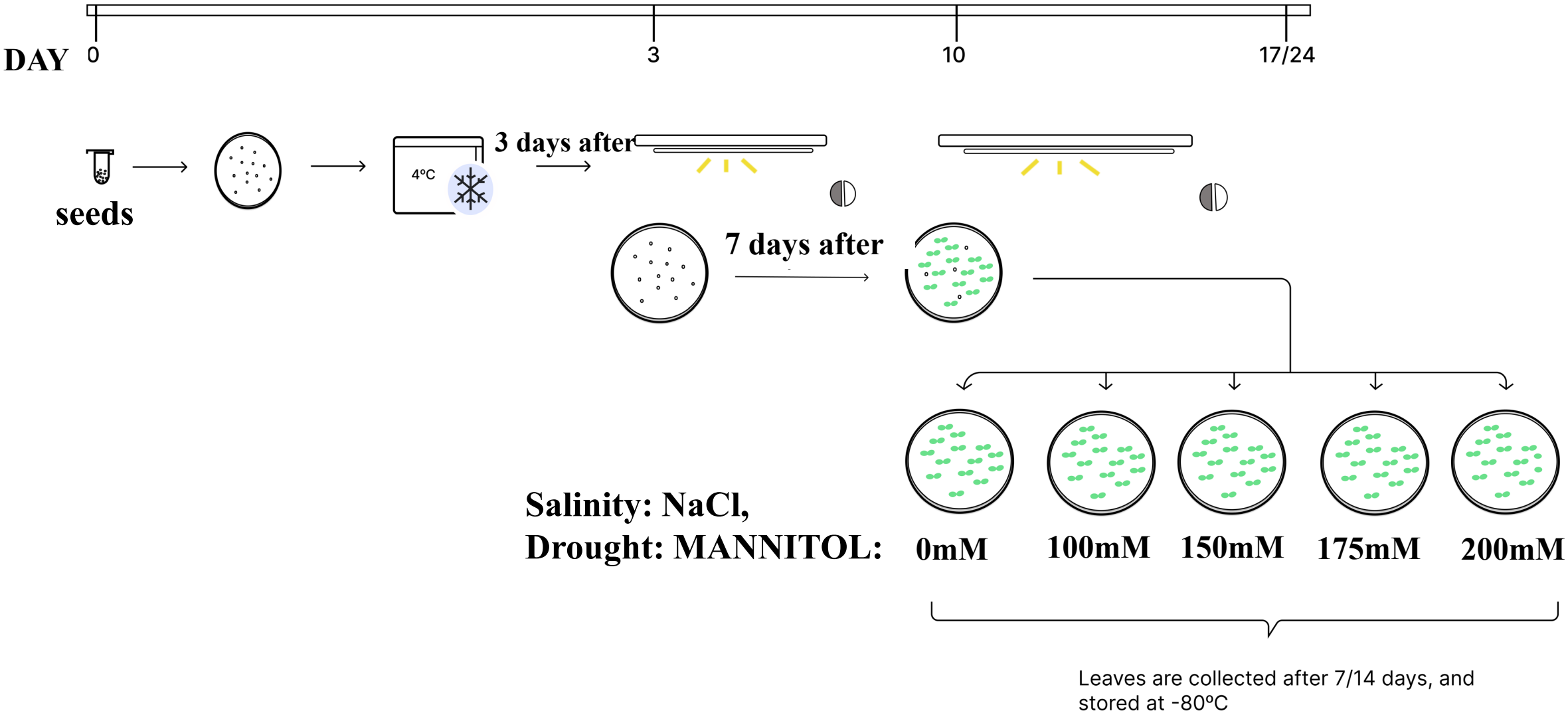
(Oviavo, 2021 Masters Thesis)

Aim: To provide evidence of regulatory functions of SIP428 in Abiotic Stress through ROS signaling

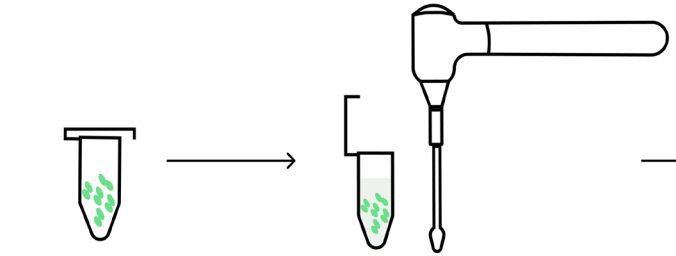
Hypothesis



Methodology: Abiotic Stress Treatment

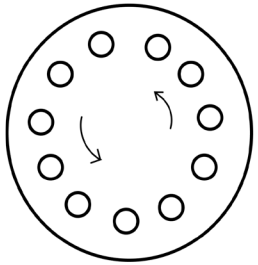


Method: Guaiacol Peroxidase and Catalase Activity Assay

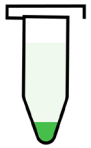


0.1g of leaves stored at -80ºC

Homogenization in phosphate buffer and EDTA



Centrifugation at 13,000g for 20 minutes

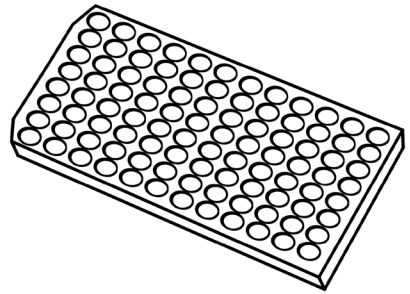


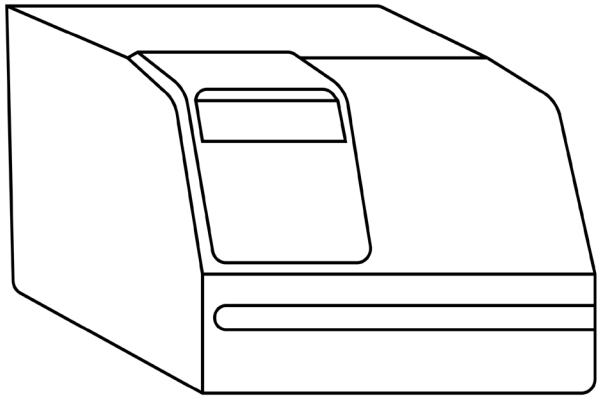
Protein quantification of supernatant

+

Catalase assay solution:
H_2O_2, pH 7.2 phosphate buffer

Guaiacol peroxidase assay solution:
guaiacol, H_2O_2, pH 6.1 phosphate buffer

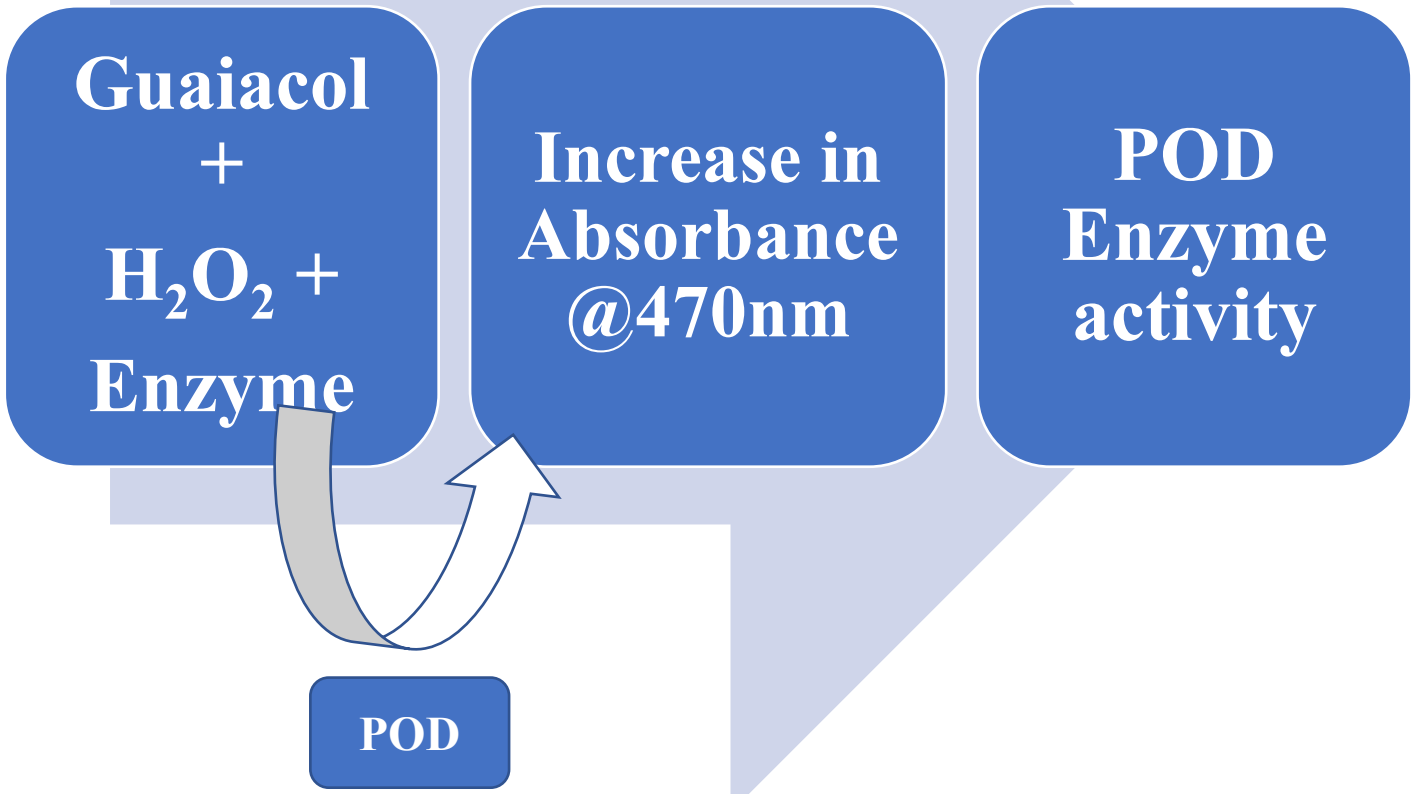


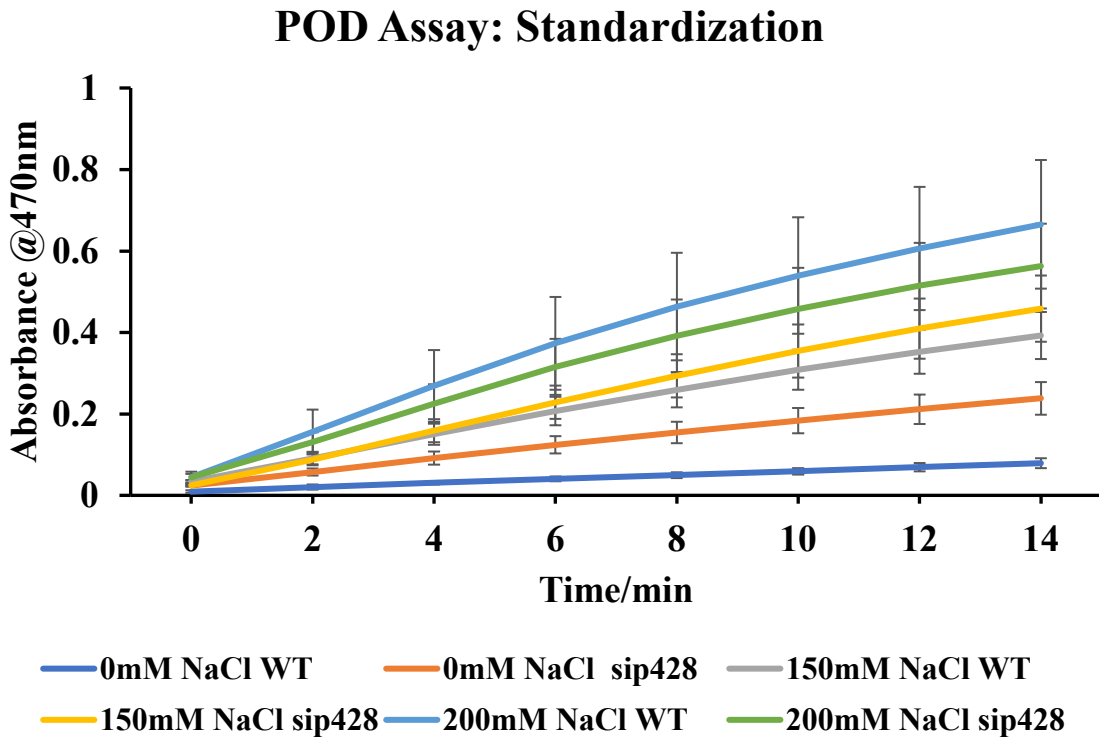


Enzyme Activity Analysis

$$Enzyme\ Activity\ (U/mg) = \frac{\Delta A}{\Delta t}\frac{V_c\ V_b}{\varepsilon\ d}$$

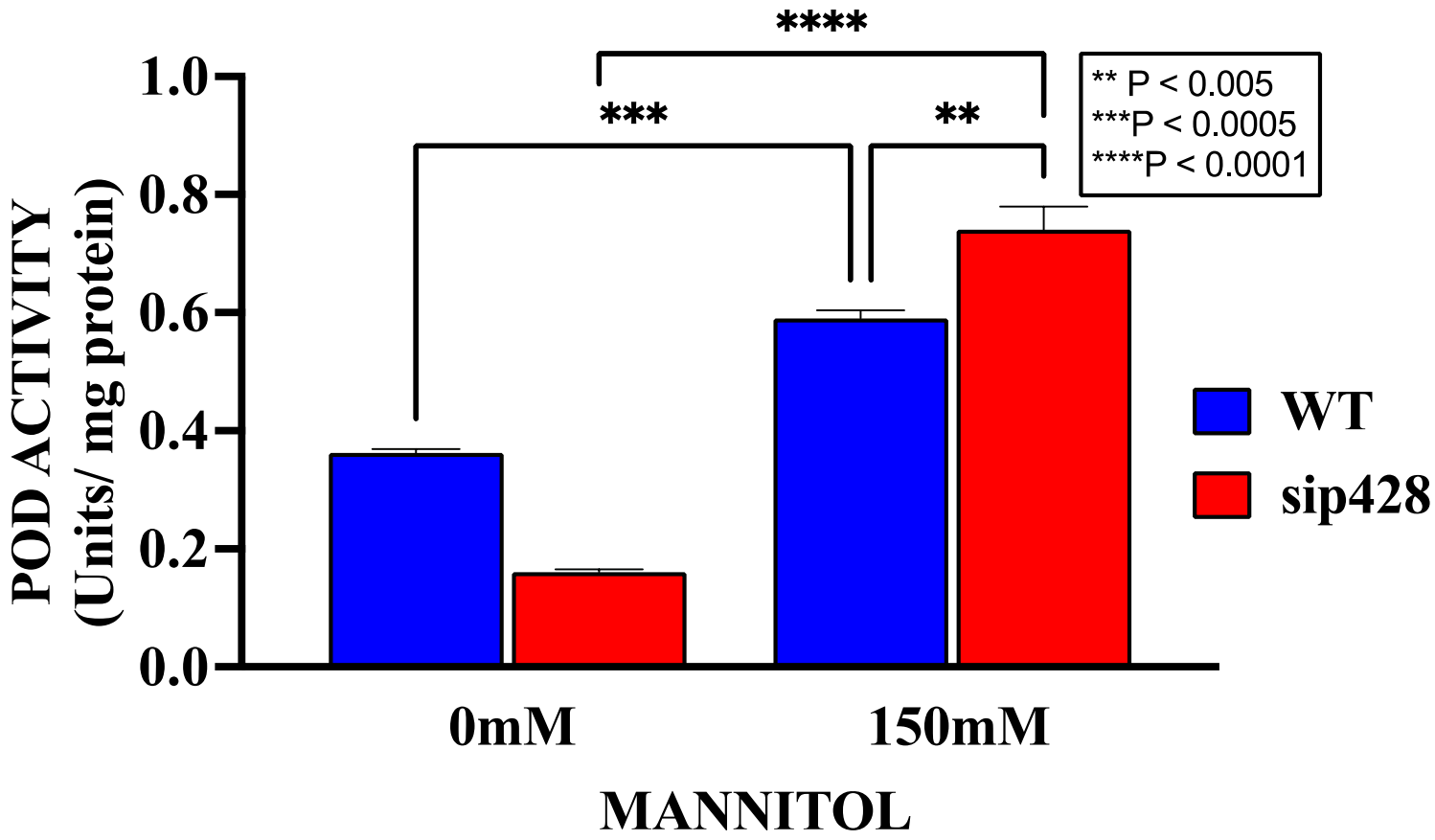
Guaiacol Peroxidase (POD) Assay





Guaiacol Peroxidase Assay: Guaiacol + H_2O_2 $\xrightarrow{\text{POD}}$ *Tetra-guaiacol + H_2O*

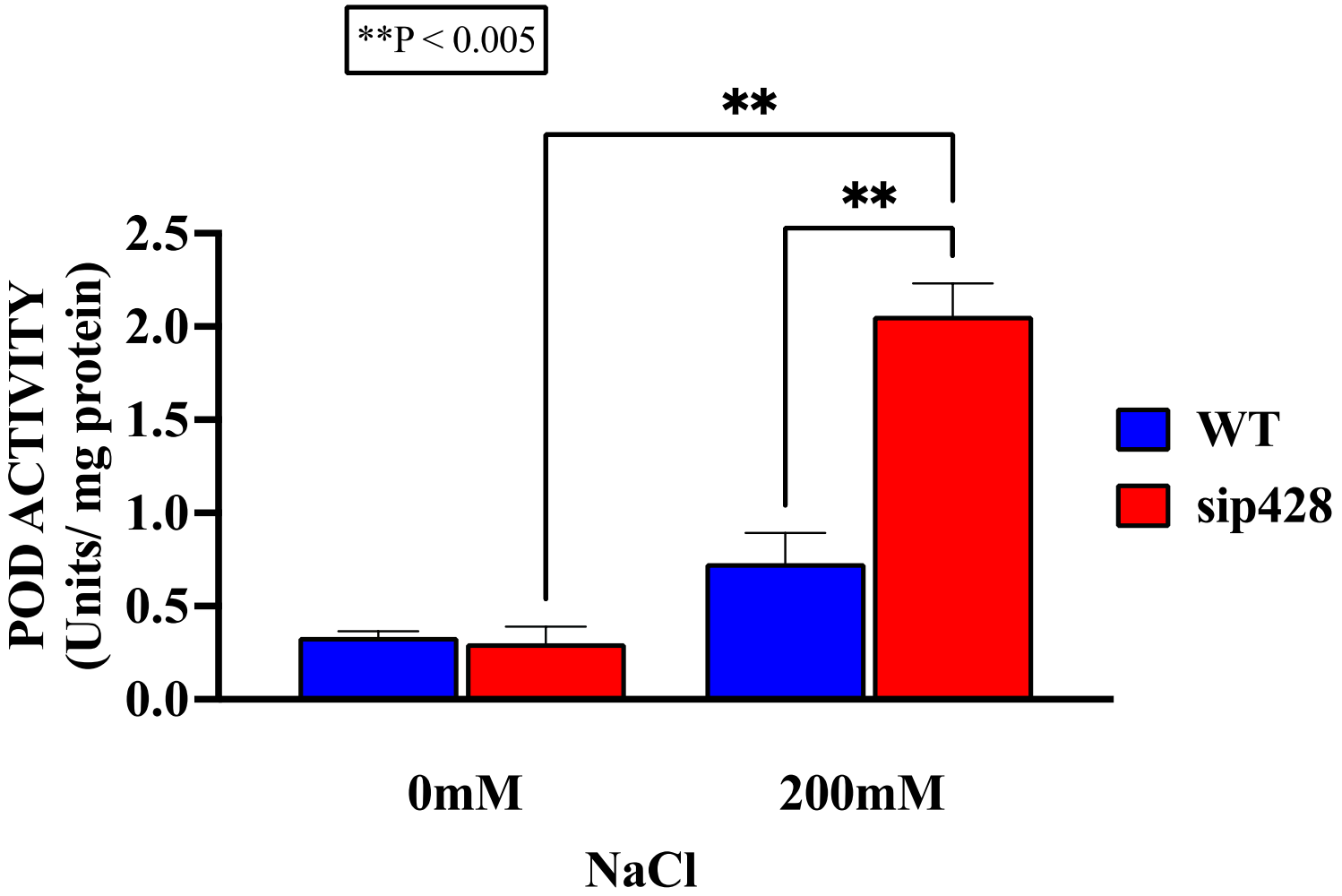
Drought Stress: Peroxidase (POD) Activity



Peroxidase Activity in Mannitol-Stressed Plants

N= 3 + S.E. One-way Anova was used to investigate significant differences within groups at $p < 0.05$.

Salinity Stress: Peroxidase (POD) Activity



Peroxidase Activity in NaCl-Stressed Plants. (N= 4 + S.E). One-way Anova was used to show significance within groups at $p < 0.05$.

Catalase (CAT) Assay

H_2O_2 + Enzyme + Phosphate Buffer

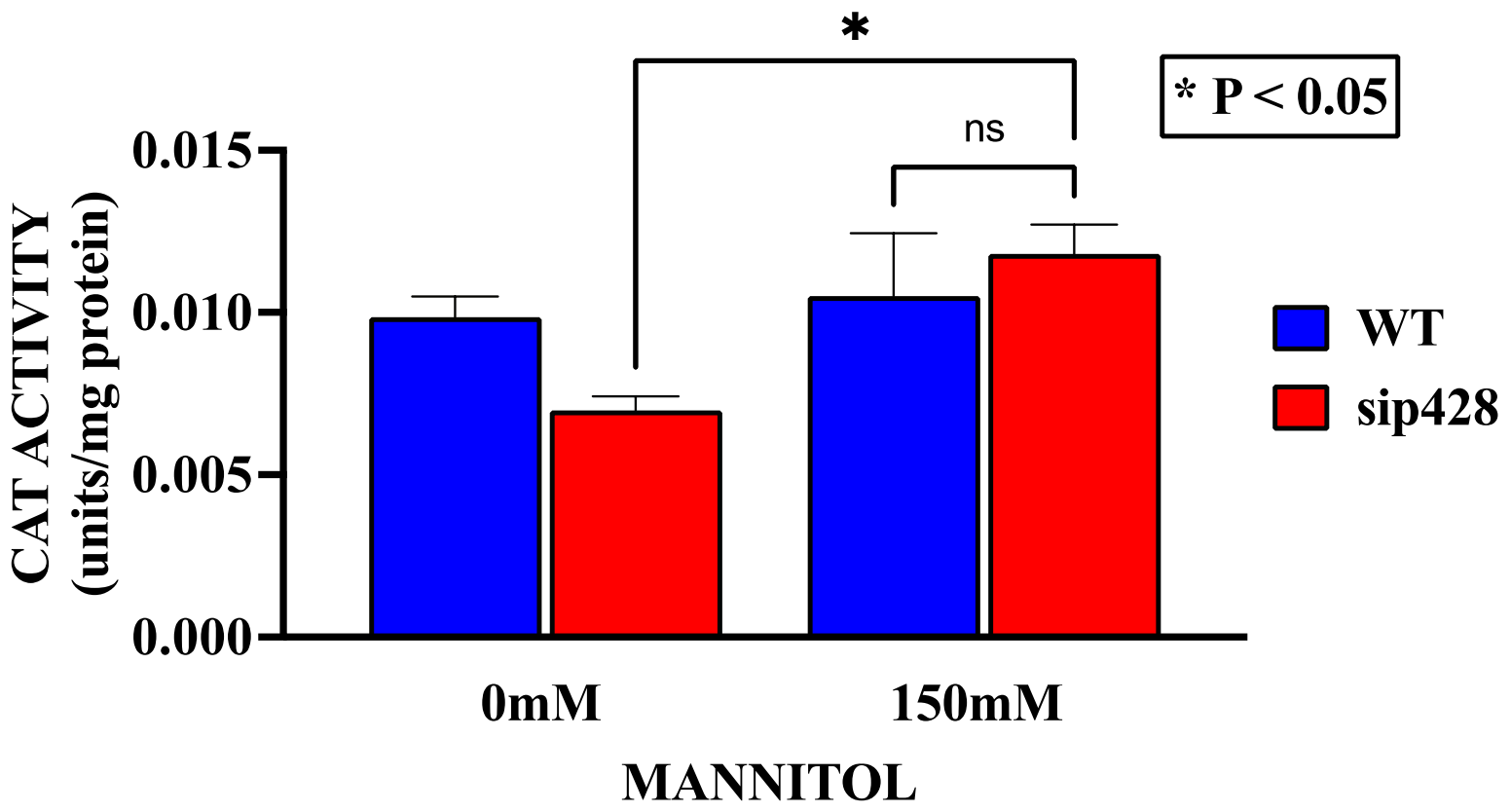
Catalase

Decrease in Absorbance @ 240nm

CATALASE Activity

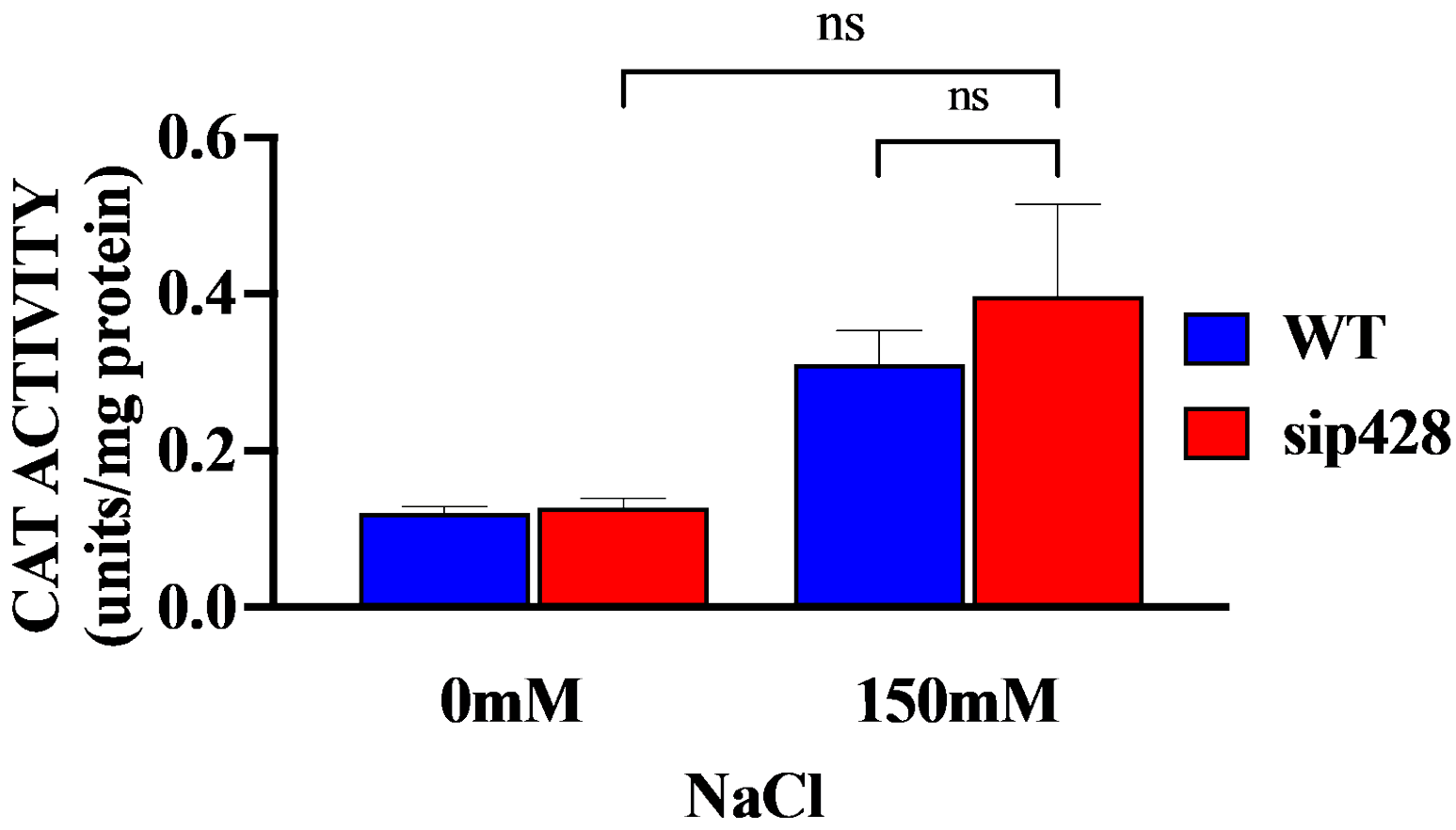
Catalase Assay: $2\,H_2O_2 \xrightarrow{CAT} O_2 + H_2O$

Drought Stress: Catalase (CAT) Activity



Catalase Activity in Mannitol-Stressed Plants (N= 4 + S.E). One-way Anova was used to show significance within groups at $p < 0.05$.

Salinity Stress: Catalase (CAT) Activity



Catalase Activity in NaCl-Stressed Plants (N= 4 + S.E). One-way Anova was used to show significance within groups at $p < 0.05$.

Conclusion and Future Directions

- **Drought Stress**: SIP428 plays negative regulatory functions in ROS signaling via Peroxidase activities

- ✓ **Salinity Stress**: SIP428 plays negative regulatory functions in ROS signaling using Guaiacol peroxidase

- ✓ There is the need to repeat biochemical analysis of CATALASE

ROS Detoxification

- Via Non-Enzymatic Antioxidant System (Proline, Carotenoids, Flavonoids)

Stress-related and responsive genes

- To understand the regulatory gene network involved in SIP428 functions

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QUESTIONS?