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Apr 25th, 9:20 AM - 9:40 AM

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

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*East Tennessee State University*

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Onabanjo, Mariam and Kumar, Dhirendra PhD., "Understanding the role of SABP2-interacting proteins (SIP) 428: an NAD<sup>+</sup>-Dependent Deacetylase Enzyme in Abiotic Stress Signaling of *Nicotiana tabacum*" (2023). *Appalachian Student Research Forum*. 111.  
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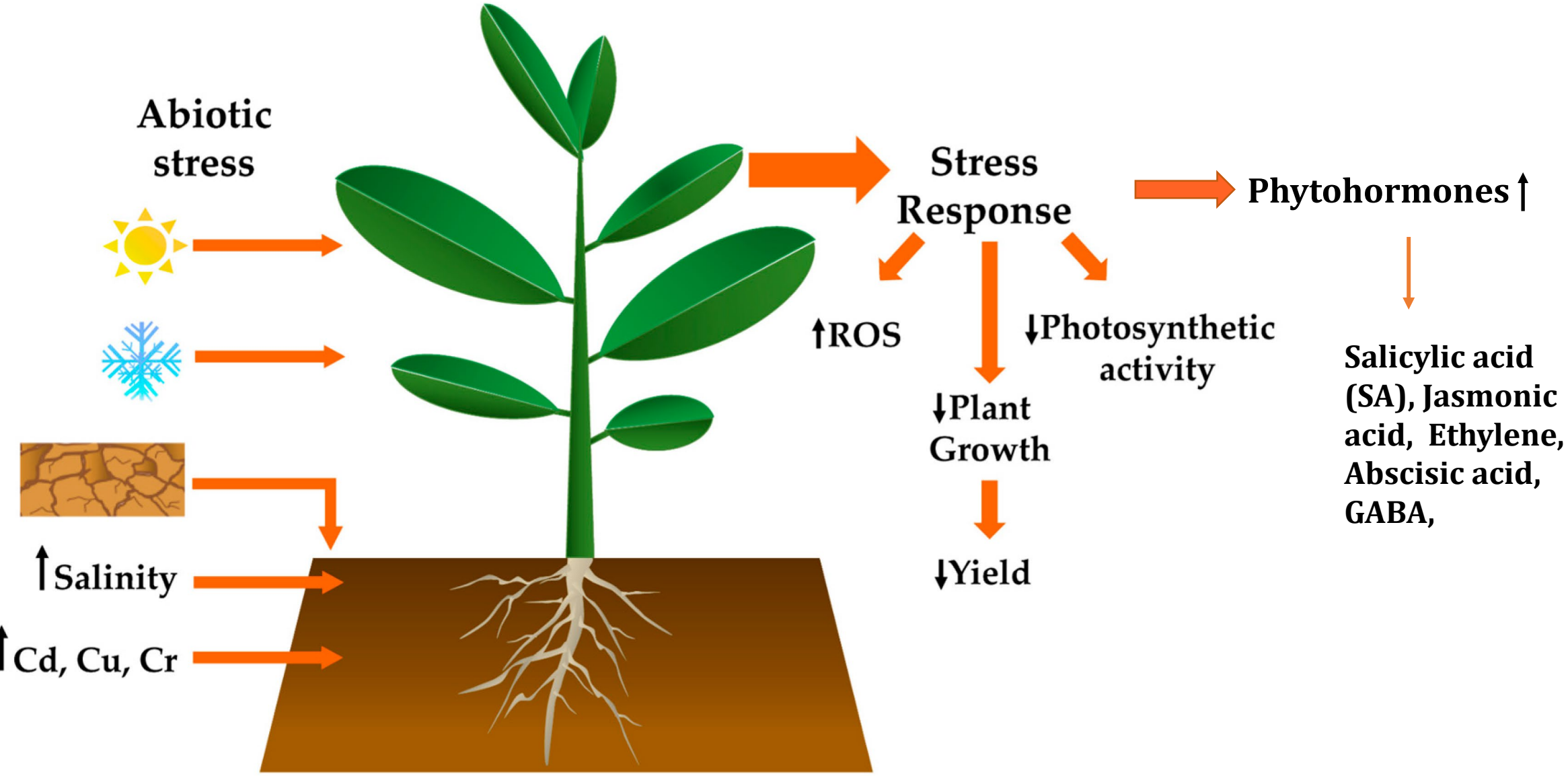
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**Understanding the roles of SIP428,  
a NAD<sup>+</sup> dependent deacetylase enzyme in  
mediating abiotic stress signaling in  
*Nicotiana tabacum***

**Mariam Onabanjo**

**Department of Biological Sciences  
East Tennessee State University**

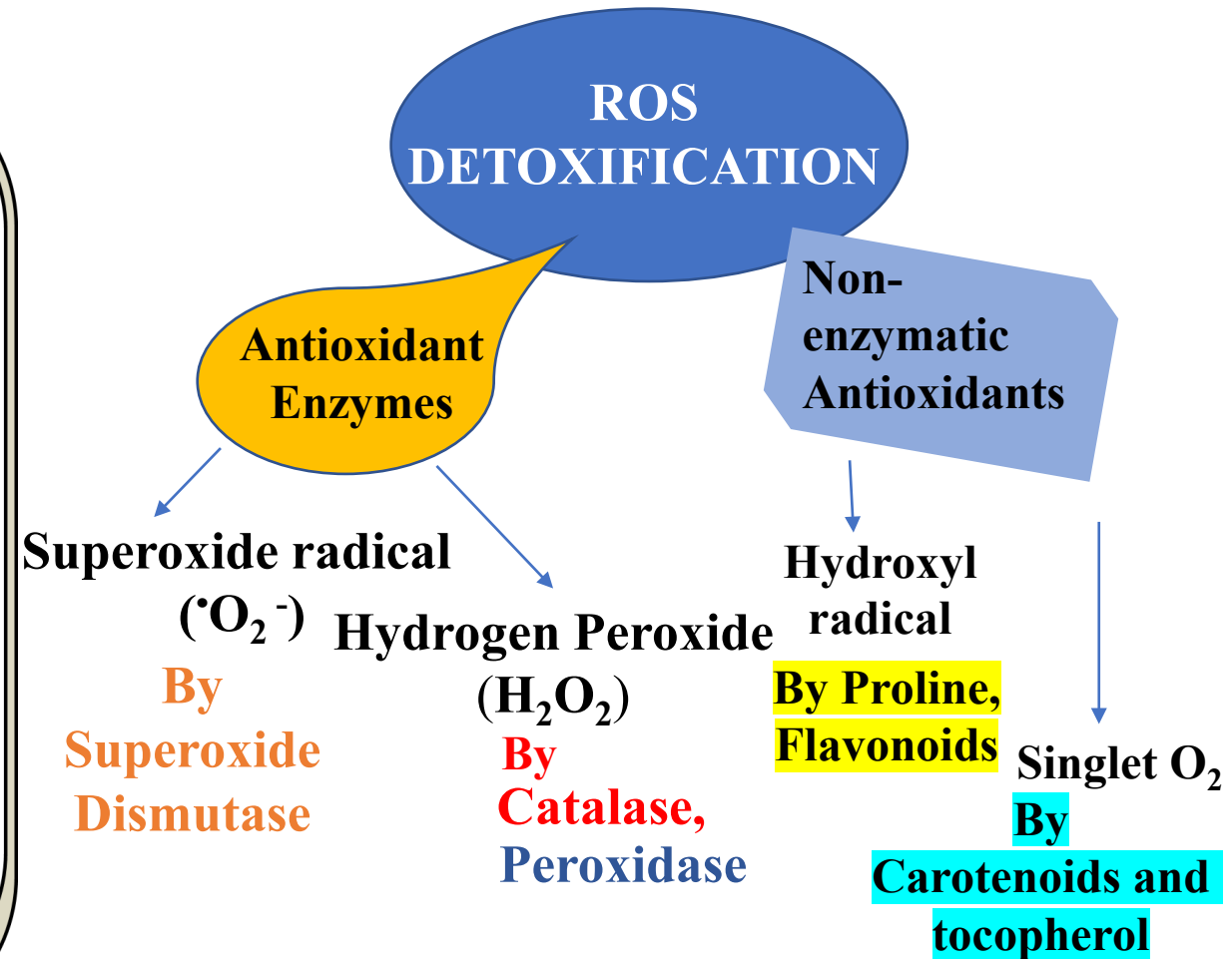
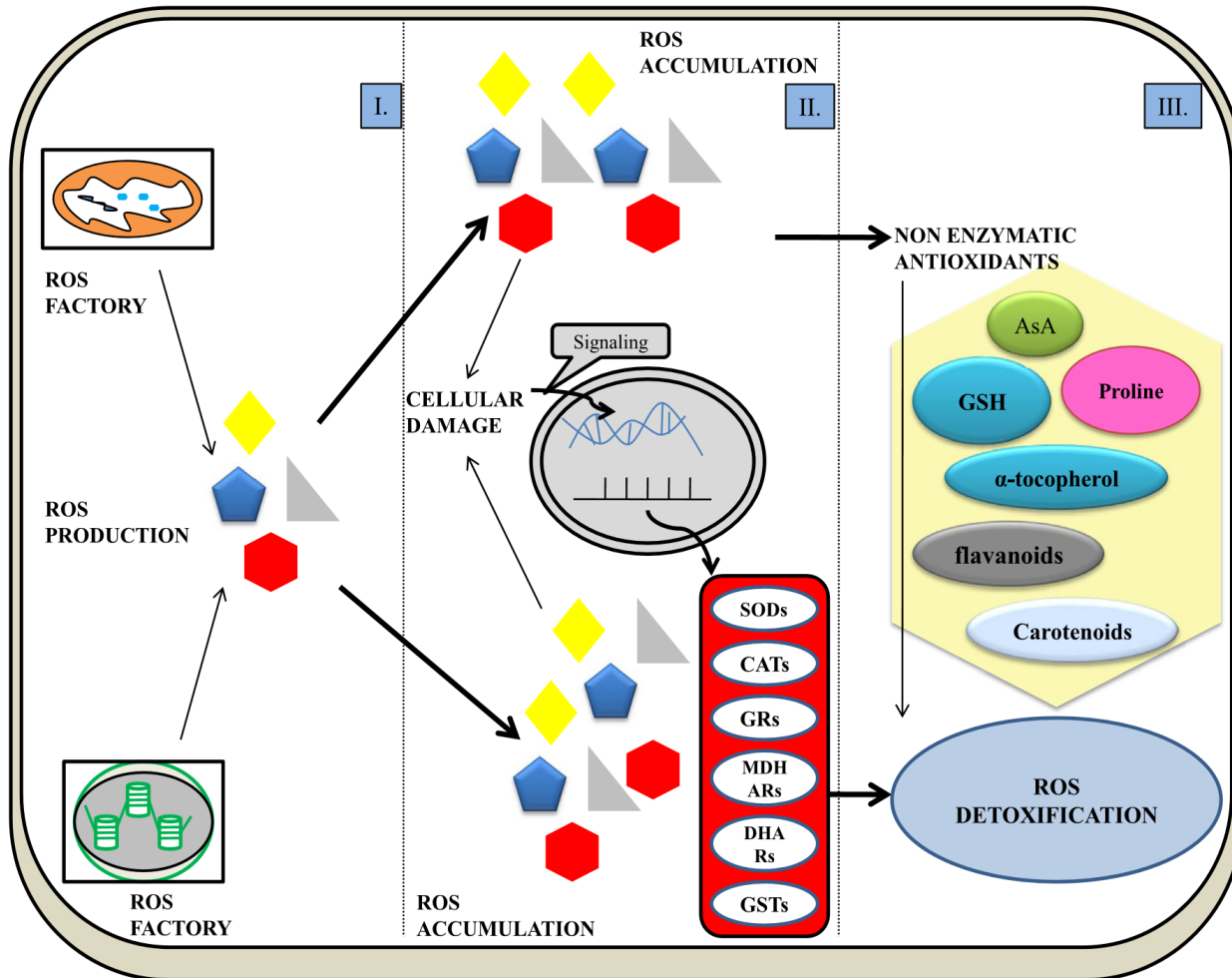
# Plants and Abiotic Stress



# Reactive Oxygen Species (ROS) Signaling

ROS are reactive O<sub>2</sub> species produced from incomplete reduction of O<sub>2</sub>.

**Superoxide radical ( $\cdot\text{O}_2^-$ ), Hydroxyl radical ( $\text{OH}\cdot$ ), Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ), Singlet oxygen ( $^1\text{O}_2$ ),**

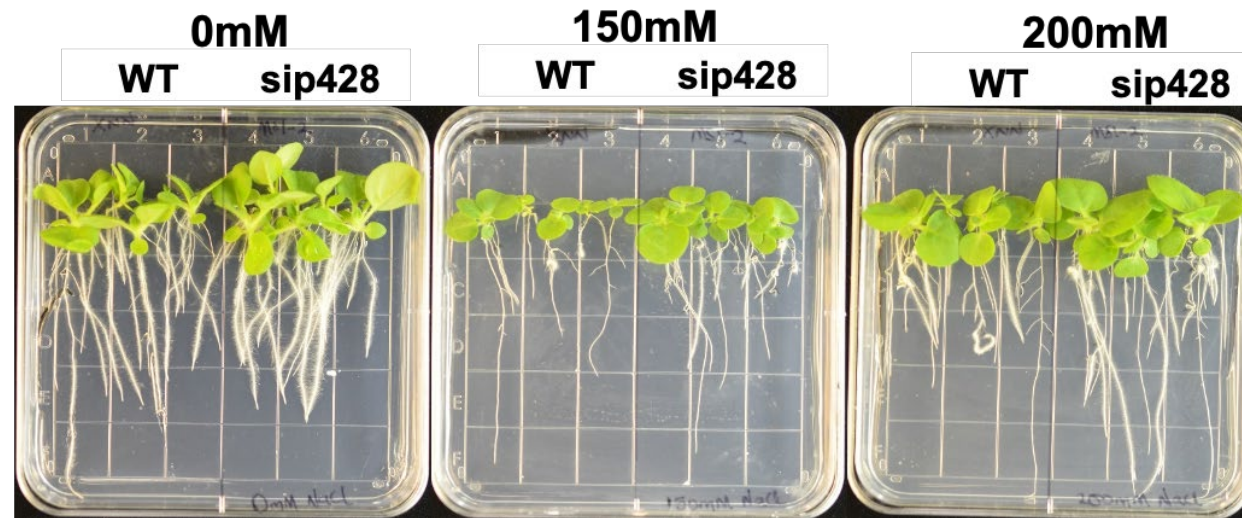
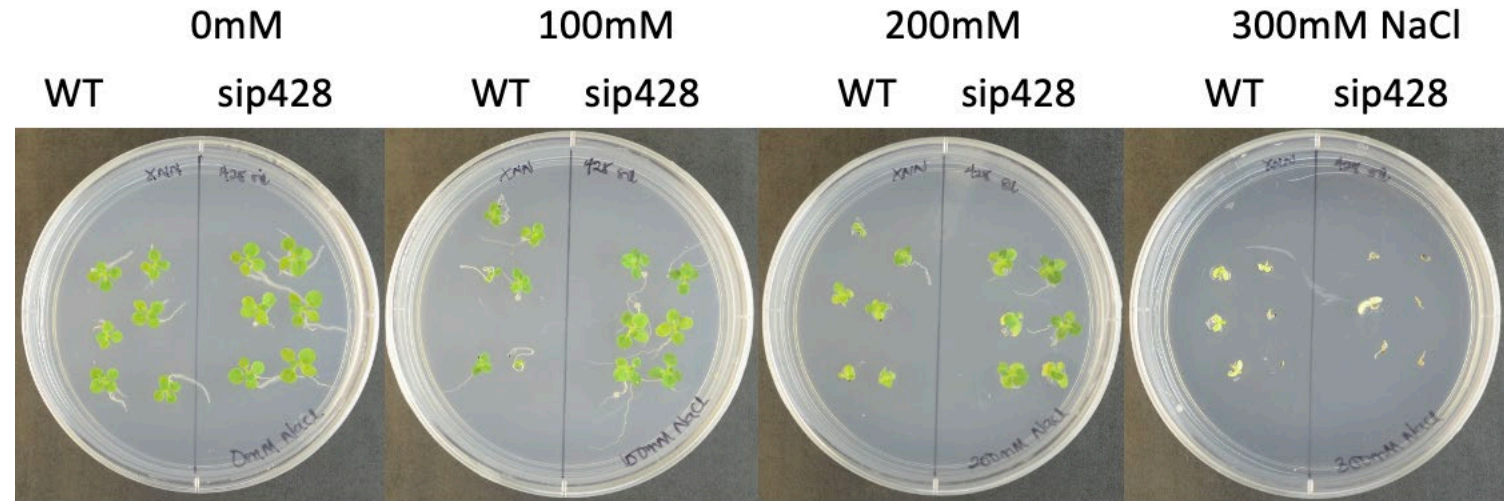


# SIP428 in *Nicotiana tabacum*

- 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<sup>+</sup> 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



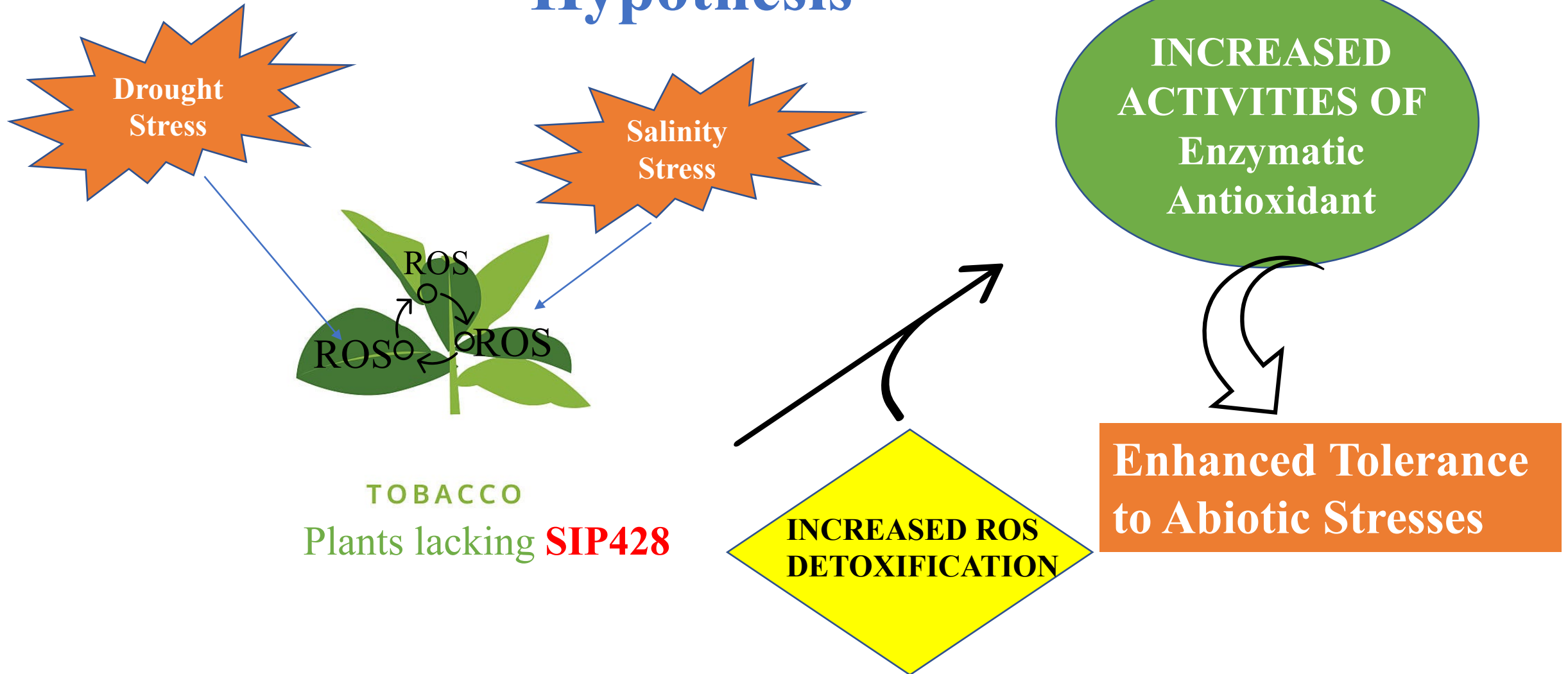
SIP428-silenced Plant and root growth under Salt Stress

(Oviavo, 2021 Masters Thesis)



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

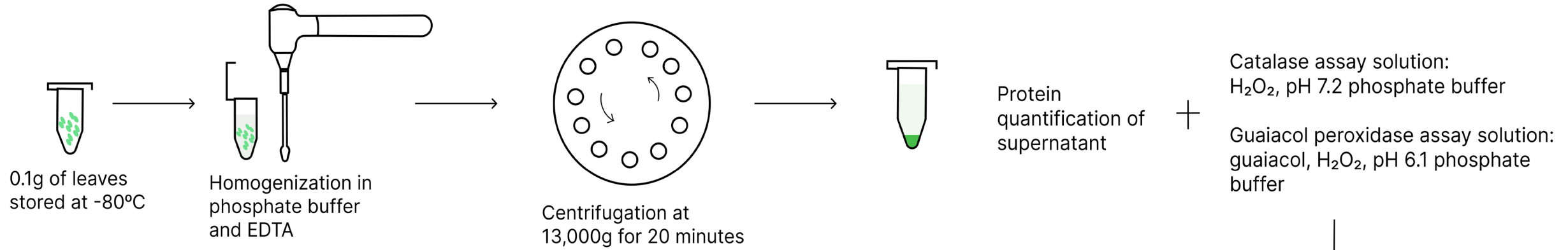
## Hypothesis





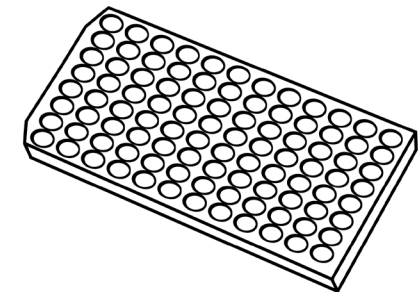
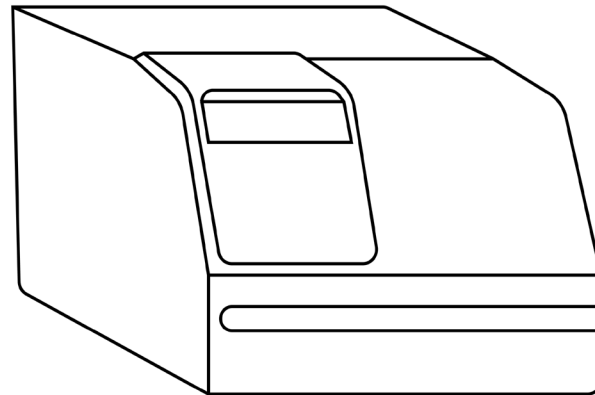


# Method: Guaiacol Peroxidase and Catalase Activity Assay



## Enzyme Activity Analysis

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



# Guaiacol Peroxidase (POD) Assay

Guaiacol  
+  
 $H_2O_2$  +  
Enzyme

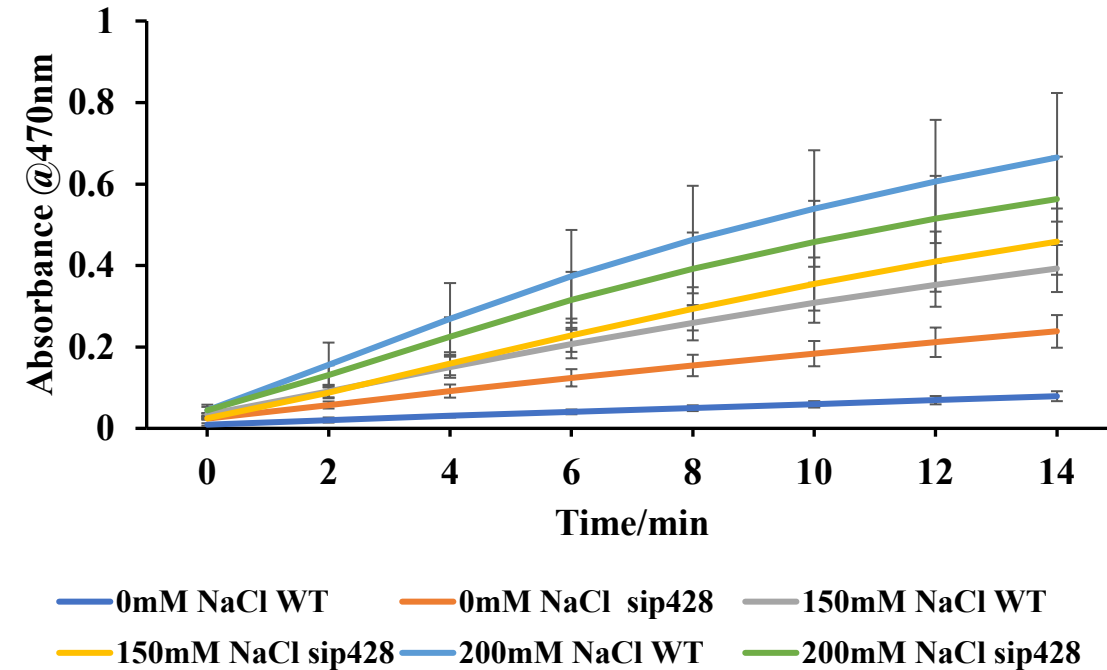
Increase in  
Absorbance  
@470nm

POD  
Enzyme  
activity

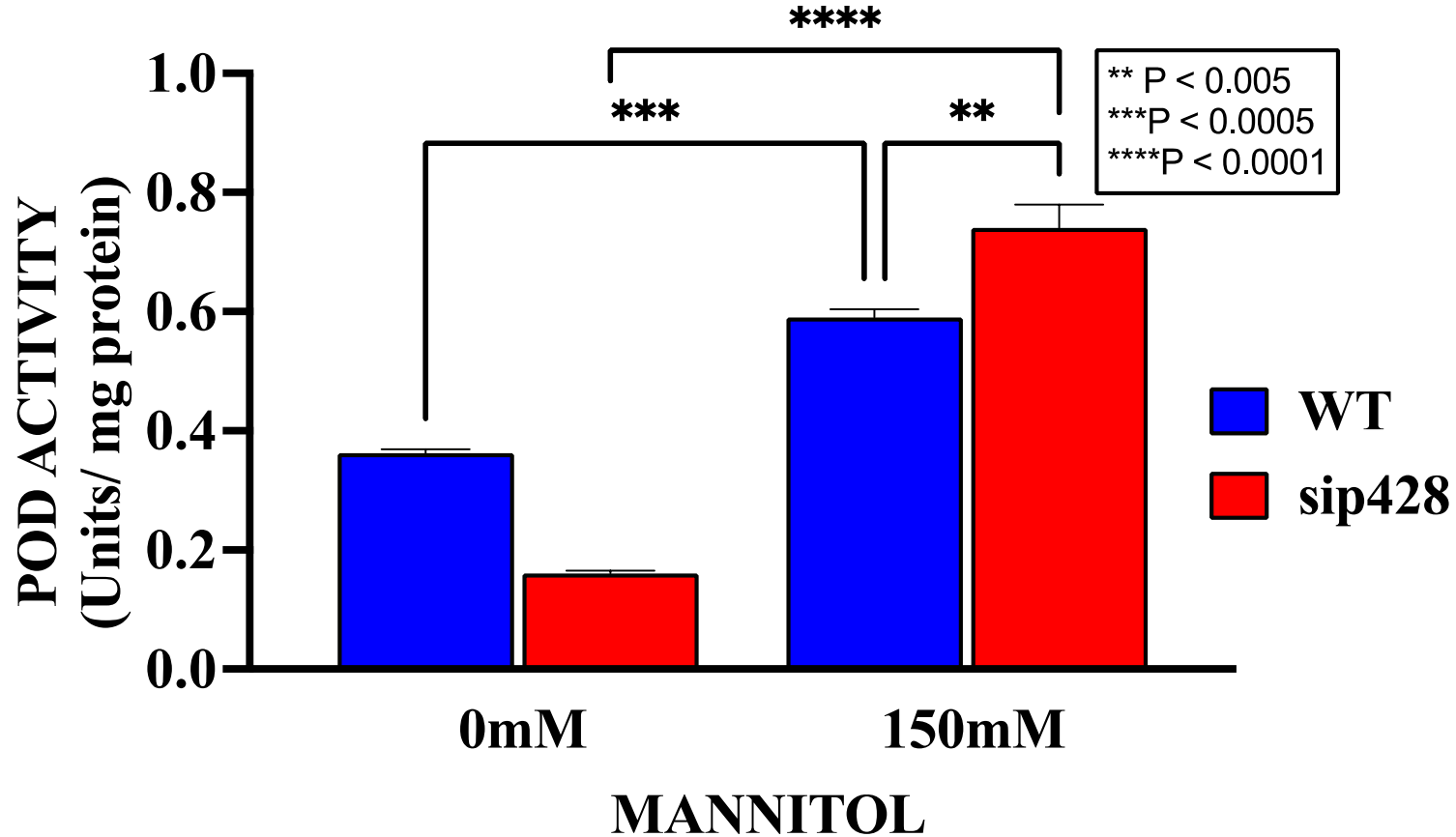
POD

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

POD Assay: Standardization



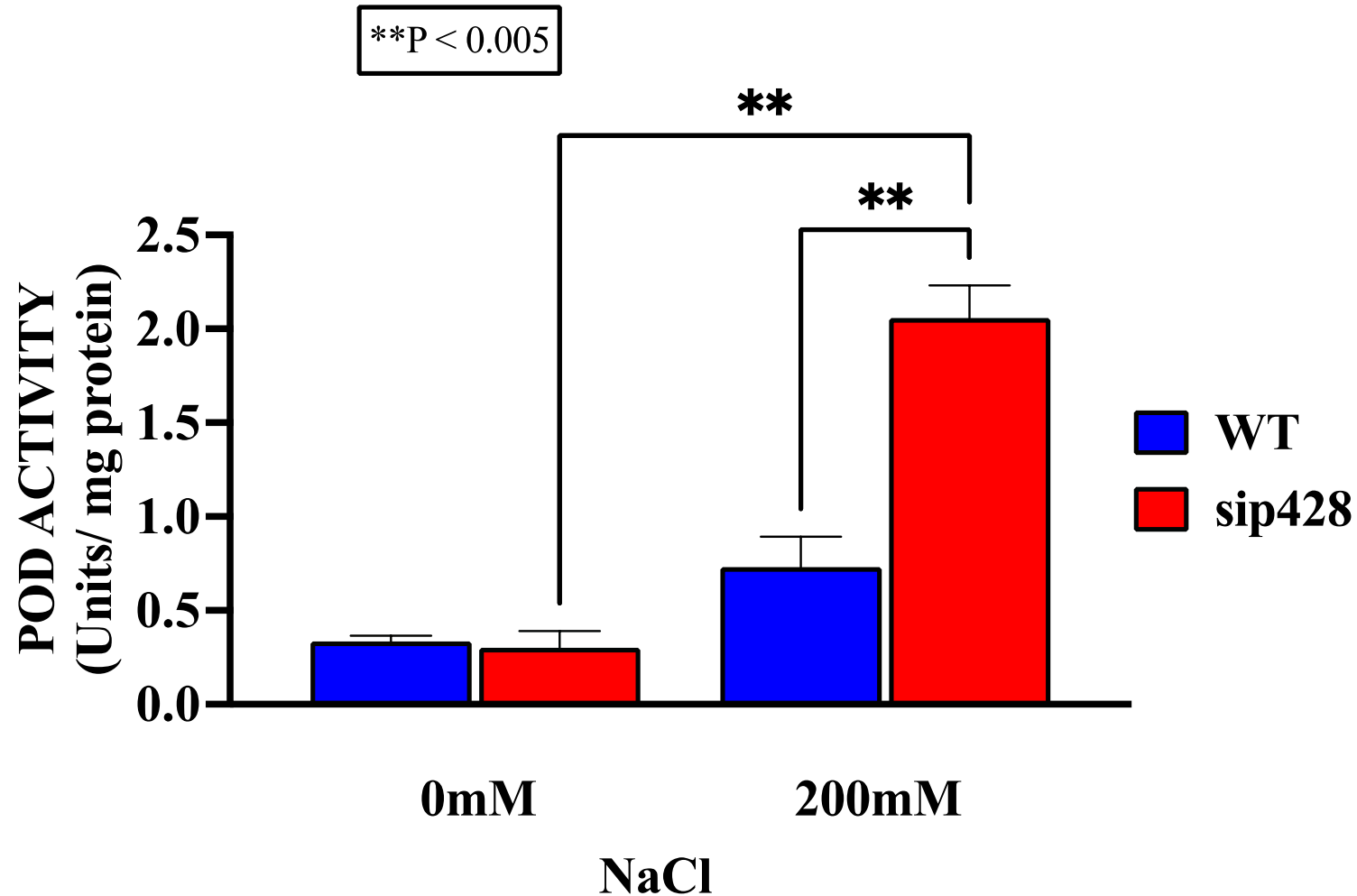
# Drought Stress: Peroxidase (POD) Activity



## 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  $p < 0.05$ .

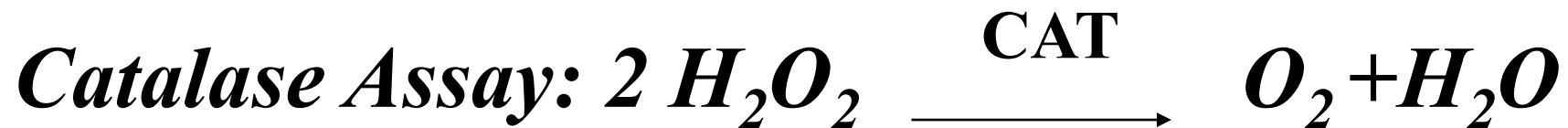
# Catalase (CAT) Assay

$H_2O_2$  + Enzyme +  
Phosphate Buffer

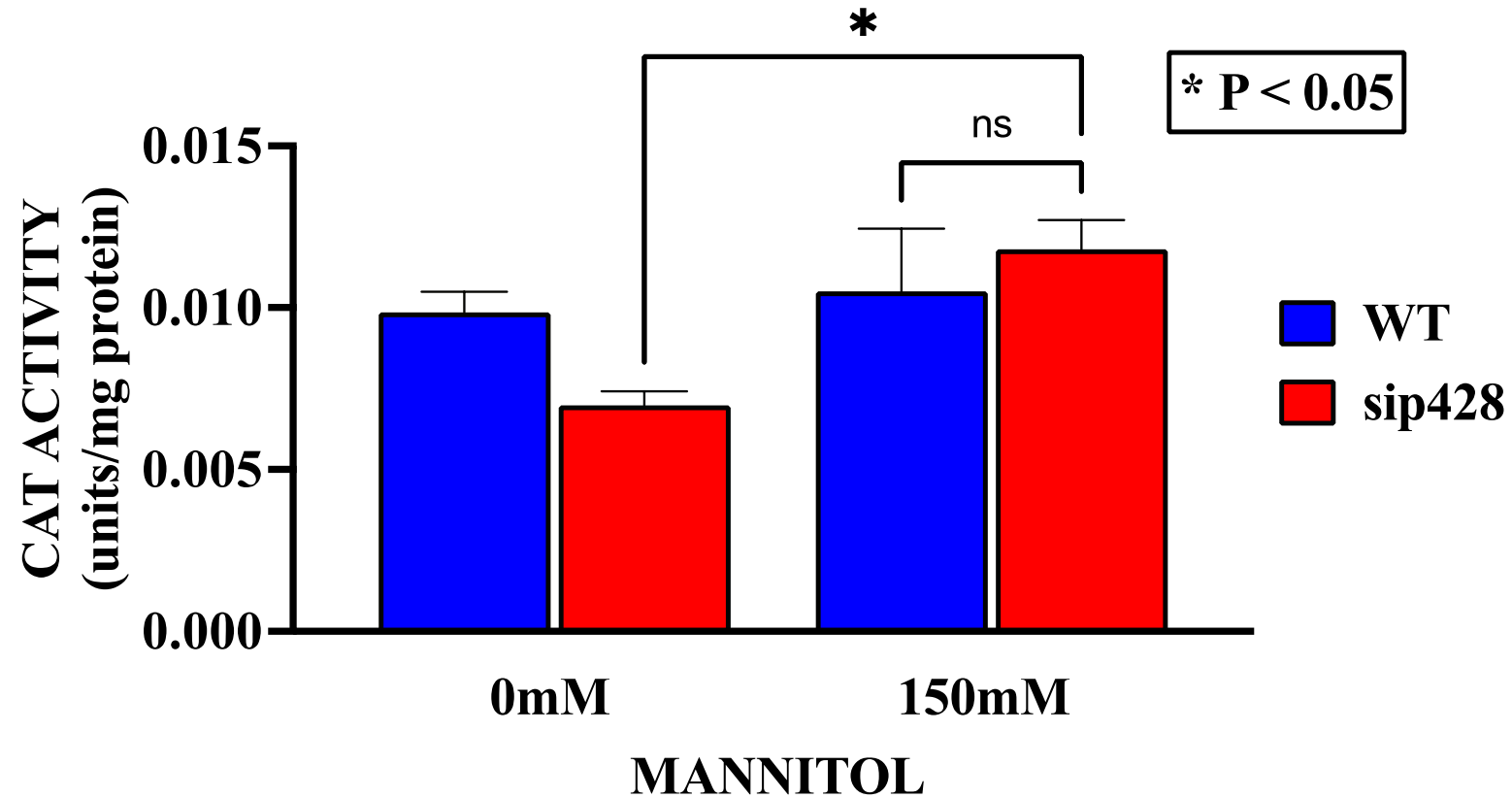
Catalase

Decrease in  
Absorbance @ 240nm

CATALASE Activity



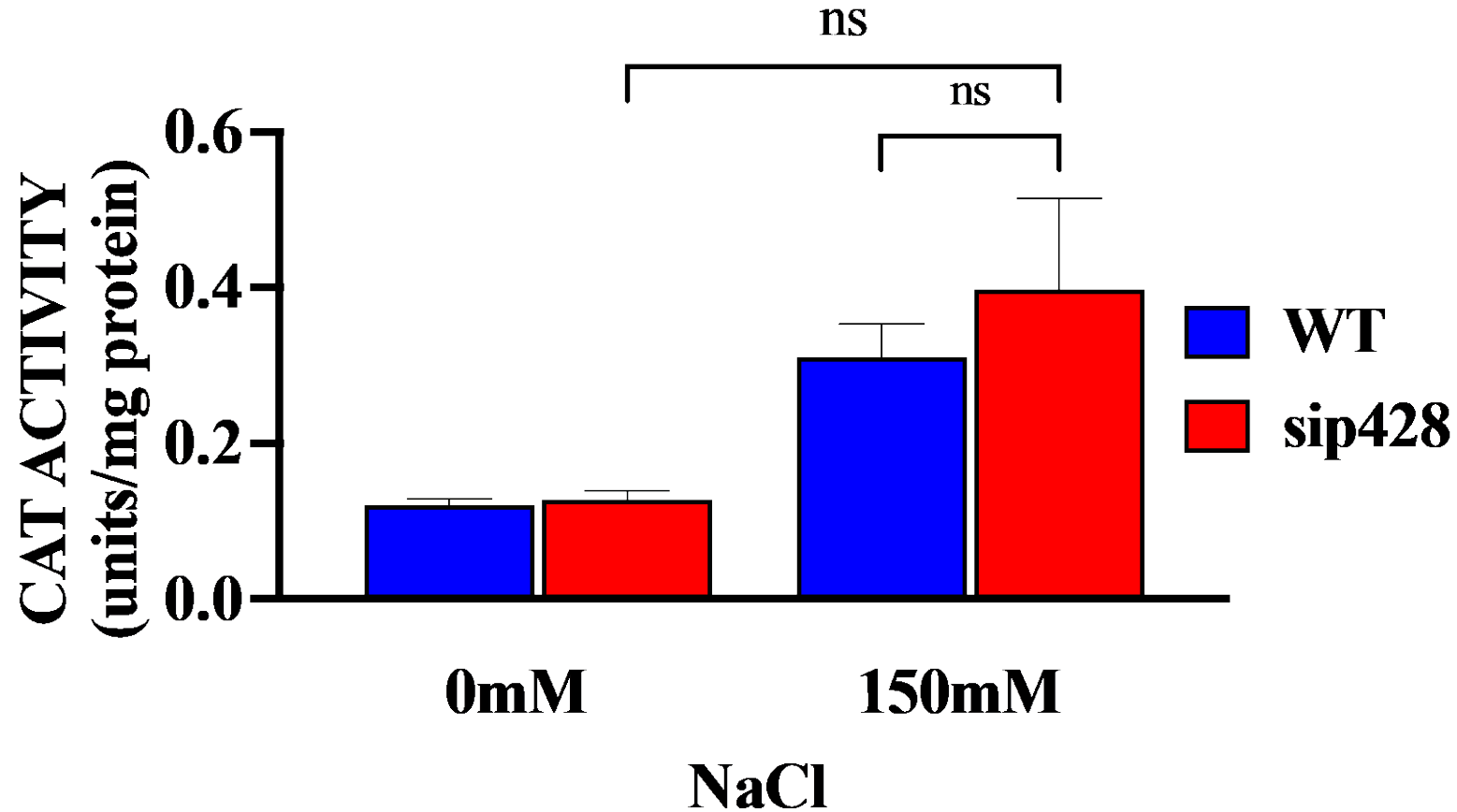
# Drought Stress: Catalase (CAT) Activity



**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

# ACKNOWLEDGEMENTS

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Dr. Ranjan Chakraborty

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QUESTIONS?

