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

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Understanding the Role of SABP2-interacting Protein (SIP) 428: an NAD^+-Dependent Deacetylase Enzyme in Abiotic Stress Signaling of *Nicotiana tabacum*

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ABSTRACT

Understanding the Role of SABP2-interacting Protein (SIP) 428: an NAD^+-Dependent Deacetylase Enzyme in Abiotic Stress Signaling of *Nicotiana tabacum*

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Abiotic stresses are constantly rising and pose a very high risk to global agricultural productivity and food security. Some plants have evolved several innate pathways for defense against these stresses. Hence, understanding stress signaling pathways can help develop crop plants with higher stress tolerance. The salicylic acid-mediated signaling pathway is important in plants experiencing biotic and abiotic stresses. In previous studies, SABP2-Interacting Protein (SIP-428) has been shown to be a negative regular of plant growth under abiotic stress. This study aimed to investigate the roles of SIP-428 in the ROS signaling of tobacco plants. We investigated transgenic RNAi-silenced lines of SIP-428 and wild-type tobacco plants for the activities of guaiacol peroxidase and catalase enzymes in Mannitol and NaCl-stressed plants for 7 and 14 days. Our results showed that SIP-428 plays a significant role in ROS signaling in Mannitol and NaCl-stressed plants via the activities of guaiacol peroxidase.

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CHAPTER 1. INTRODUCTION

Plants are highly vulnerable to both biotic and abiotic stresses because of their inability to move from place to place. As a result, several immune responses and defense signaling pathways are induced in response to these unfavorable conditions. Abiotic stresses such as osmotic, salinity, metals, heavy rainfall, UV-B radiation, and fluctuations in temperature are global threats to agricultural productivity (Khan et al. 2015).

Natural and anthropogenic climate change drivers such as volcanic eruptions, solar irradiance, greenhouse gases, and fertilizers from agriculture and land use practices are constantly increasing global mean temperature. This affects precipitation patterns and may lead to osmotic or inundation (Ullah et al. 2021). Increased temperature influences soil moisture content, rate of evaporation, and water-holding capacity of the soil, therefore restricting the movement and availability of water and nutrients in the soil profile (Onwuka 2018) in the last 25 years of both surface and groundwater flooding (Rahman et al. 2018; Bannari and Al-Ali 2020).

The impacts of climate change such as drought, temperature, flooding, and tropical storms are expected to continue to escalate until 2060 (ENS 2022). Food security and climate change are two major global problems that the world is trying to salvage. With the increasing population, the need to improve stress tolerance in plants via genetic modification or editing to support the global demand for food is expected. The development of crops that show resistance or tolerance to abiotic stresses is highly desirable.

Plants respond to stresses by either altering the gene expression, cellular metabolism, growth rates, or by crop yields (Gull et al. 2019). They can be categorized into biotic and abiotic stresses. Biotic stresses are those caused by living organisms such as viruses, bacteria, fungi,

nematodes, insects, arachnids, and weeds. These organisms deprive their host of its nutrients, thereby leading to growth defects/death, and are major causes of pre- and postharvest losses.

Plant growth regulators (PGRs) or phytohormones are naturally occurring chemicals produced in plants that regulate physiological processes involved in the growth and development of plants. They regulate plant stress by initiating responses under stress (Sabagh et al. 2021). The signaling pathways connect the responses from these regulators and help initiate a biochemical and physiological response after sensing the stress environment. It becomes quite important to identify responsive genes/proteins against abiotic stresses for a better understanding of the abiotic stress response mechanisms in crop plants.

Plants' Defense Against Abiotic Stress

Abiotic stresses affect the plants negatively and lead to about ~ 50% reduction in yield. In response, some plants have evolved interconnected regulatory pathways composed of signaling molecules and gene regulation factors to counter the effect of these stresses (Zhang et al. 2022). There are five general defenses against abiotic stresses in plants, namely: cuticle (acts as an outermost shield that restricts liquid and gas fluxes), unsaturated fatty acids, ROS scavengers, molecular chaperones, and compatible solutes that serve to protect them from various adverse conditions (He et al. 2018).

Phytohormones play a crucial role in mediating abiotic stress tolerance in plants. These include Auxins, Salicylic Acid (SA), Abscisic Acid (ABA), Jasmonic Acid (JA), ethylene, gibberellins, and cytokinin. Generally, these phytohormones act in synergy rather than individually, they can act antagonistically, or additively with other phytohormones and or other signaling factors to regulate plant physiological processes (Zhang et al. 2022).

Auxin is known to be responsible for the regulation of genes associated with biosynthesis, catabolism, and signaling pathways of other hormones while modulating defense and developmental responses (Bari and Jones 2009). Abscisic acid is another important hormone in stress-related responses and other plant growth processes. During drought stress, ABA-mediated stomatal closure decreases the transpiration rate, thereby reducing water loss (Aslam et al. 2022). Advancements in plant genomics have allowed the identification and functional characterization of ABA-dependent genes that are drought-responsive. Reports have also shown that ABA can regulate calcium-dependent protein kinases (CPK) signaling by inducing CPK6 expression under drought stress. CPKs phosphorylate ABA-related TFs, and ABFs/AREBs (ABA-responsive element-binding factors) to enhance their transcriptional functions (Zhang et al. 2020; Park et al. 2008; Lu et al. 2009).

Salicylic acid (another plant hormone) has been widely studied for its contribution to antioxidant metabolism, ROS-signaling, and ROS detoxification during abiotic stress in plants (Hernández et al. 2017). SA has also been shown to be involved in the synthesis and/or signaling of other phytohormones, especially the antagonistic relation between SA and abscisic acid (ABA) or jasmonic acid (JA).

Salicylic Acid in Plant Defense Against Abiotic Stress

SA is a phenolic compound (2-hydroxyl benzoic acid) present in prokaryotes and eukaryotes including plants with differing basal levels among plant species. SA and its derivatives have been widely used as pain relievers in different eras of the world. In 1828, Johann A. Buchner became the first scientist to purify a synthetic derivative of SA (aspirin) from a plant. To date, SA has been characterized from plants belonging to diverse groups, and its roles in the regulation of plant growth and development, especially their responses to pathogen attack,

environmental stresses such as salt and osmotic stress, and others also have been properly documented (Zhao et al. 2017).

SA is synthesized via two distinct pathways: the Phenylalanine ammonia-lyase (PAL) pathway (in the cytoplasm) and Isochorismate synthase (ICS) pathway (in the chloroplast) both originating from chorismate (Lefevere et al. 2020). In the PAL pathway, SA is synthesized from phenylalanine. PAL is an upstream enzyme that leads to many other possibly defense-related compounds. In tobacco plants, SA is synthesized primarily via the PAL pathway (Ogawa et al, 2006) and it was found that the genes encoding the PAL pathway but not the ICS pathway to be upregulated when the leaves of tobacco plants were infected with the tobacco mosaic virus. After synthesis, SA is enzymatically modified either by glycosylation (SAG- SA β-Glucoside, SGE-SA glucose ester), methylation (MeSA- Methyl salicylate or MeSAG), hydroxylation (SA 3-hydroxylase (S3H) and SA 5-hydroxylase (S5H) or via amino-acid conjugation (SA-Asp-Salicyloyl-L-aspartic acid) to allow fine-tuning of its accumulation, function, and/or mobility. Glycosylated forms of SA make the SA inactive and may allow larger SA storage in the vacuoles due to its reduced toxicity. Methylated forms are volatile with better membrane permeability and allow more distal transport (Dempsey et al. 2011). Endogenous and exogenous application of SA and its conjugates have been implicated in alleviating abiotic stresses; drought (Safari et al. 2022), salinity (Khairy and Roh 2016; Patel et al. 2020), heavy metals (Gondor et al. 2022), osmotic, heat stress (Yang et al. 2022) and cold stress (Ignatenko et al. 2019) conferring tolerance to these plants under stress.

Salicylic acid binding protein 2 (SABP2) is an esterase that catalyzes the conversion of MeSA to SA. The role of SABP2 has been established in tobacco plants (Vlot et al. 2008). SABP2 was purified from tobacco plants and was shown to have a high affinity for SA (Du and

Klessig 1997; Kumar and Klessig 2003). The biochemical analysis of the structure of SABP2 shows that it belongs to the alpha/beta hydrolase family, including a catalytic triad with Ser-81, His-238, and Asp-210 (Forouhar et al. 2005). It is needed for the robust expression of systemic acquired resistance (SAR). SABP2-silencing increased susceptibility to virulent pathogens in tobacco plants (Kumar and Klessig 2003). In 2006, Kumar et al. established the fact that SABP2 was an important enzyme in SAR development in tobacco plants. Overexpression of the *LcSABP* SABP2-like gene, cloned from *Lycium chinense* increased drought tolerance in transgenic tobacco via increased activities of antioxidant enzymes (SOD, CAT & APX) (Li et al. 2019). Several interacting proteins of SABP2 have been identified and one of them is the SABP2-Interacting protein, SIP428 (Haq et al. 2020).

SABP2-Interacting Protein (SIP)428

In 2020, Haq et al. described the SIP428 as a Silent Information Regulator (SIR)- 2 family NAD+-dependent deacetylase like enzyme. Sir2 gene family is conserved from bacteria to mammals, and it was reported to have gene silencing and chromosome stability functions (Brachmann et al. 1995). Some Sir2 orthologs (Sirtuins) are histone deacetylase enzymes (NAD+ dependent) that catalyze the β-nicotinamide adenine dinucleotide (βNAD+)-dependent N^{ε}-acyl-lysine deacetylation on histone and non-histone protein substrates (Soccio et al. 2018; Zheng 2020).

Plant Sirtuins are known to have a protective role in genome instability and cell oxidative damage, which are important for plant growth (Soccio et al. 2018), and in other cellular functions, including life DNA repair, metabolism, stress resistance, proliferation, and energy production (Zheng 2020). Sirtuins in plants have been shown to have different enzymatic activity and localization, but their functional characterization is incomplete in most plants. More studies

involved majorly *Arabidopsis thaliana* and *Oryza sativa*, and only two genes (*AtSRT1*, *AtSRT2*, *OsSRT1*, *OsSRT2*) have been identified in both. Downregulation of *OsSRT1* enhanced histone H3K9 acetylation on transposable elements and promoters of hypersensitive response-related genes, which led to DNA fragmentation and cell death, under salinity and drought (Zheng et al. 2016), *AtSRT1*is a negative regulator of plant tolerance to stress and glycolysis but stimulates mitochondrial respiration (Liu et al. 2017).

Histone deacetylation/acetylation controls gene expression by modifying chromatin structure and function, therefore, it is important for many cellular processes such as post-translational modifications of protein at lysine residues. Acylation or deacetylation of the lysine side chain on histone proteins affects their stereoelectronic properties, therefore, facilitates, or weakens DNA's accessibility to transcription machinery (Zheng 2020). Sirtuins are ubiquitous to many subcellular compartments; yeasts and human Sirtuins are localized in the cytoplasm, nucleus, and mitochondria (North and Verdin 2004); SIP-428 was found to be localized in the mitochondria (Thakuri 2018).

ROS Signaling and Abiotic Stress in Plants

Abiotic stresses like salinity, drought, osmotic, heat, and cold can induce oxidative stress in plants by the accumulation of very reactive oxygen compounds called ROS. ROS (Reactive Oxygen Species) are oxygen radicals and their derivatives produced because of the partial or incomplete oxidation of oxygen. They include superoxide anion, $O_2^{\bullet-}$; hydroperoxyl radical, $HO_2^{\bullet}$; alkoxy radical, $RO^{\bullet}$; and hydroxyl radical, $^{\bullet}OH$ and non-radical molecules, hydrogen peroxide, H_2O_2, and singlet oxygen, $^1O^2$ (Hasanuzzaman et al. 2020). In plants, the major sites of production of ROS are chloroplasts, mitochondria, peroxisomes, plasma membrane, and apoplast. ROS targets biomolecules such as proteins, lipids, and DNA by breaking their bonds,

mutation, or proteolytic degradation of these molecules, causing cellular damage (Das and Roychoudhury 2014).

Although ROS are produced under normal cellular metabolism, the plant's innate defense mechanism is constantly regulating ROS production by the actions of a robust antioxidant system. These antioxidants detoxify the ROS, making them less toxic, and these include enzymatic antioxidants localized in different compartments of the cell; superoxide dismutase (SOD, localized in the chloroplasts and mitochondria detoxifying $O_2^{\bullet-}$), catalase (CAT found in the mitochondria and peroxisomes, detoxifies H_2O_2), ascorbate peroxidase (APX, localized in the chloroplast, cytosol, mitochondria, and peroxisomes, detoxifies H_2O_2), Monodehydroascorbate reductase (MDHAR, localized in the chloroplast, cytoplasm, and mitochondria, generates ascorbic acid), dehydroascorbate reductase (DHAR), glutathione reductase (GR localized in the chloroplast, ER, cytoplasm, and mitochondria), and guaiacol peroxidase (GPX localized in the chloroplast, cytoplasm, and mitochondria, detoxifies H_2O_2). Others are non-enzymatic antioxidants including carotenoids, α-tocopherol, flavonoids, proline, reduced glutathione, GSH, and ascorbic acid (most abundant and widely studied). ROS also act as secondary messengers and/ or signaling molecules that use the mitogen-activated protein kinase (MAPK) pathway to transport signals to the nucleus via redox reactions to increase tolerance against abiotic stresses (Hasanuzzaman et al. 2020).

Evidence has established a close connection between ROS metabolism and epigenetic regulation during plant growth and environmental acclimation (Wang et al. 2016). Transgenic tobacco plants overexpressing APX from *Arabidopsis* showed higher tolerance to salinity stress (300mM NaCl) compared to wild-type plants (Badawi et al. 2007). A choline monooxygenase (*BvCMO)* from *Beta vulgaris* overexpression in transplastomic tobacco plants enhanced

tolerance to salt (100 and 150 mM NaCl) and osmotic stresses (300mM mannitol) (Zhang et al. 2008). Transgenic plants overexpressing a theta class glutathione transferase (GST) enhanced tobacco plants' tolerance to osmotic (mannitol) and salinity (NaCl) *in vitro* (Stavridou et al. 2019). Downregulation of Sirtuins-like genes, *OsSRT1* and *OsSRT2* in rice, induced Histone H3K9 acetylation, leading to DNA silencing of many important genes while the overexpression of these genes increased tolerance to oxidative stress (Huang et al. 2007).

Chloroplast, mitochondria, and peroxisome produce excessive ROS under salinity and osmotic stress (Guo et al. 2022).

Hypothesis

Previous studies indicate that SIP428 is a negative regulator of tobacco plant growth under abiotic stress (Oviavo 2021). It remains largely unknown how SIP428 regulates abiotic stress signaling in plants. It is expected that SIP428-silenced plants show increased or higher activities of antioxidant enzymes/ ROS scavengers than wild-type tobacco plants under abiotic stress (in NaCl or Mannitol stresses *in vivo).*

Hypothesis (H_1): SIP428 plays a regulatory role in the ROS signaling pathway of tobacco plants under abiotic stress.

To test this hypothesis, the levels of two antioxidant enzymes: guaiacol peroxidase and catalase will be assessed in SIP428-silenced and control tobacco plants after treatment either with NaCl (for salinity stress) or with Mannitol (for osmotic stress).

Alternate Hypothesis (H_2): SIP428 does not play a significant regulatory role in the ROS signaling pathway of tobacco plants under abiotic stress.

It is very likely for the levels of the two antioxidant enzymes: (guaiacol peroxidase and catalase) in control and SIP428-silenced tobacco plants after treatment

either with NaCl or with Mannitol do not show any significant difference. This could be because this investigation is based solely on ROS detoxification by the antioxidant enzymes, and both plants under abiotic stress can initiate the expression/activation of these enzymes for defense during stress signaling.

CHAPTER 2. MATERIALS AND METHODS

Plant Materials

Tobacco plants, wild-type *Nicotiana tabacum* cv. Xanthi-nc NN (XNN) and SIP428-silenced in the *Nicotiana tabacum* cv. Xanthi-nc NN background (transgenic line #MSI-2-7 SIP-428-silenced (Thakuri 2018) was used in this study. Transgenic tobacco lines silenced in SIP428 expression were previously generated via RNAi (Thakuri 2018). All tobacco plants were grown from seeds in a controlled environment in a growth chamber.

Chemicals/Reagents

Murashige and Skoog (MS), Phyto agar (PlantMedia), Sucrose (Fisher Scientific), Sodium chloride (NaCl) powder (Fisher Scientific/ Fisher Biotech), Mannitol (Fisher Scientific), Ethyl alcohol (Acros Organics), bleach solution (household), EDTA(Fisher Scientific), Bradford reagent solution (Bio-Rad), Potassium hydroxide (Fisher Scientific), Bovine Serum Albumin (BSA) powder (Sigma), Sodium phosphate monobasic and dibasic anhydrous (Fisher Scientific), Hydrogen peroxide (Fisher Scientific), Guaiacol solution (Acros Organics), and Gamborg B5 vitamin (recipe in Appendix C).

Materials/Equipment

Evolution 300 Spectrophotometer (Thermo Fisher Scientific), BioTek Synergy HT Microplate reader, Costar 96-microplate well plates, Elkay Ultra-Vu disposable Cuvettes, Tissue culture room, Conviron growth chamber, Airegard Laminar Airflow Work Station (Nuaire) with steri 350 for sterilization, Fisher brand Petri plate/ dish, Fisher Scientific Isotemp 210 water bath, Sterilelink Autoclave (Steris Amsco Century SG-120), soil, 1.5 mL Eppendorf tubes, Fisherbrand micropipettes and specialty tips, Finn pipettes multi-channel pipette, ThermoFisher nunc tubes 15mL, Gilford Vacuum receiver 3021, Fisher Scientific Accumet (AE150) pH meter,

3M Micropores (Deutschland GmbH), Labquake rotating shaker (Barnstead | Thermolyne), Fisher Vortex Genie 2, Grinders- MP FastPrep -24, IKA RW20 digital or hand held grinder, Biospec 2.0 mm diameter Zirconia, and Sorvall Biofuge pico Centrifuge.

Growth Conditions

Tobacco seeds were sown on wet autoclaved soil in 4 x 4-inch square plastic pots and covered with a transparent plastic cover. Initially, the sown seeds were kept in a cold room (at 4-8°C) for 72hrs before transferring to a plant growth chamber (PGW 36, Conviron, Canada) set at 22°C and a 16-hour light cycle maintained with a light intensity of about 200 $\mu molm^{-2}sec^{-1}$. About 7-10 days later, two seedlings (a two-cotyledon stage) were transferred to 4 x 4-inch pots and grown for an additional 3 to 4 weeks. Each young tobacco plant was transferred to a single 7" pot and allowed to grow for 7-10 days. For stress treatments, specific stressors (NaCl or mannitol) were applied to the soil and plants were grown for 2-3 weeks before using them for biochemical analysis.

Growth Conditions: Tissue Culture

Wild-type *Nicotiana tabacum* cv. Xanthi-nc NN (XNN) and SIP428-silenced seeds (50-100) were surface-sterilized by adding 1mL 70% ethanol followed by shaking for 1 minute. This and the subsequent steps were carried out in a Laminar Airflow Workstation. The ethanol solution was removed and 1mL of 20% bleach (commercial household) was added to the seeds. The tubes were subjected to gentle shaking for 20 mins on a rotating shaker. The bleach solution was removed, and the seeds were rinsed with 1mL sterilized autoclaved water for 1 minute. The rinsing step was repeated 3 times or more. The surface-sterilized seeds were transferred to an autoclaved filter paper and allowed to dry in the laminar flow hood. The seeds were individually transferred to MS media plates (see appendix #B for recipe) using sterilized forceps while also

spacing them at approximately 1cm apart. Finally, these plates were sealed with parafilm or micropores tape and wrapped in aluminum foil, and placed in a cold room (4°C) for 72 hrs. After 72 hours, the aluminum foil from the plates was removed and the plates were transferred to a lighted plant growth room. The seeds were allowed to germinate. After germination (7 days after cold treatment), seedlings with two cotyledons were transferred to MS media plates containing abiotic stress-inducing chemicals.

Abiotic Stress Treatment

Salinity Stress

Various concentrations (0-200mM) of NaCl-containing MS media plates were prepared and used in this experiment. Young seedlings (7-10 days old) were transferred to the MS media plates containing various concentrations of NaCl and incubated under light for an additional 7-10 days. The plants were observed for 7-14 days respectively. On day 7 and day14, the leaves were harvested (~100mg) and snap-frozen in liquid N_2 and stored at -80°C for enzyme analysis. The concentrations of NaCl used in this study were previously standardized (Oviavo 2021).

Osmotic Stress

Various concentrations of mannitol (0-200mM) were added to the MS media plates to induce osmotic stress in plants growing for seven (7) and fourteen (14) days respectively. These concentrations were standardized in a previous study (Oviavo 2021). The biochemical analysis of the activities of catalase and peroxidase enzymes were measured using spectrophotometric assays as described in the methods below. Plant leaves are collected at 7 and 14 days respectively after stress.

Leaf Extract Preparation for Antioxidant Enzyme Assays

Fresh leaf tissues (100mg) were collected from plants (WT and SIP428-silenced lines) and frozen in liquid nitrogen to prevent proteolytic activity and stored at -80°C. These leaf tissues were homogenized by grinding in 1ml extraction buffer (0.1M phosphate buffer, pH 7.0, containing 0.5mM EDTA), while keeping everything on ice. The samples were centrifuged at 13,000×g for 20 min at 4°C and the supernatants were transferred to new tubes, kept on ice, and used for further analysis of the enzyme activities (crude leaf extracts were prepared fresh and assays conducted within a few hours and extracts were always kept on ice).

Protein Quantification Using Bradford Reagents

The amount of protein present in each sample was determined using Bradford Reagent to measure absorbance at 495nm and a BSA standard curve to measure its concentration (mg/ml). For Bradford Test, 800µl of milliQ water was added to 1.5 ml Eppendorf tubes and a known volume of leaf extract was transferred to the same tube and mixed by inverting several times. Bradford reagents (200µl) was then added to the tubes and mixed by vortexing. After five minutes, the absorbance was measured at 595nm using a spectrophotometer. The blank was the solution/ tube with no protein sample (Bradford 1976). The equation of the BSA standard curve (Fig. 1) was used to quantify the unknown protein concentration (see Appendix #B for full recipe).

Catalase Assay (CAT) Mix

Potassium phosphate buffer (50 mM, pH 7.0); Buffer was prepared using Milli-Q water (Biocel Millipore Milli-Q) and stored at 4°C until usage, Fisher Scientific H_2O_2 solution (20 mM), and quantified protein from leaf extract.

CAT Assay Procedure

CAT activity was determined according to Aebi 1984. The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm in a UV/Vis spectrophotometer or Gen 5 microplate reader. The 1 mL CAT assay (in a spectrophotometer) or 200µl (in a microplate reader) mixture contained 1 mL extract (mixture) in 50 mM potassium phosphate buffer (pH 7.0), 20 mM H_2O_2, milliQ water and a known protein concentration from each sample or replicates (see appendix #B for full recipe). The control (blank) was the solution without protein. The extinction coefficient of H_2O_2 (40 mM^{-1} cm^{-1} at 240 nm) was used to calculate the catalase activity which was expressed in terms of (millimoles of H_2O_2 per minute) units per milligram protein.

Peroxidase Assay (POD) Mix

Potassium phosphate buffer (60 mM, pH 6.1); Buffer was prepared using Milli-Q water (Biocel Millipore Milli-Q) and stored at 4°C until usage, Fisher Scientific H_2O_2 solution (2 mM), Acros Organics Guaiacol Solution (16 mM) and quantified protein from leaf extract.

POD Assay Procedure

POD activity was measured by the increase in absorbance as guaiacol gets oxidized into tetra guaiacol at 470 nm (Castillo et. al. 1984). The 1 mL POD assay (while using a spectrophotometer) or 200µl (when using a microplate reader). The 1 mL reaction mixture contained 60mM potassium phosphate buffer (pH 6.1), 16mM guaiacol, 2 mM H_2O_2, and a known protein concentration from each sample or replicates (see appendix #B for full recipe). The control (blank) reaction mixture was without protein. The extinction coefficient of tetra guaiacol (26.6 mM^{-1} cm^{-1} at 470 nm) was used to calculate the peroxidase activity that was expressed in terms of Units per milligram protein.

Enzyme Activity

The activities of peroxidase and catalase enzyme were calculated using the formula (2.1):

Enzyme Activity (Units/mg protein) = $A \frac{V_C}{\varepsilon\, d\, c}$

Where, A= Absorbance at a particular point in time; ε = millimolar extinction coefficient

For the CAT activity at 240 nm the specific extinction coefficient, ε= 40 mM-1cm-1

For POD activity, tetra-guaiacol formation at 470 nm, the specific extinction coefficient, ε= 26.6 mM-1cm-1

d = light path (cm)= 0.56cm (96-well plates microplate), 1cm (spectrophotometer cuvettes)

V_c = reaction volume (ml) = 0.2 ml (microplate reader) and 1ml (spectrophotometer)

c = protein concentration of leaf extracts

Chemical reactions occurring in each assay solution:

For Catalase Assay: $2\ H_2O_2 \xrightarrow{\textbf{CAT from sample}} O_2 + H_2O$

For Guaiacol Peroxidase Assay: Substrate (Guaiacol) + $H_2O_2 \xrightarrow{\textbf{POD}}$ Tetra-guaiacol + H_2O

Statistical Analysis

Data were analyzed using one-way ANOVA, and the means were compared using the Tukey's test at a confidence level of 0.05. GraphPad Prism 9.0 Software was used for conducting

this analysis. The graphs for mean comparison were drawn by Microsoft Excel version 16.71 software.

CHAPTER 3. RESULTS AND DISCUSSION

BSA Standard Curve for Protein Quantification

A known concentration of BSA was prepared (10mg/ml) and several dilutions of this concentration was made (0.5 - 4mg/ml). The sample without the BSA served as a blank control. The mean absorbance of three replicates of each concentration was measured at 595 nm and the trend and equation of line was added using Excel. Fig. 1 shows a graph of the mean absorbance at 595 nm against different standard concentrations of BSA representing the linear relationship between enzyme concentration and absorbance (Beer-Lambert's law). The equation was used to quantify the concentration of proteins in leaf samples.

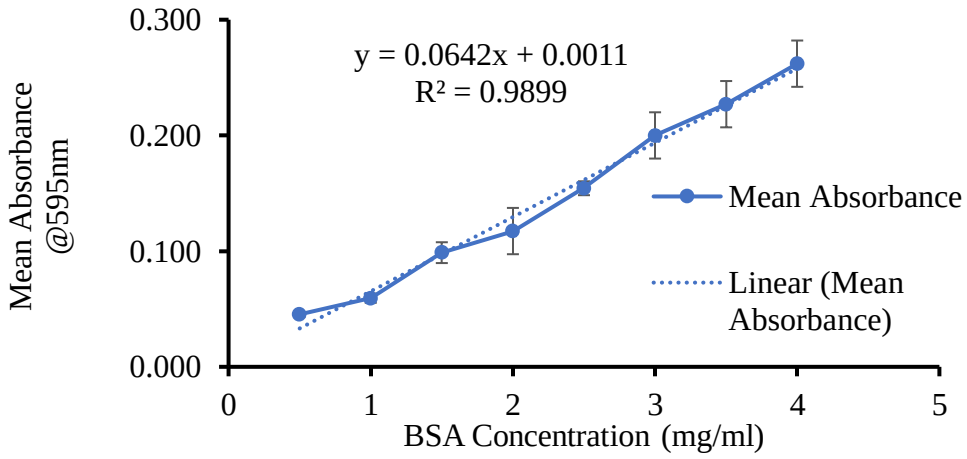


Figure 1. BSA Standard Curve.
A graph of mean absorbance (n=3± S.E) at 595nm using various concentrations of BSA was prepared.

Osmotic Stress

Peroxidase Activity in Mannitol- Stressed Plants

The peroxidase activity in mannitol-stressed plants were examined in tobacco plants (WT - control) and sip428 - SIP428-silenced plants) as described above. After exposure to mannitol

for seven days, SIP428-silenced plants showed lower peroxidase activities than WT plants in both 150mM and 200mM Mannitol (Fig. 2). Interestingly, they show significantly higher peroxidase activity in 100mM and 175mM Mannitol than WT (Fig. 3). At 14 days, significantly higher peroxidase activities were observed in 150mM mannitol-stressed SIP428-silenced plants compared to the WT plants (Fig. 4) and (Fig. 5) at $p < 0.05$ (Data from two independent experiments). This suggests that the regulatory role of SIP428 in ROS signaling of plants under osmotic stress might be dependent on the concentration and length of exposure to mannitol, because 14 days SIP428-silenced plants under 150mM mannitol showed higher peroxidase activity while 7 days only showed significant enzyme activities in 100mM and 175mM but not in 150mM mannitol.

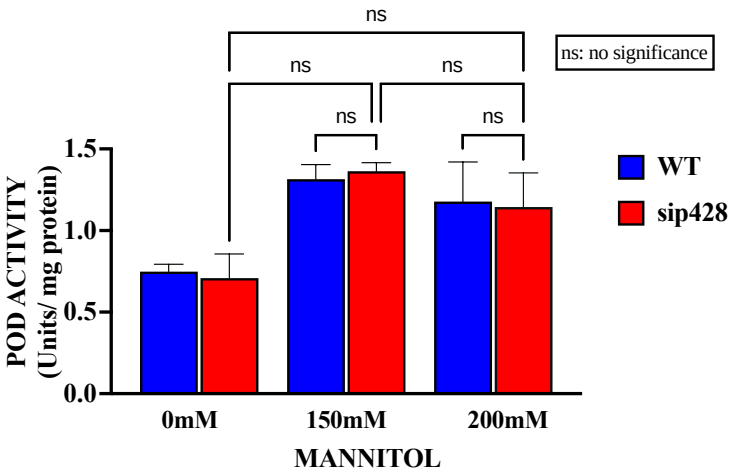


Figure 2. Peroxidase activity in Mannitol stress (0, 150mM & 200mM) for 7 days. N=2, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9; ns means not significant.

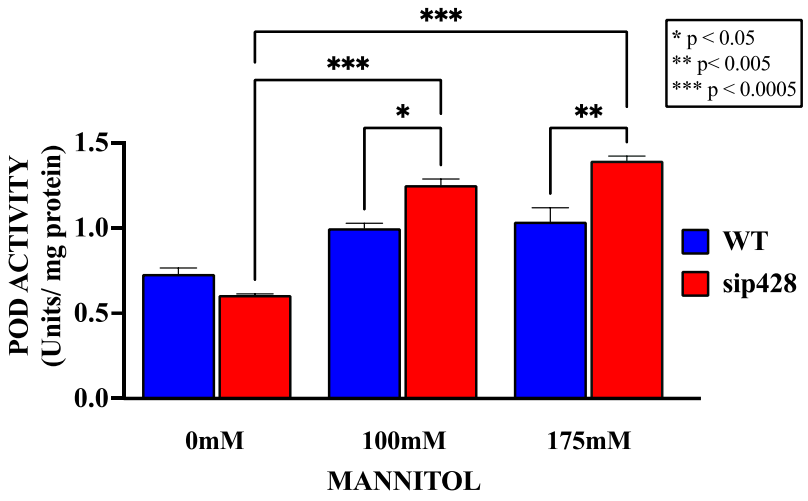


Figure 3. Peroxidase activity in Mannitol stress (0, 100mM & 175mM) for 7 days.
N=2, mean+ SEM. Statistical significance between groups was determined via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

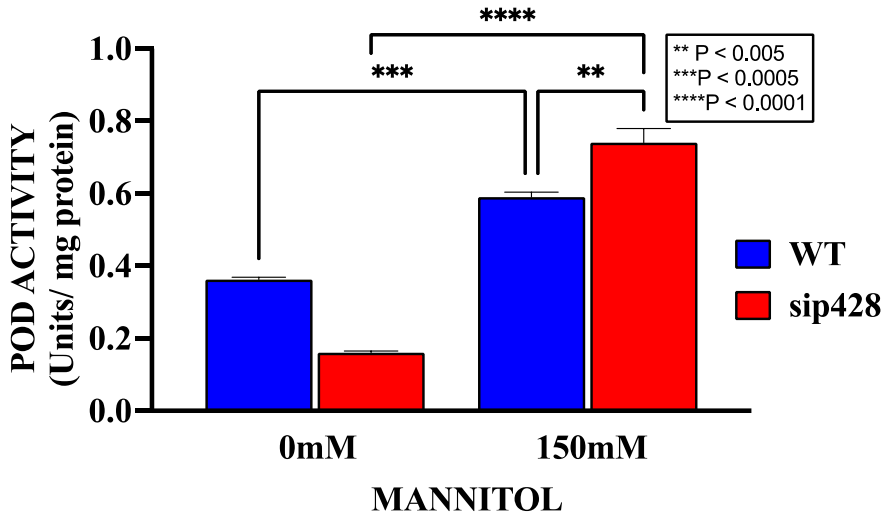


Figure 4. Peroxidase activity in Mannitol stress (0 & 150mM) for 14 days.
N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0.

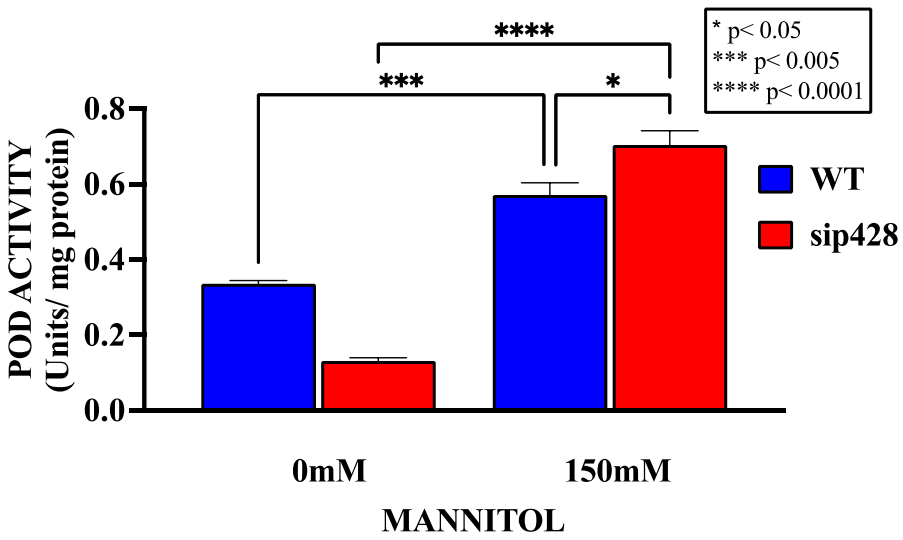


Figure 5. Peroxidase activity in 14 days Mannitol-stressed plants (100 and 150mM Mannitol) N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

Catalase Activity in 7- and 14-days Mannitol-Stressed Plants

The catalase activity in mannitol-stressed plants were investigated to understand the significance of SIP428 in ROS detoxification in WT (control) and SIP428-silenced tobacco plants via the use of Catalase (antioxidant enzyme). The Catalase activity in 7-days mannitol-stressed, SIP428-silenced were lower than those of WT, but these were not significantly different statistically from those of wild-type plants at $p<0.05$ (Fig. 6). In 14 days, mannitol-stressed SIP428-silenced plants', the catalase activities were higher than those of wild-type plants in 200mM mannitol, however this wasn't statistically significant at $p<0.005$ as presented in (Fig. 8) but the differences between the CAT activities of SIP428-silenced plants in 0 and 150mM mannitol were statistically significant at $p<0.005$ as seen in (Fig. 7).

Upon exposure to osmotic stress, plants adjust their morphology (enhanced shoots and roots) and developmental changes (ion transport, carbon metabolism and solutes synthesis) in response. Mannitol is often used induced osmotic stress in plants *in vitro*, and it can act as an osmolyte or compatible solute, or both when accumulated. As a compatible solute, it prevents

inactivation of metabolism by accumulating in the cytosol. High accumulation can become toxic triggering the antioxidant machinery including SOD, CAT, POD, and other enzymes (Hasanuzzaman et al. 2020). Osmotic stress induces oxidative stress by increasing the levels of H_2O_2 and singlet oxygen. Under 150mM mannitol, the activities of polyamines, SOD and POD were significantly increased in potato cultivars (*in vitro*) (Sajid and Aftab 2022). OsSRT1 (an orthologous sirtuin present in rice) was found to target transposable elements, regulates H3K9 acetylation and expression stress-related and metabolism genes (Zhong et al. 2013). Osmotic stress (176mM) increased the activities of CAT, SOD, APX, and POD in broccoli sprouts (Kiani et al. 2018).

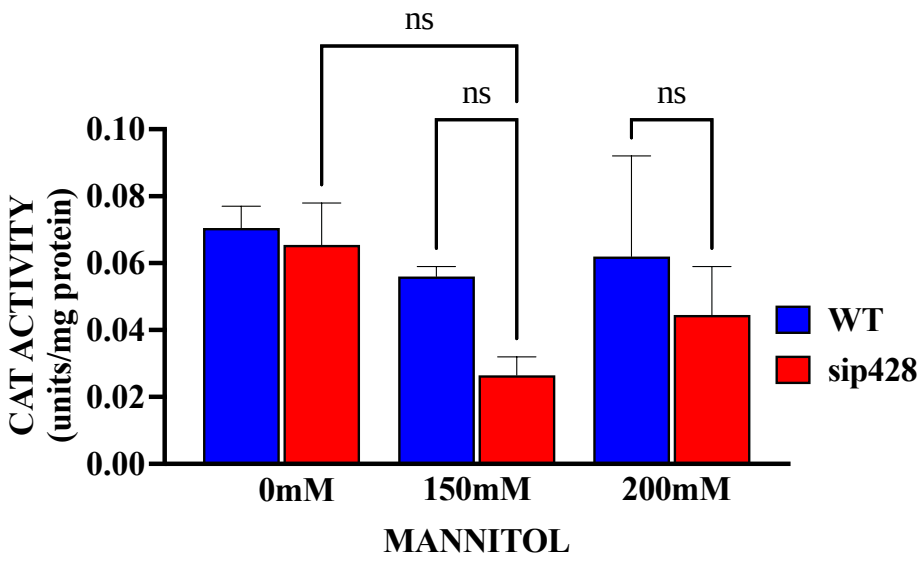


Figure 6. Catalase activity in 7 days mannitol stressed plants.
N=4, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9

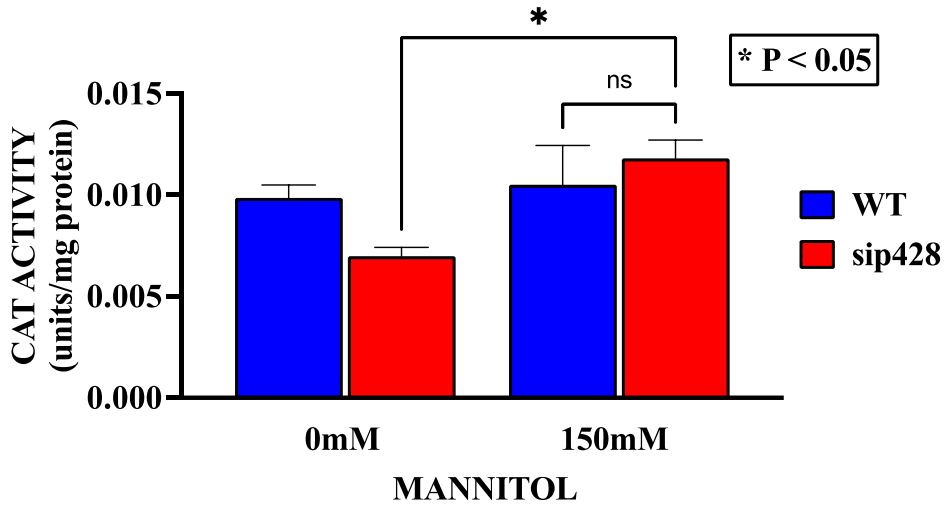


Figure 7. Catalase activity in 14 days mannitol stressed plants.
N=4, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0. ns= no significance

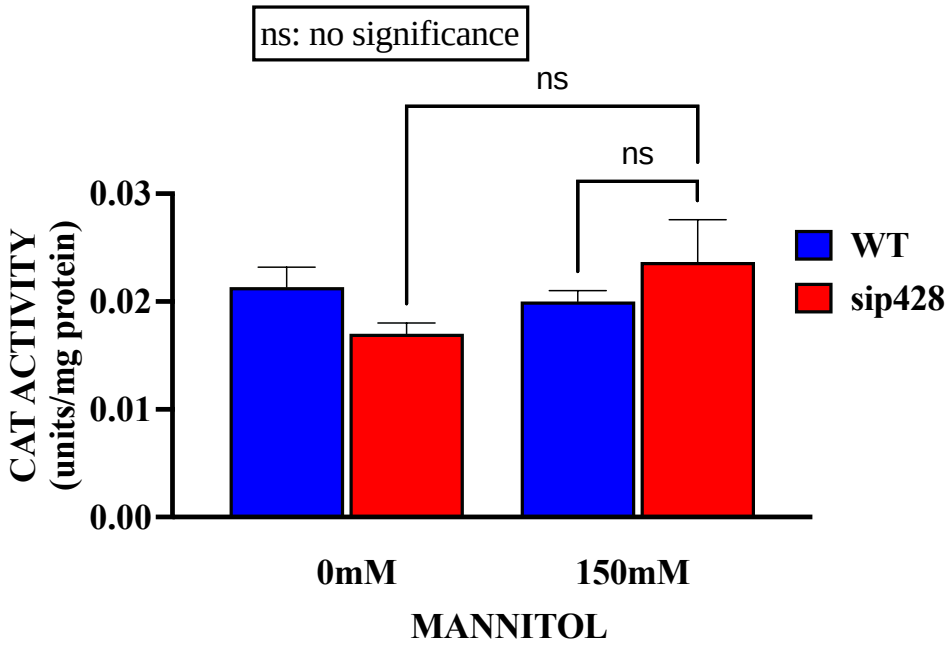


Figure 8. Catalase activity in Mannitol stressed plants for 14 days.
N=4, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

Salinity Stress

Peroxidase Activity in NaCl- Stressed Plants

The peroxidase activity in NaCl-stressed plants were investigated for the regulatory functions of SIP428 in salinity stress of tobacco: WT (control) and SIP428-silenced (sip428) plants as described in the methods section. When SIP428-silenced plants were exposed to NaCl-stress for 7 days, they exhibited lower peroxidase activities than WT plants in 200mM NaCl, but this was statistically significant at $p<0.05$ (Fig. 9). However, it was important to note that after reducing the NaCl to 100mM, the peroxidase activity in SIP428-silenced plants became significantly higher than those in WT plants (Fig. 10). Interestingly, SIP428-silenced plants that were exposed to 200 mM NaCl-stress for 14 days had significantly higher peroxidase activities than WT plants (Fig. 11 & 12: two independent experiments; In Fig. 11: There is a significant difference in the POD activities between sip428 and WT plants under 200mM NaCl and between sip428 plants in 0 & 200mM NaCl, In Fig. 12: The differences in the POD activity between WT and sip428 plants were not significant; however, there is a significant increase between sip428 plants in 0 & 200mM NaCl). Suggesting that SIP428 might also be playing a role in ROS-mediated signaling in plants under salinity stress induced with higher NaCl concentration (200 mM) via the activities of peroxidase activity when exposed for 14 days. While this is not clear when the plants were exposed for only a short duration (7 days). High salinity stress increases ion toxicity (Na^+) and oxidative damage as secondary effects, and it might take a while for the antioxidant machinery's defense to get initiated because of increased ROS and oxidative agents.

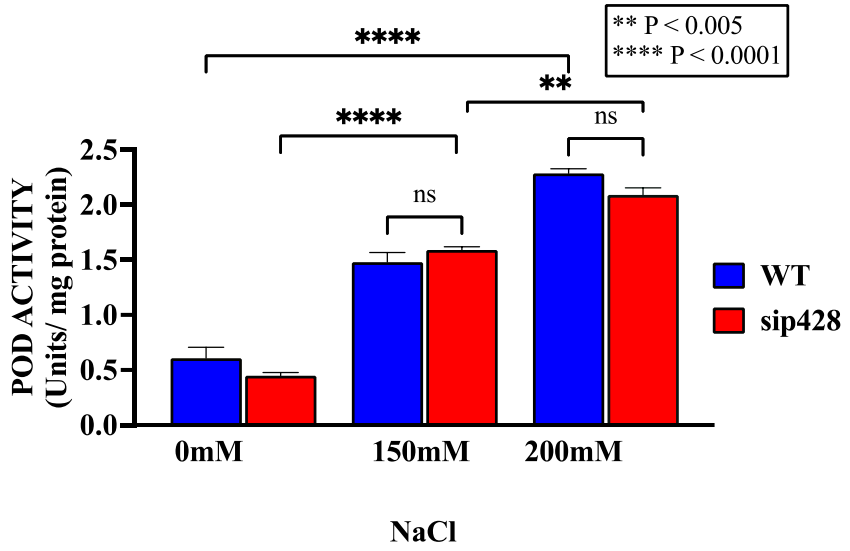


Figure 9. Peroxidase activity of in NaCl stressed plants (0, 150mM & 200mM) for 7 days. N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism

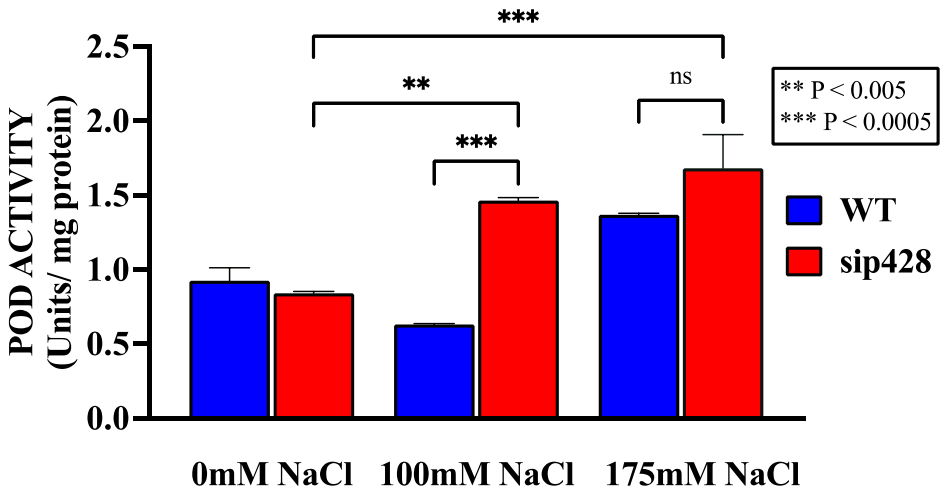


Figure 10. Peroxidase activity in NaCl stress (0, 100mM & 175mM) for 7 days. N=4, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0.

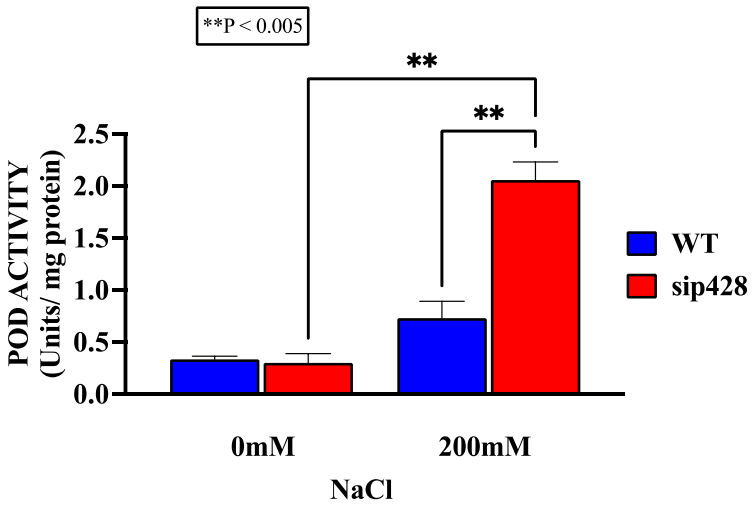


Figure 11. Peroxidase activity in NaCl stressed plants (0 & 200mM) for 14 days.
A representative experiment with N=2, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

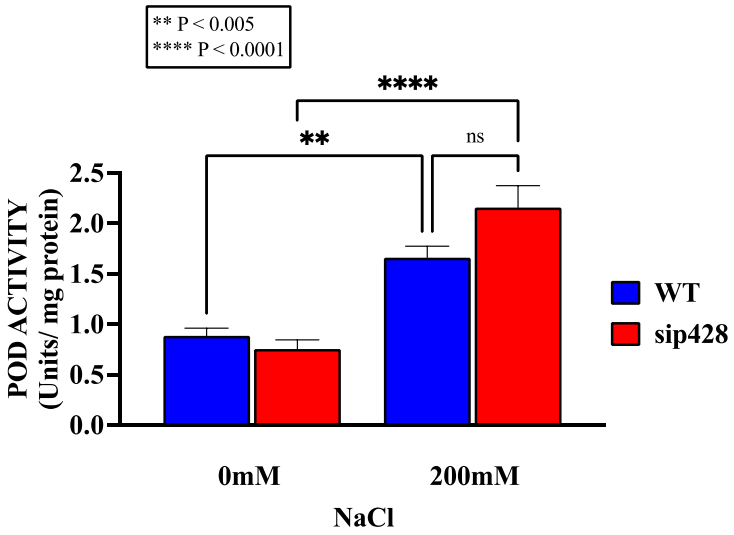


Figure 12. Peroxidase activity in NaCl stress (0 & 200mM) for 14 days.
This is another independent experiment showing the same trend; N=4, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0.

Catalase Activity in NaCl- Stressed Plants

The catalase activity in NaCl-stressed SIP428-silenced tobacco plants were investigated to understand the significance of SIP428 in ROS detoxification. SIP428-silenced plants had

significantly higher catalase activity in 100mM NaCl (Fig. 13) when exposed for 7 days, but catalase activity reduced significantly in 200mM NaCl (Fig. 14). As seen in Fig. 13 and 14, there was a slight increase in catalase activity in 14 days- NaCl stressed SIP428-silenced plants in 150mM NaCl, however this increase was not significantly different from those of wild-type plants in the same concentration or transgenic plants in 0mM NaCl. It can be inferred from these results that SIP428 might have regulatory function in stress signaling via the use of catalase for ROS detoxification significantly in low concentration (100mM) and for shorter number of days (7 days). At 150mM NaCl, no significant differences were noticed between SIP428-silenced and WT plants (Fig 15).

During high salinity stress, the uptake of Na^+ increases while decreasing the uptake of K^+, and this disrupts the ionic homeostasis in plants, inhibiting the absorption of other nutrients and ions, which results in nutrient deficiency, ion toxicity (Mickelbart et al. 2015) and ROS accumulation (Hasanuzzaman et al. 2020). Plants undergo metabolic reprogramming to maintain osmotic homeostasis and activation of signaling pathways by changing the functions of their primary and secondary metabolites. Primary metabolites such as sugars and amino acids can act as osmolytes (hexoses, disaccharides, and oligosaccharides) and osmo-protectants (proline) under salinity stress (Zhang et al. 2011). The gene expression and enzyme activities of SOD, POD, and CAT increased when two *Asparagus* cultivars were subjected to salinity stress (Guo et al. 2022).

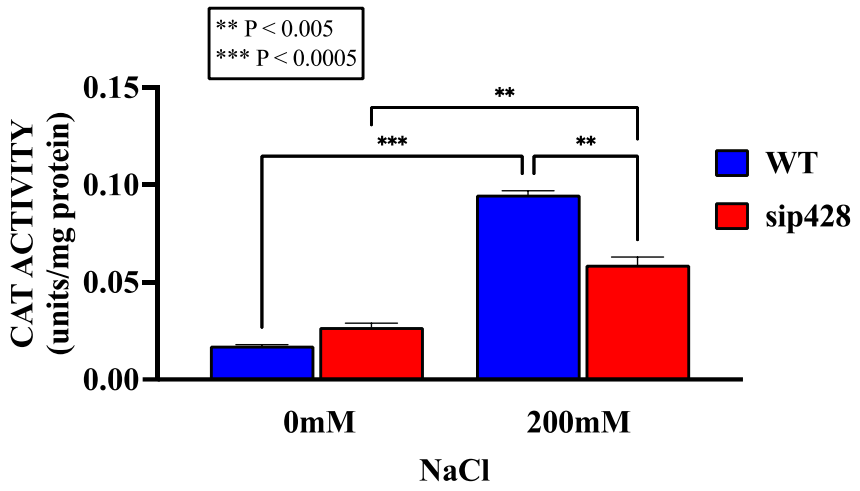


Figure 13. Catalase activity in NaCl stress (0 & 200mM) for 7 days.
N=2, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

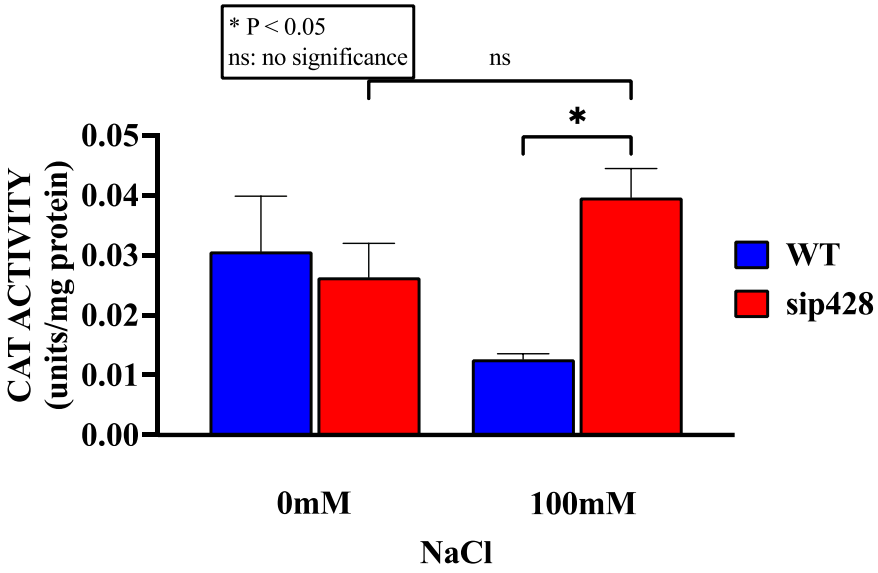


Figure 14. Catalase activity in NaCl stress (0 & 100mM) for 7 days.
N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.

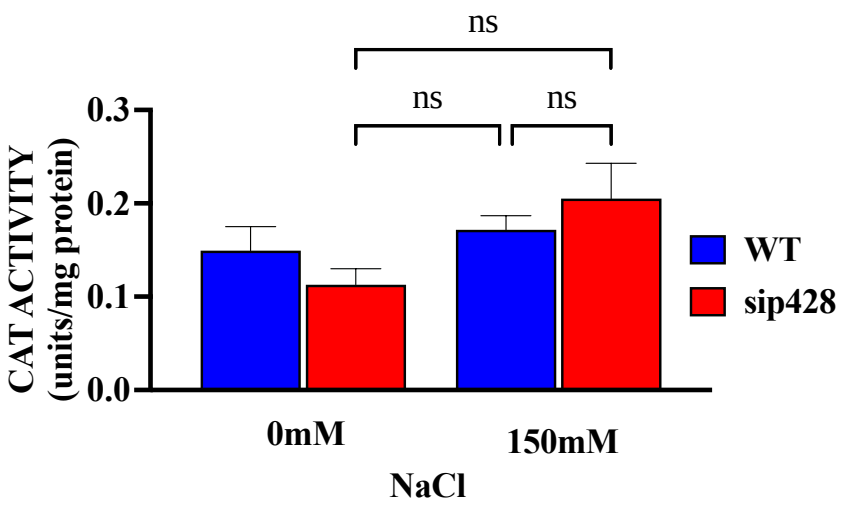


Figure 15. Catalase activity in NaCl stress (0 & 150mM) for 14 days.
An independent experiment using N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0.

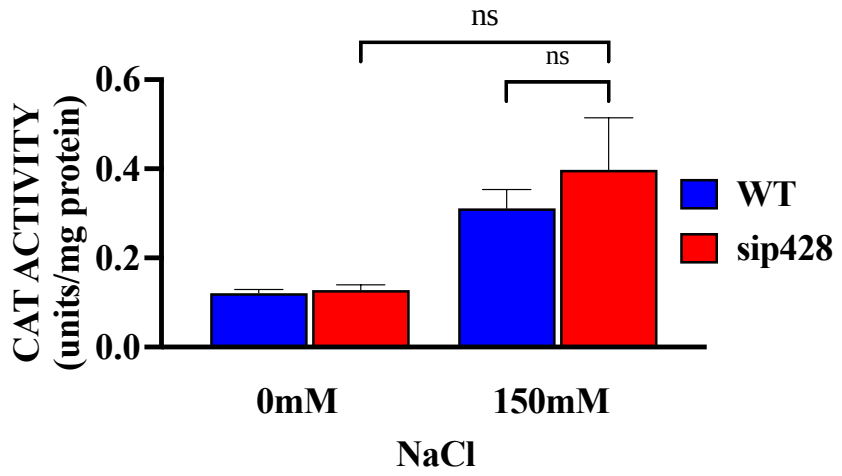


Figure 16. Catalase activity in NaCl stress (0 & 150mM) for14 days.
This is another representative experiment showing a similar trend; N=3, mean + SEM. Statistical significance between groups was investigated via one-way ANOVA at $p < 0.05$ using Tukey's post Hoc test with GraphPad Prism 9.0

CHAPTER 4. SUMMARY AND FUTURE DIRECTIONS

Plants regularly experience abiotic stresses caused by the ever-changing environment resulting in reduced crop yield and productivity. Salinity and osmotic stress are two major abiotic stresses that can reduce water potential in plants, increase ion toxicity, and ROS accumulations, leading to oxidative stress. In response, plants have evolved defense mechanisms involving transcriptional modifications via several signaling pathways (Patel et al. 2020b). Understanding these pathways will allow plant molecular biologists and biochemists to genetically engineer plants that are more tolerant to most abiotic stresses.

This research investigated the regulatory role of SIP428, an NAD^+-dependent deacetylase in ROS-mediated salinity and osmotic signaling in tobacco plants. The enzymatic activities of two important antioxidant enzymes, CAT & POD, that detoxify ROS, specifically H_2O_2 in the chloroplasts were measured in the leaves of stressed plants. The results presented in this study suggest that SIP428 is playing a significant role in mediated stress tolerance via modulating the peroxidase activities. This effect was more apparent in plants treated with higher levels of stressors (both osmotic and salinity) and for a longer duration of exposure. When plants undergo abiotic stress, they generate and accumulate a large amount of ROS that their antioxidant defense system cannot detoxify. This increased level of ROS leads to oxidative stress which can trigger apoptotic-like programmed cell death (Tripathy and Oelmüller 2012).

Studies have reported that the activities of antioxidant enzymes vary based on the amount of stressors, time of exposure, and the developmental stages of plants. For example, 150mM NaCl and 268mM mannitol induced contrasting antioxidant responses for *POD*, *APX*, and *CAT* genes as they increased significantly at high salinity (150mM NaCl) but decreased with mannitol (268 mM Mannitol) (Cunha et al. 2016) and that these antioxidant activities ameliorate abiotic

stress by correlating their increase with decreasing ROS content (Farooq et al. 2021). Changes in the redox status of the cell regulate many transcription factors and enzymes which are quite important for cellular signaling and in epigenetic modifications such as DNA methylation, chromatin structure and remodeling and histone modifications (Ramakrishnan et al. 2022).

SIP428 belongs to the Sirtuins family that use cellular levels of NAD^+ and NADH/ NAD^+ ratio for their deacetylase activity when they undergo oxidative stress. Under oxidative stress, some protein kinases can introduce post-translational modifications into the *SIRT2*, causing the dysfunction of the gene; phosphorylation of this gene increases acetylation (Lau et al. 2014). After NAD^+ -dependent deacetylation by Sirtuins, nicotinamide and 2-0-acetyl-ADP ribose are generated as by-products (Tanner et al. 2000); and studies have shown that these products can inhibit the enzymatic functions of these deacetylases. Under abiotic stress conditions, this ADP-ribosylase activity was linked to the DNA damage repair pathway by the activation of the enzyme, poly (ADP-ribose) polymerase 1 (PARP1) (Mitra and Dey 2022). Increased ROS accumulation under stress causes DNA breakage and this is sensed by PARP1, which transfers ADP-ribose units to these damaged DNA regions, assembling other damage repair pathways (Murata et al. 2019). However, high PARP activity can lead to cell death because PARP depletes NAD^+ content, which forces plants to synthesize more NAD^+ and as a result more energy (ATP) is expended towards its production for other signaling pathways. (Ramakrishnan et al. 2022) Therefore, silencing this deacetylase protein (SIP428) could have reduced PARP-mediated NAD^+ utilization, leading to increased tolerance to oxidative stress. As silencing PARP in both *Arabidopsis thaliana* and oilseed rape (*Brassica napus*) increased tolerance to osmotic, heat, and light stresses (Vanderauwera et al. 2007).

Also, since SIP428 is an important interacting protein in the SABP2 pathway, its silencing can affect SA availability, which can also increase the activities of antioxidant enzymes and improve osmotic stress (La et al. 2019) and or salinity stress (Lee et al. 2010). In 2012, Choi et al. showed using *Arabidopsis* that HDA1 class deacetylase represses the gene expression in SA biosynthesis and SA-mediated defense-associated pathogenesis-related (PR) proteins. Also, when oxidative stress was induced via pathogen attack, the activities of HDA19 (deacetylation of the promoter regions), was significantly reduced and this led to promoter acetylation, and increased SA accumulation and PR protein expression, initiating a defense response. Endogenous SA can be measured to determine if SIP428 modulates its levels to mitigate abiotic stress tolerance.

Further work is needed to identify ROS metabolism-related genes during osmotic and salinity stress through genome-wide, transcriptome, and metabolomic analysis. Changes in gene expression patterns of stress-regulated cis-acting elements, and stress-responsive transcription factors have been linked to responses during abiotic stress as the translation of these genes can be used to prevent from oxidative damage (Singh et al. 2019). Also, it is important to note that ROS signaling is in three parts; generation, accumulation, and detoxification (Huang et al. 2019). We investigated ROS detoxification in this current study, however, SIP428 might be playing a more significant role in either generation or accumulation based on its subcellular localization. Thakuri in 2018 investigated the subcellular localization of SIP428 using confocal microscopy and subcellular fractionation and concluded that it was localized in the mitochondria as was reported for *AtSRT2*. However, recent *in silico* analysis predicted SIP428 to be localized in the chloroplast (#Appendix C). Since the chloroplast generates more ROS under stress, the presence of SIP428 in the chloroplast might play a significant role in ROS generation under abiotic stress. Another

mechanism that can be investigated further is the ROS detoxification using non-enzymatic antioxidants, especially proline and ascorbic acid pathways as these two play very important roles in scavenging ROS during abiotic stress (Sun et al. 2020).

REFERENCES

Aebi H. 1984. Catalase *in vitro*. Methods in Enzymology, 105:121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Aslam MM, Waseem M, Jakada BH, Okal EJ, Lei Z, Sohaib H, Saqib A, Yuan W, Xu W, Zhang, Q. 2022. Mechanisms of Abscisic Acid-Mediated Drought Stress Responses in Plants. Int. J. Mol. Sci. 23:1084. https://doi.org/10.3390/ijms23031084

Badawi GH, Kawano N, Yamauchi Y, Shimada E, Sasaki R, Kubo A, Tanaka K. 2004. Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol. Plant. 121:231–238. doi: 10.1111/j.0031-9317.2004.00308.x.

Bannari A, Al-Ali ZM. 2020. Assessing climate change impact on soil salinity dynamics between 1987-2017 in arid landscape using Landsat TM, ETM+ and OLI data. Int. J. Remote Sens. 12(17). https://doi.org/10.3390/RS12172794

Bari R, Jones, JD. 2009. Role of plant hormones in plant defense responses. Plant Mol. Bio. 69(4):473–488. https://doi.org/10.1007/s11103-008-9435-0

Brachmann CB, Herm Man JM, Devine SE, Cameron EE, Pillus L, Boeke JD. 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 9:2888 – 2902.

Bradford MM. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 72: 248-254.

Castillo FJ, Penel C, Greppin H. 1984. Peroxidase Release Induced by Ozone in Sedum album Leaves: Involvement of Ca^{2+}. Plant Physiol. 74(4):846-851. Doi: 10.1104/pp.74.4.846..

Choi SM, Song HR, Han SK., Han M, Kim CY, Park J, Lee YH, Jeon JS, Noh YS, Noh B. 2012. HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. Plant J. 71(1):135-46. doi: 10.1111/j.1365-313X.2012.04977.x.

Cunha JR, Lima Neto MC, Carvalho FE, Martins MO, Jardim-Messeder D, Margis-Pinheiro M, Silveira JG. 2016. Salinity and osmotic stress trigger different antioxidant responses related to cytosolic ascorbate peroxidase knockdown in rice roots. Env. Exp. Bot. 131:58–67. https://doi.org/10.1016/j.envexpbot.2016.07.002

Das K, Roychoudhury A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front. Env. Sci. 2:53(1). https://doi.org/10.3389/fenvs.2014.00053

de Carvalho MHC. 2008. Drought stress and reactive oxygen species. Production, scavenging and signaling. Plant Signal. Behav. 3(3):156–165. doi: 10.4161/psb.3.3.5536

Dempsey DA, Vlot AC, Wildermuth MC, Klessig DF. 2011. Salicylic Acid Biosynthesis and Metabolism. The Arabidopsis Book. 9:e0156. https://doi.org/10.1199/tab.0156

Du H, Klessig DF. 1997. Identification of a Soluble, High-Affinity Salicylic Acid-Binding Protein in Tobacco. Plant Physiol. 113(4):1319-1327. doi: 10.1104/pp.113.4.1319.

ENS. (2022). Environmental New Service: Report on Climate Disasters. Available at https://ens-newswire.com/wmo-climate-disasters-to-increase-until-at-least-the-2060s/ February 20, 2022

Farooq M, Ahmad R, Shahzad M, Sajjad Y, Hassan A, Shah MM, Naz S, Khan SA. 2021. Differential variations in total flavonoid content and antioxidant enzymes activities in pea under different salt and drought stresses. Scientia Horticulturae, 287. https://doi.org/10.1016/j.scienta.2021.110258. Elsevier

Forouhar F, Yang Y, Kumar D, Chen Y, Fridman E, Park SW, Chiang Y, Acton TB, Montelione GT, Pichersky E, Klessig DF, and Tong L. 2005. Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. The Procs. Natl. Acad. Sci. (PNAS)USA*;* 102(5):1773-8. doi: 10.1073/pnas.0409227102.

Gondor OK, Pál M, Janda T, Szalai G. 2022. The role of methyl salicylate in plant growth under stress conditions. J. Plant Physiol. 277:153809 https://doi.org/10.1016/j.jplph.2022.153809

Gull A, Lone AA, Ul Islam Wani N. 2019. Chapter: Biotic and Abiotic Stresses in Plants: Abiotic and Biotic Stress in Plants. DOI: http://dx.doi.org/10.5772/intechopen.85832. IntechOpen Books.

Guo X, Ahmad N, Zhao S, Zhao C, Zhong W, Wang X, Li G. 2022. Effect of Salt Stress on Growth and Physiological Properties of Asparagus Seedlings. Plants 11(21). https://doi.org/10.3390/plants11212836

Haq I, Krishna B, Thakuri C, Hobbs T, Davenport ML, Kumar D. 2020. Tobacco SABP2-interacting protein SIP428 is a SIR2 type deacetylase. Plant Physiol. Biochem. [Internet]. 152:72–80. https://doi.org/10.1016/j.plaphy.2020.04.034

Hasanuzzaman M, Bhuyan MH, Zulfiqar F, Raza A, Mohsin SM, al Mahmud J, Fujita M, Fotopoulos V. 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 9(8):1–52. MDPI. https://doi.org/10.3390/antiox9080681

He M, He CQ, Ding NZ. 2018. Abiotic stresses: General defenses of land plants and chances for engineering multi-stress tolerance. Front Plant Sci. 9:1771-1789. https://doi.org/10.3389/fpls.2018.01771

Hernández JA, Diaz-Vivancos P, Barba-Espín G, Clemente-Moreno MJ. 2017. On the role of salicylic acid in plant responses to environmental stresses: R. Nazar et al. (eds.), *Salicylic Acid: A Multifaceted Hormone* (pp. 17–34). Springer Singapore. https://doi.org/10.1007/978-981-10-6068-7_2

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y. 2019. Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci. 10:800-810. https://doi.org/10.3389/fpls.2019.00800

Huang L, Sun Q, Qin F, Li C, Zhao Y, Zhou DX. 2007. Down-regulation of a Silent Information Regulator2-related histone deacetylase gene, *OsSRT1*, induces DNA fragmentation and cell death in rice. Plant Physiol. 144(3):1508–1519. https://doi.org/10.1104/pp.107.099473

Ignatenko A, Talanova V, Repkina N, Titov A. 2019. Exogenous salicylic acid treatment induces cold tolerance in wheat through promotion of antioxidant enzyme activity and proline accumulation. Acta Physiol. Plant. 41(6):80-90. https://doi.org/10.1007/s11738-019-2872-3

Khairy A, Roh K. 2016. Effect of Salicylic Acid, Benzoic Acid, and p-Coumaric Acid on Growth, Chlorophyll, Proline, and Vitamin C of Salinity-Stressed Tobacco (Nicotiana tabacum). Intl J. Plant Soil Sci. 9(4):1–10. https://doi.org/10.9734/ijpss/2016/22396

Khan MI, Fatma M, Per TS, Anjum NA, Khan NA. 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci. 6:462-479 https://doi.org/10.3389/fpls.2015.00462

Kiani S, Babaeianjelodar N, Bagheri N, Najafizarrini H. 2018. Effect of mannitol stress on morphological, biochemical and polyphenol parameters in broccoli sprouts (*Brassica oleracea var. Italica*). App Ecol Environ Res. 16(2):2043–2058. https://doi.org/10.15666/aeer/1602_20432058

Kumar D, Klessig DF. 2003. High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. Procs Natl Acad Sci. USA. 100(26):16101–16106. http://www.jstor.org/stable/3149137

Kumar D, Gustafsson C, Klessig DF. 2006. Validation of RNAi silencing specificity using synthetic genes: salicylic acid-binding protein 2 is required for innate immunity in plants. Plant J. 45(5):863-8. doi: 10.1111/j.1365-313X.2005.02645.x.

La VH, Lee BR, Zhang Q, Park SH, Islam MT, Kim TH. 2019. Salicylic acid improves drought-stress tolerance by regulating the redox status and proline metabolism in *Brassica rapa*. Hortic Environ Biotechnol. 60(1):31–40. https://doi.org/10.1007/s13580-018-0099-7

Lau AW, Liu P, Inuzuka H, Gao D. 2014. SIRT1 phosphorylation by AMP-activated protein kinase regulates p53 acetylation. Am. J. Cancer Res. 4(3):245-55. www.ajcr.us /ISSN:2156-6976/ajcr0000323 PMID: 24959379; PMCID: PMC4065405.

Lee S, Kim SG, Park CM. 2010. Salicylic acid promotes seed germination under high salinity by modulating antioxidant activity in *Arabidopsis.* New Phytol. 188(2):626–637. https://doi.org/10.1111/j.1469-8137.2010.03378.x

Lefevere H, Bauters L, Gheysen G. 2020. Salicylic Acid Biosynthesis in Plants. Front Plant Sci. 11:338. doi: 10.3389/fpls.2020.00338

Li Q, Wang G, Guan C, Yang D, Wang Y, Zhang Y, Ji J, Jin C, An T. 2019. Overexpression of *LcSABP*, an orthologous gene for salicylic acid binding protein 2, enhances drought stress tolerance in transgenic tobacco. Front Plant Sci. 10:200-220 https://doi.org/10.3389/fpls.2019.00200

Liu X, Wei W, Zhu W, Su L, Xiong Z, Zhou M, Zheng Y, Zhou DX. 2017. Histone Deacetylase AtSRT1 Links Metabolic Flux and Stress Response in Arabidopsis. *Molecular Plant, 10*(12), 1510–1522. https://doi.org/10.1016/j.molp.2017.10.010

Lu G, Gao C, Zheng X, Han B. 2009. Identification of OsbZIP72 as a positive regulator of ABA response and drought tolerance in rice. Planta. 229(3):605–615. https://doi.org/10.1007/s00425-008-0857-3

Mickelbart MV, Hasegawa PM, Bailey-Serres J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat. Rev. Genet. 16 (4):237–251. Nature Publishing Group. https://doi.org/10.1038/nrg3901

Mitra N, Dey, S. 2022. Understanding the catalytic abilities of class IV sirtuin OsSRT1 and its linkage to the DNA repair system under stress conditions. Plant Sci. 323. https://doi.org/10.1016/j.plantsci.2022.111398

Murata MM, Kong X, Moncada E, Chen Y, Imamura H, Wang P, Berns MW, Yokomori K, Digman MA. 2019. NAD+ consumption by PARP1 in response to DNA damage triggers metabolic shift critical for damaged cell survival. Mol. Bio. Cell. 30(20):2584–2597. https://doi.org/10.1091/mbc.E18-10-0650

North BJ, Verdin, E. 2004. Sirtuins: Sir2-related NAD-dependent protein deacetylases. Genome Biol. 5:224. https://doi.org/10.1186/gb-2004-5-5-224

Ogawa D, Nakajima N, Seo S, Mitsuhara I, Kamada H, Ohashi Y. 2006. The phenylalanine pathway is the main route of salicylic acid biosynthesis in tobacco mosaic virus-infected tobacco leaves. Plant Biotech. 23:395–398

Onwuka B. 2018. Effects of Soil Temperature on Some Soil Properties and Plant Growth. Adv. Plants Agric. Res. 8(1): 34-37. https://doi.org/10.15406/apar.2018.08.00288

Oviavo N. 2021 "Characterization of SABP2-Interacting Proteins (SIP) 428: an NAD+-Dependent Deacetylase Enzyme in Plant Abiotic Stress Signaling" (2021). Electronic Theses and Dissertations. Paper 3961. https://dc.etsu.edu/etd/3961

Park HY, Seok HY, Park BK, Kim SH, Goh, C. H., Lee, B. ha, Lee, C. H., & Moon, Y. H. (2008). Overexpression of Arabidopsis ZEP enhances tolerance to osmotic stress. *Biochemical and Biophysical Research Communications*, *375*(1), 80–85. https://doi.org/10.1016/j.bbrc.2008.07.128

Patel MK, Kumar M, Li W, Luo Y, Burritt DJ, Alkan N, Tran LS. 2020. Enhancing Salt Tolerance of Plants: From Metabolic Reprogramming to Exogenous Chemical Treatments and Molecular Approaches. Cells 9(11): 2492- 2518. https://doi.org/10.3390/cells9112492

Rahman AK, Ahmed KM, Butler AP, Hoque MA. 2018. Influence of surface geology and micro-scale land use on the shallow subsurface salinity in deltaic coastal areas: a case from southwest Bangladesh. Env. Earth Sci. 77(12): 423-431. https://doi.org/10.1007/s12665-018-7594-0

Ramakrishnan M., Papolu PK, Satish L, Vinod KK, Wei Q, Sharma A, Emamverdian A, Zou LH, Zhou M. 2022. Redox status of the plant cell determines epigenetic modifications under abiotic stress conditions and during developmental processes. J. Adv. Res. 42:99–116. https://doi.org/10.1016/j.jare.2022.04.007

Sabagh AE, Mbarki S, Hossain A, Iqbal MA, Islam MS, Raza A, Llanes A, Reginato M, Rahman MA, Mahboob W, Farooq M. et al. 2021. Potential Role of Plant Growth Regulators in Administering Crucial Processes Against Abiotic Stresses. Front. Agron. 3: 648694.28. doi: 10.3389/fagro.2021.64

Safari M, Mousavi-Fard S, Rezaei Nejad A, Sorkheh K, Sofo A. 2022. Exogenous salicylic acid positively affects morpho-physiological and molecular responses of *Impatiens walleriana* plants grown under drought stress. Int. J. Environ. Sci.Technol. 19(2):969–984. https://doi.org/10.1007/s13762-020-03092-2

Sajid ZA, Aftab F. 2022. Improvement of Polyethylene Glycol, Sorbitol, Mannitol, and Sucrose-Induced Osmotic Stress Tolerance through Modulation of the Polyamines, Proteins, and Superoxide Dismutase Activity in Potato. Int. J. Agron. no 5158768.14 https://doi.org/10.1155/2022/5158768

Singh A, Kumar A, Yadav S, Singh IK. 2019. Reactive oxygen species-mediated signaling during abiotic stress. Plant Gene. 18: 100173.7. https://doi.org/10.1016/j.plgene.2019.100173

Soccio M, Laus MN, Alfarano M, Pastore D. 2018. Measuring activity of native plant sirtuins - The wheat mitochondrial model. Front. Plant Sci. 9:961- 975 https://doi.org/10.3389/fpls.2018.00961

Stavridou E, Michailidis M, Gedeon S, Ioakeim A, Kostas S, Chronopoulou E, Labrou NE, Edwards R, Day A, Nianiou-Obeidat I, Madesis P. 2019. Tolerance of transplastomic tobacco plants overexpressing a theta class glutathione transferase to abiotic and oxidative stresses. Front. Plant Sci. 9:1861-1898. https://doi.org/10.3389/fpls.2018.01861

Sun H, Sun X, Wang H, Ma X. 2020. Advances in salt tolerance molecular mechanism in tobacco plants. Hereditas. 157(5):1-6. BioMed Central Ltd. https://doi.org/10.1186/s41065-020-00118-0

Tanner KG, Landry J, Sternglanz R, Denu, JM. 2000. Silent information regulator 2 family of NAD-dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose. Proc. Natl. Acad. Sci. USA, PNAS. 97(26):14178–14182. https://doi.org/10.1073/pnas.250422697

Thakuri BK. 2018:SIP-428, a SIR2 Deacetylase Enzyme and Its Role in Biotic Stress Signaling Pathway". Electronic Theses and Dissertations. Paper 3505. https://dc.etsu.edu/etd/3505.

Tripathy BC, Oelmüller R. 2012. Reactive oxygen species generation and signaling in plants. Plant Signal. Behav. 7(12):1621–1633. https://doi.org/10.4161/psb.22455

Ullah A, Bano A, Khan N. 2021. Climate Change and Salinity Effects on Crops and Chemical Communication Between Plants and Plant Growth-Promoting Microorganisms Under Stress. Front. in Sustain. Food Syst. 5:618092.16. https://doi.org/10.3389/fsufs.2021.618092

Vanderauwera S, De Block M, Van De Steene N, Van De Cotte B, Metzlaff M, & Van Breusegem F. 2007. Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction. Proc. Natl. Acad. Sci. USA, PNAS 18(38):15150-15155.

Vlot AC, Liu PP, Cameron RK, Park SW, Yang Y, Kumar D, Zhou F, Padukkavidana T, Gustafsson C, Pichersky E, Klessig, DF. 2008. Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in Arabidopsis thaliana. Plant J. 56(3):445–456. https://doi.org/10.1111/j.1365-313X.2008.03618.x

Yang J, Duan L, He H, Li Y, Li X, Liu D, Wang J, Jin G, Huang S. 2022. Application of Exogenous KH2PO4 and Salicylic Acid and Optimization of the Sowing Date Enhance Rice Yield Under High-Temperature Conditions. J. Plant Growth Regul. 41(4):1532–1546. https://doi.org/10.1007/s00344-021-10399-y

Zhang H, Liu D, Yang B, Liu WZ, Mu B, Song H, Chen B, Li Y, Ren D, Deng H, Jiang YQ. 2020. Arabidopsis CPK6 positively regulates ABA signaling and drought tolerance through phosphorylating ABA-responsive element-binding factors. J. Exp. Bot. 71(1):188–203. https://doi.org/10.1093/jxb/erz432

Zhang H, Zhu J, Gong Z, Zhu JK. 2022. Abiotic stress responses in plants. Nat. Rev. Genet. 23(2): 104–119. Nature Research. https://doi.org/10.1038/s41576-021-00413-0

Zhang J, Zhang Y, Du Y, Chen S, Tang H. 2011. Dynamic metabonomic responses of tobacco (*Nicotiana tabacum*) plants to salt stress. J. Proteome Res. 10(4):1904–1914. https://doi.org/10.1021/pr101140n

Zhang Q, Gong M, Xu X, Li H, Deng W. 2022. Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. Cells. 11(17): 2761-2776. MDPI. https://doi.org/10.3390/cells11172761

Zheng W. 2020. Review: The plant Sirtuins. Plant Sci. (293):110434.8. Elsevier Ireland Ltd. https://doi.org/10.1016/j.plantsci.2020.110434

Zheng Y, Ding Y, Sun X, Xie S, Wang D, Liu X, Su L, Wei W, Pan L, Zhou, DX. 2016. Histone deacetylase HDA9 negatively regulates salt and drought stress responsiveness in Arabidopsis. *Journal of Experimental Botany*, *67*(6):1703–1713. https://doi.org/10.1093/jxb/erv562

Zhong X, Zhang H, Zhao Y, Sun Q, Hu Y. 2013 The Rice NAD+-Dependent Histone Deacetylase *OsSRT1* Targets Preferentially to Stress- and Metabolism-Related Genes and Transposable Elements. PLoS ONE 8(6): e66807.8. doi:10.1371/journal.pone.0066807

APPENDICES

Appendix A: Abbreviations

SABP2: Salicylic Acid Binding Protein 2

SIP428: SABP2-Interacting protein 428

XNN/ WT: Wild-type plants (*Nicotiana tabacum* cv Xanthi nc)

NaCl: Sodium Chloride

H_2O_2: Hydrogen peroxide

SA: Salicylic Acid

ROS: Reactive oxygen species

CAT: Catalase

POD: Guaiacol Peroxidase

g: gram

L: Litres, metric unit of volume

M: Molar, unit of concentration molarity

μL: microliter (10^{-6} L)

mL: milliliters (10^{-3} L)

μM: micro-Molar (10^{-6} M)

mM: milli Molar (10^{-3} M)

pH: potential of hydrogen (measures acidity or basicity of a solution)

SEM/ SE: Standard Error of Mean

Appendix B: Buffers and Reagents

½ x MS Media plates: Dissolve the following reagents in each proportion listed

Murashige and Skoog (MS) powder: 2.2g/L

Sucrose: 1% total volume

Phyto agar: 8g/L

MilliQ water: Adjust to total volume (L)

Gamborg B5 Vitamin solution (1000x)

To make 100ml total volume of ½ x MS Media plates: In a beaker, 90mL of milliQ water was combined with 0.22g MS powder and 1g Sucrose; this was mixed properly using the magnetic stirrer. With the help of a pH meter, the pH was adjusted to 5.9 using 1M KOH. This mixture/ solution was transferred to an autoclave bottle/ flask (500ml) and 0.8g Phyto agar was added and mixed. The final volume was adjusted to 100ml with water and stirred thoroughly. This solution was autoclaved for 15mins, and an autoclave tape is kept on the bottle for color confirmation.

In a 55°C water bath, the autoclaved media was allowed to cool for 10-15mins. Before pouring into petri-plates (100mm x 15mm) under the Laminar Airflow hood, 100 µl (1000x) vitamin solution, thawed in 37°C water bath was transferred into the media, and stirred thoroughly.

½ x MS media plate with NaCl

Molecular weight of NaCl = 58.44 $gmol^{-1}$

100mM NaCl: 5.84g/L

150mM NaCl: 8.77g/L

175mM NaCl: 10.23g/L

200mM NaCl: 11.69g/L

For 250ml total volume of ½ x MS Media plates with 100mM/150mM/175mM/200mM NaCl:

In a beaker, 240ml of milliQ water was combined with 0.55g MS powder, 2.5g Sucrose and 1.46g NaCl (100mM), 2.19g NaCl (150mM), 2.56g NaCl (175mM), or 2.92g NaCl

(200mM) and mixed properly with a stirrer. The pH of the solution was adjusted to 5.9 with 1M KOH. And finally, 2g Phyto agar was transferred into the solution in an autoclave bottle and the final volume was adjusted to 250ml with water. After stirring properly, the solution was placed in the autoclave for 15mins.

Media was placed in a 55°C water bath for 10-15mins to cool. 250µl thawed (1000x) vitamin solution was transferred into the media and mix thoroughly just before pouring into petri-plates (150mm x 30mm) under the Laminar Airflow hood.

½ x MS media plates with Mannitol

Molecular weight of Mannitol = 182.17g/mol

100mM Mannitol: 18.22g/L

150mM Mannitol: 27.33g/L

175mM Mannitol: 31.88g/L

200mM Mannitol: 36.43g/L

For 250ml total volume of ½ x MS Media plates with 100mM/150mM/175mM/200mM Mannitol:

In a beaker, 240ml of milliQ water was transferred using a measuring cylinder, and 0.55g MS powder, 2.5g Sucrose was combined with 4.56g Mannitol (100mM), 6.83g Mannitol (150mM), 7.97g Mannitol (175mM), or 9.11g Mannitol (200mM) and mix properly. The pH of the solution was adjusted to 5.9 with 1M KOH. And finally, 2g Phyto agar was transferred into the solution in an autoclave bottle and the final volume was adjusted to 250ml with water. After stirring properly, the solution was placed in the autoclave for 15mins.

Media was placed in a 55°C water bath for 10-15mins to cool. 250µl thawed (1000x) vitamin solution was transferred into the media and mix thoroughly just before pouring into petri-plates (150mm x 30mm) under Laminar Airflow hood.

Gamborg B5 Vitamin (1000x vit solution):

The following reagents were disssolved in approximately 100ml MilliQ water. Filter-sterilized and aliquoted in 10mL tubes. These were stored at -20°C and thawed at 37°C before usage.

Thiamine-HCl	1gm (1mg/ml)
Nicotinic acid	100mg (0.5mg/ml)
Myo-inositol	10gm
Pyridoxine- HCl	100mg (0.5mg/ml)

$NaHPO_4$ (Sodium Phosphate) Buffers (Store in -4°C)

$NaHPO_4$ Monobasic (anhydrous) molecular weight: 119.98$gmol^{-1}$

To make 0.2M Monobasic: Dissolve 23.99g $NaHPO_4$ Monobasic in 1000ml water

$NaHPO_4$ Dibasic (Anhydrous) molecular weight: 141.96 $gmol^{-1}$

To make 0.2M Dibasic: Dissolve 28.39g $NaHPO_4$ Dibasic in 1000mL water

0.1M pH 7.0 buffer: 39mL of 0.2M NaHPO4 monobasic + 61ml of 0.2M $NaHPO_4$ dibasic + 100mL milliQ water.

0.1M pH 6.1 buffer: 85mL of 0.2M $NaHPO_4$ monobasic + 15mL of 0.2M $NaHPO_4$ dibasic + 100ml milliQ water.

0.1M pH 7.2 buffer: 28mL 0.2M NaHPO4 monobasic + 72mL 0.2M NaHPO4 dibasic + 100mL milliQ water.

After stirring properly, autoclave for 15mins and Store at -4°C.

Assay chemicals

H_2O_2: Hydrogen peroxide (8.8M Stock solution),

20mM for CAT assay: dilute 440 times with pH 7.2 buffer. Store at -4°C

To make 200mM stock: add 80µL to 3.52mL pH 7.2 buffer.

2mM for POD assay: dilute 4,400 times with pH 6.1 buffer. Store at -4°C

To make 20mM stock: add 8µL to 3.52mL pH 6.1 buffer.

Guaiacol: 8M Stock solution,

16mM for POD: dilute 500 times with Milli-Q water. Store at -4°C

To make 160mM stock: add 200µL to 10mL milliQ water.

EDTA: 500mM Stock,

10mM for Extraction: dilute 50 times with water. Store at room temperature

To make 10mM stock: add 0.2µL to 10mL milliQ water.

Protein extraction from Frozen leaves

1mL of 0.1M pH 7.0 buffer and 0.5µL 0f 10mM EDTA were transferred into tubes with frozen leaf samples (100mg) on ice. The mixture was homogenized by grinding using either the hand-held, digital, or fast protein grinder while keeping on ice. These homogenized samples were centrifuged at 13,000 x for 20mins in the cold room.

Pellets and supernatant were separated, and the supernatant was used for protein quantification and enzyme assays. Please note that samples were always kept on ice.

Protein quantification using Bradford reagent

In a 1.5ml Eppendorf, 800µL of water was transferred, then (1-3µl) protein sample was added from the supernatant. 200µL of Bradford reagent was then added to the tube and vortexed for mixing. All experiments were kept at normal room temperature for 5mins. The spectrophotometer was blanked at 595nm with the mixture without protein sample. The absorbance of all samples was calculated at 595nm.

The equation of the line from the BSA standard curve was used to quantify protein concentration (per µL protein) and a known concentration was selected (µg/µL) for each assay.

Catalase Assay

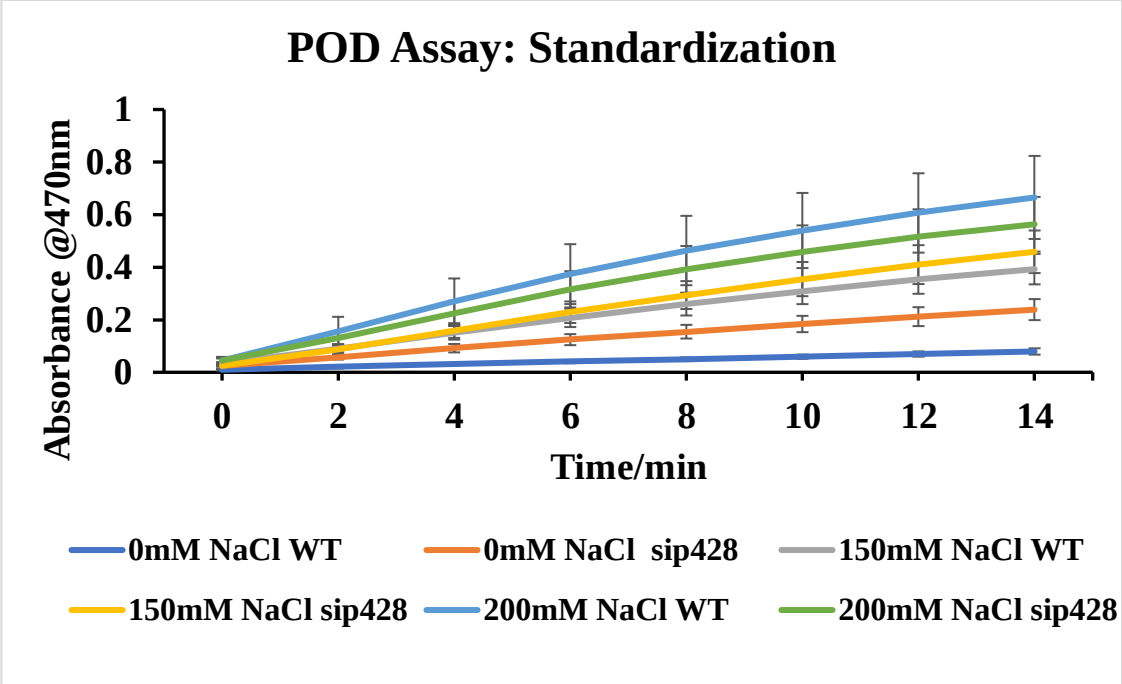
Spectrophotometer: 1mL Assay solution contained 900 µL (100mM pH 7.2 buffer and 20mM H_2O_2) and 100 µL enzyme (50µg/µL). The reaction was linear for 30minutes; @ 5mins interval.

Microplate Reader: 200µL assay solution contained 100µL (100mM pH 7.2 buffer), 20µL (200mM H_2O_2) and 80µL (water + enzyme (2µg/µL (final standardized concentration), 20µg/µL or 40 µg/µL)). These were made in excess since they would be transferred into 96- microplate well plates using the multi-channel pipettes. The reaction was linear for 10-30minutes; **10 mins @ 1min intervals was standardized for this assay.**

Guaiacol Peroxidase Assay

Spectrophotometer: 1mL Assay solution contained 900 µL (100mM pH 6.1 buffer, 160mM guaiacol and 20mM H_2O_2) and 100 µL enzyme (50µg/µL). The reaction was linear for 30minutes; @ 5mins interval.

Microplate Reader: 200µL assay solution contained 120µl (100mM pH 6.1 buffer), 20µL (20mM H_2O_2), 20µL (160mM guaiacol) and 40µl (water + enzyme (2µg/µL, 5µg/µL (final standardized concentration) or 10µg/µL)). These were made in excess because they would get transferred into 96- microplate well plates using the multi-channel pipettes. The reaction was linear for 15-30minutes; **20 mins @ 2min interval was standardized for this assay.**



A graph showing the linear relationship/ increase in absorbance at 470nm over 15mins.

Appendix C: Data and Tables

BSA Standard Curve

BSA Concentration (mg/ml)	A1 @ 595nm	A2 @ 595nm	A3 @ 595nm	Mean Absorbance	SE
0				0	
0.5	0.042	0.047	0.047	0.045	**0.002**
1	0.053	0.057	0.068	0.059	**0.004**
1.5	0.108	0.107	0.081	0.099	**0.009**
2	0.131	0.148	0.073	0.117	**0.023**
2.5	0.144	0.156	0.163	0.154	**0.006**
3	0.171	0.189	0.24	0.200	**0.021**
3.5	0.226	0.189	0.266	0.227	**0.022**
4	0.226	0.257	0.303	0.262	**0.022**

Table 1. Shows data for BSA Standard curve, Where A1, A2, and A3 signify BSA absorbance @ 595nm for replicate experiments 1,2 and 3 respectively.

OSMOTIC STRESS

PEROXIDASE ACTIVITY IN 7 DAYS MANNITOL-STRESSED PLANTS

FIG 2: POD MANN 7 DAYS						
	MANNITOL					
	0mM		**150mM**		**200mM**	
REPLICAT	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.856	0.703	1.416	1.225	0.935	0.935
2	0.561	0.794	1.311	1.404	1.353	1.42
MEAN	**0.71**	**0.75**	**1.36**	**1.31**	**1.14**	**1.18**
SE	**0.15**	**0.05**	**0.05**	**0.09**	**0.21**	**0.24**

EXPERIMENT 2. Data shows mean and SEM of the peroxidase activity in replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0, 150mM and 200mM) for 7 days.

Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI o	Below t	Summa	Adjusted P Value			
0mM Mannitol WT vs. 0mM Manni	0.04	-0.8107 to 0.	No	ns	>0.9999	A-B		
0mM Mannitol WT vs. 150mM Mar	-0.566	-1.417 to 0.2	No	ns	0.2174	A-C		
0mM Mannitol WT vs. 150mM Mar	-0.615	-1.466 to 0.2	No	ns	0.1682	A-D		
0mM Mannitol WT vs. 200mM Mar	-0.429	-1.280 to 0.4	No	ns	0.4313	A-E		
0mM Mannitol WT vs. 200mM Mar	-0.3955	-1.246 to 0.4	No	ns	0.5021	A-F		
0mM Mannitol sip 428 vs. 150mM	-0.606	-1.457 to 0.2	No	ns	0.1764	B-C		
0mM Mannitol sip 428 vs. 150mM	-0.655	-1.506 to 0.1	No	ns	0.1364	B-D		
0mM Mannitol sip 428 vs. 200mM	-0.469	-1.320 to 0.3	No	ns	0.3561	B-E		
0mM Mannitol sip 428 vs. 200mM	-0.4355	-1.286 to 0.4	No	ns	0.4184	B-F		
150mM Mannitol WT vs. 150mM M	-0.049	-0.8997 to 0.	No	ns	0.9999	C-D		
150mM Mannitol WT vs. 200mM M	0.137	-0.7137 to 0.	No	ns	0.9828	C-E		
150mM Mannitol WT vs. 200mM M	0.1705	-0.6802 to 1.	No	ns	0.9578	C-F		
150mM Mannitol sip428 vs. 200mM	0.186	-0.6647 to 1.	No	ns	0.941	D-E		
150mM Mannitol sip428 vs. 200mM	0.2195	-0.6312 to 1.	No	ns	0.8931	D-F		
200mM Mannitol WT vs. 200mM M	0.0335	-0.8172 to 0.	No	ns	>0.9999	E-F		
Test details	Mean 1	Mean 2	Mean D	SE of d	n1	n2	q	DF
0mM Mannitol WT vs. 0mM Manni	0.7485	0.7085	0.04	0.214	2	2	0.26	6
0mM Mannitol WT vs. 150mM Mar	0.7485	1.315	-0.566	0.214	2	2	3.75	6
0mM Mannitol WT vs. 150mM Mar	0.7485	1.364	-0.615	0.214	2	2	4.07	6
0mM Mannitol WT vs. 200mM Mar	0.7485	1.178	-0.429	0.214	2	2	2.84	6
0mM Mannitol WT vs. 200mM Mar	0.7485	1.144	-0.396	0.214	2	2	2.62	6
0mM Mannitol sip 428 vs. 150mM	0.7085	1.315	-0.606	0.214	2	2	4.01	6
0mM Mannitol sip 428 vs. 150mM	0.7085	1.364	-0.655	0.214	2	2	4.33	6
0mM Mannitol sip 428 vs. 200mM	0.7085	1.178	-0.469	0.214	2	2	3.1	6
0mM Mannitol sip 428 vs. 200mM	0.7085	1.144	-0.436	0.214	2	2	2.88	6
150mM Mannitol WT vs. 150mM M	1.315	1.364	-0.049	0.214	2	2	0.32	6
150mM Mannitol WT vs. 200mM M	1.315	1.178	0.137	0.214	2	2	0.91	6
150mM Mannitol WT vs. 200mM M	1.315	1.144	0.171	0.214	2	2	1.13	6
150mM Mannitol sip428 vs. 200mM	1.364	1.178	0.186	0.214	2	2	1.23	6
150mM Mannitol sip428 vs. 200mM	1.364	1.144	0.22	0.214	2	2	1.45	6
200mM Mannitol WT vs. 200mM M	1.178	1.144	0.034	0.214	2	2	0.22	6

One-way ANOVA Experiment 2. Data shows the mean peroxidase activity± standard error of two replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) from which the peroxidase activity is measured from, at different concentrations of Mannitol (0mm, 150mm, and 200mm) showing statistical significance (at p-value < 0.05).

FIG 3: POD MANN 7 DAYS						
	MANNITOL					
	0mM		100mM		175mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT	MSI-2-7	WT
1	0.614	0.695	1.218	1.028	1.422	1.119
2	0.602	0.766	1.287	0.969	1.367	0.955
MEAN	**0.61**	**0.73**	**1.25**	**1.00**	**1.39**	**1.04**
SE	**0.01**	**0.04**	**0.03**	**0.03**	**0.03**	**0.08**

Experiment 3. Data shows mean and SEM of the peroxidase activity in replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0, 100mM and 175mM) for 7 days.

Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI o	Below t	Summary	Adjusted P Value			
0mM Mannitol WT vs. 0mM Mannito	0.1225	-0.1167 to 0.	No	ns	0.418	A-B		
0mM Mannitol WT vs. 100mM Mann	-0.268	-0.5072 to -0	Yes	*	0.0305	A-C		
0mM Mannitol WT vs. 100mM Mann	-0.522	-0.7612 to -0	Yes	**	0.001	A-D		
0mM Mannitol WT vs. 175mM Mann	-0.3065	-0.5457 to -0	Yes	*	0.0163	A-E		
0mM Mannitol WT vs. 175mM Mann	-0.664	-0.9032 to -0	Yes	***	0.0003	A-F		
0mM Mannitol sip 428 vs. 100mM M	-0.3905	-0.6297 to -0	Yes	**	0.0049	B-C		
0mM Mannitol sip 428 vs. 100mM M	-0.6445	-0.8837 to -0	Yes	***	0.0003	B-D		
0mM Mannitol sip 428 vs. 175mM M	-0.429	-0.6682 to -0	Yes	**	0.003	B-E		
0mM Mannitol sip 428 vs. 175mM M	-0.7865	-1.026 to -0.5	Yes	***	0.0001	B-F		
100mM Mannitol WT vs. 100mM Ma	-0.254	-0.4932 to -0	Yes	*	0.0386	C-D		
100mM Mannitol WT vs. 175mM Ma	-0.0385	-0.2777 to 0.	No	ns	0.9829	C-E		
100mM Mannitol WT vs. 175mM Ma	-0.396	-0.6352 to -0	Yes	**	0.0045	C-F		
100mM Mannitol sip428 vs. 175mM I	0.2155	-0.02370 to 0	No	ns	0.0764	D-E		
100mM Mannitol sip428 vs. 175mM I	-0.142	-0.3812 to 0.	No	ns	0.2975	D-F		
175mM Mannitol WT vs. 175mM Ma	-0.3575	-0.5967 to -0	Yes	**	0.0076	E-F		
Test details	Mean 1	Mean 2	Mean D	SE of dif	n1	n2	q	DF
0mM Mannitol WT vs. 0mM Mannito	0.7305	0.608	0.1225	0.0601	2	2	2.88	6
0mM Mannitol WT vs. 100mM Mann	0.7305	0.9985	-0.268	0.0601	2	2	6.31	6
0mM Mannitol WT vs. 100mM Mann	0.7305	1.253	-0.522	0.0601	2	2	12.3	6
0mM Mannitol WT vs. 175mM Mann	0.7305	1.037	-0.307	0.0601	2	2	7.21	6
0mM Mannitol WT vs. 175mM Mann	0.7305	1.395	-0.664	0.0601	2	2	15.6	6
0mM Mannitol sip 428 vs. 100mM M	0.608	0.9985	-0.391	0.0601	2	2	9.19	6
0mM Mannitol sip 428 vs. 100mM M	0.608	1.253	-0.645	0.0601	2	2	15.2	6
0mM Mannitol sip 428 vs. 175mM M	0.608	1.037	-0.429	0.0601	2	2	10.1	6
0mM Mannitol sip 428 vs. 175mM M	0.608	1.395	-0.787	0.0601	2	2	18.5	6
100mM Mannitol WT vs. 100mM Ma	0.9985	1.253	-0.254	0.0601	2	2	5.98	6
100mM Mannitol WT vs. 175mM Ma	0.9985	1.037	-0.039	0.0601	2	2	0.91	6
100mM Mannitol WT vs. 175mM Ma	0.9985	1.395	-0.396	0.0601	2	2	9.32	6
100mM Mannitol sip428 vs. 175mM I	1.253	1.037	0.2155	0.0601	2	2	5.07	6
100mM Mannitol sip428 vs. 175mM I	1.253	1.395	-0.142	0.0601	2	2	3.34	6
175mM Mannitol WT vs. 175mM Ma	1.037	1.395	-0.358	0.0601	2	2	8.41	6

One-way ANOVA Experiment 3. Data shows the One-way analysis of variance between two replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different mannitol concentrations (0mM, 100mM, and 175mM) showing statistical significance (at p-value < 0.05).

PEROXIDASE ACTIVITY IN 14 DAYS MANNITOL-STRESSED PLANTS

FIG 4: POD MANN 14DAYS				
	MANNITOL			
	0mM		150mM	
REPLICATES	MSI-2-7	WT	MSI-2-7	WT
1	0.154	0.376	0.698	0.577
2	0.168	0.356	0.819	0.577
3	0.161	0.356	0.705	0.618
MEAN	**0.16**	**0.36**	**0.74**	**0.59**
SE	**0.00**	**0.01**	**0.03**	**0.01**

Experiment 4. Data shows mean and SEM of the peroxidase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0mM and 150mM) for 14 days.

Number of families	1							
Number of comparisons per fan	10							
Alpha	0.05							
Tukey's multiple comparisons te	Mean Diff.	95.00% CI o	Below thresh	Summary	Adjusted P Value			
0mM Mannitol WT vs. 0mM M	0.02667	-0.08187 to 0	No	ns	0.9221	A-B		
0mM Mannitol WT vs. 0mM M	0.2317	0.1231 to 0.3	Yes	***	0.0003	A-C		
0mM Mannitol WT vs. 150mM	-0.2083	-0.3169 to -0	Yes	***	0.0006	A-D		
0mM Mannitol WT vs. 150mM	-0.3407	-0.4492 to -0	Yes	****	<0.0001	A-E		
0mM Mannitol WT vs. 0mM M	0.205	0.09646 to 0.	Yes	***	0.0007	B-C		
0mM Mannitol WT vs. 150mM	-0.235	-0.3435 to -0	Yes	***	0.0002	B-D		
0mM Mannitol WT vs. 150mM	-0.3673	-0.4759 to -0	Yes	****	<0.0001	B-E		
0mM Mannitol sip 428 vs. 150	-0.44	-0.5485 to -0	Yes	****	<0.0001	C-D		
0mM Mannitol sip 428 vs. 150	-0.5723	-0.6809 to -0	Yes	****	<0.0001	C-E		
150mM Mannitol WT vs. 150m	-0.1323	-0.2409 to -0	Yes	*	0.0164	D-E		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff	n1	n2	q	DF
0mM Mannitol WT vs. 0mM M	0.3627	0.336	0.02667	0.03298	3	3	1.14	10
0mM Mannitol WT vs. 0mM M	0.3627	0.131	0.2317	0.03298	3	3	9.93	10
0mM Mannitol WT vs. 150mM	0.3627	0.571	-0.2083	0.03298	3	3	8.93	10
0mM Mannitol WT vs. 150mM	0.3627	0.7033	-0.3407	0.03298	3	3	14.6	10
0mM Mannitol WT vs. 0mM M	0.336	0.131	0.205	0.03298	3	3	8.79	10
0mM Mannitol WT vs. 150mM	0.336	0.571	-0.235	0.03298	3	3	10.1	10
0mM Mannitol WT vs. 150mM	0.336	0.7033	-0.3673	0.03298	3	3	15.8	10
0mM Mannitol sip 428 vs. 150	0.131	0.571	-0.44	0.03298	3	3	18.9	10
0mM Mannitol sip 428 vs. 150	0.131	0.7033	-0.5723	0.03298	3	3	24.5	10
150mM Mannitol WT vs. 150m	0.571	0.7033	-0.1323	0.03298	3	3	5.68	10

One-Way ANOVA Experiment 4. Data shows the one-way analysis of variance between the peroxidase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced

plants) at different mannitol concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

FIG 5:POD MANN 14 DAYS				
	MANNITOL			
	0mM		150mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT
1	0.125	0.34	0.661	0.636
2	0.12	0.348	0.78	0.538
3	0.148	0.32	0.669	0.539
MEAN	**0.13**	**0.34**	**0.70**	**0.57**
SE	**0.01**	**0.01**	**0.04**	**0.03**

Experiment 5. Data shows mean and SEM of three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0mM and 150mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff	95.00% (	Below th	Summary	Adjusted P Value			
0mM Mannitol WT vs. 0mM Mannitc	0.2017	0.1060 t	Yes	***	0.0007	A-B		
0mM Mannitol WT vs. 150mM Mani	-0.228	-0.3237 t	Yes	***	0.0003	A-C		
0mM Mannitol WT vs. 150mM Mani	-0.378	-0.4737 t	Yes	****	<0.0001	A-D		
0mM Mannitol sip 428 vs. 150mM M	-0.4297	-0.5254 t	Yes	****	<0.0001	B-C		
0mM Mannitol sip 428 vs. 150mM M	-0.5797	-0.6754 t	Yes	****	<0.0001	B-D		
150mM Mannitol WT vs. 150mM Ma	-0.15	-0.2457 t	Yes	**	0.0045	C-D		
Test details	Mean 1	Mean 2	Mean Di	SE of diff.	n1	n2	q	DF
0mM Mannitol WT vs. 0mM Mannitc	0.3627	0.161	0.2017	0.02988	3	3	9.55	8
0mM Mannitol WT vs. 150mM Mani	0.3627	0.5907	-0.228	0.02988	3	3	10.8	8
0mM Mannitol WT vs. 150mM Mani	0.3627	0.7407	-0.378	0.02988	3	3	17.9	8
0mM Mannitol sip 428 vs. 150mM M	0.161	0.5907	-0.43	0.02988	3	3	20.3	8
0mM Mannitol sip 428 vs. 150mM M	0.161	0.7407	-0.58	0.02988	3	3	27.4	8
150mM Mannitol WT vs. 150mM Ma	0.5907	0.7407	-0.15	0.02988	3	3	7.1	8

One-Way ANOVA Experiment 5. Data shows the one-way analysis of variance between the peroxidase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different mannitol concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

CATALASE ACTIVITY IN 7 DAYS-MANNITOL STRESSED PLANTS

FIG 6: CAT MANN 7 DAYS						
	MANNITOL					
	0mM		150mM		200mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT	MSI-2-7	WT
1	0.053	0.077	0.032	0.059	0.059	0.092
2	0.078	0.064	0.021	0.053	0.03	0.032
MEAN	0.066	0.071	0.027	0.056	0.045	0.062
SE	0.013	0.007	0.005	0.003	0.015	0.030

Experiment 6. Data shows mean and SEM of catalase activity in two replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0mM, 150mM, and 200mM) for 7 days.

Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI o	Below thresh	Summar	Adjusted P Value			
0mM Mannitol WT vs. 0mM Manni	0.005	-0.07936 to 0	No	ns	0.9998	A-B		
0mM Mannitol WT vs. 150mM Mar	0.0145	-0.06986 to 0	No	ns	0.9774	A-C		
0mM Mannitol WT vs. 150mM Mar	0.044	-0.04036 to 0	No	ns	0.4024	A-D		
0mM Mannitol WT vs. 200mM Mar	0.0085	-0.07586 to 0	No	ns	0.9979	A-E		
0mM Mannitol WT vs. 200mM Mar	0.026	-0.05836 to 0	No	ns	0.8116	A-F		
0mM Mannitol sip428 vs. 150mM M	0.0095	-0.07486 to 0	No	ns	0.9965	B-C		
0mM Mannitol sip428 vs. 150mM M	0.039	-0.04536 to 0	No	ns	0.507	B-D		
0mM Mannitol sip428 vs. 200mM M	0.0035	-0.08086 to 0	No	ns	>0.9999	B-E		
0mM Mannitol sip428 vs. 200mM M	0.021	-0.06336 to 0	No	ns	0.9055	B-F		
150mM Mannitol WT vs. 150mM M	0.0295	-0.05486 to 0	No	ns	0.7323	C-D		
150mM Mannitol WT vs. 200mM M	-0.006	-0.09036 to 0	No	ns	0.9996	C-E		
150mM Mannitol WT vs. 200mM M	0.0115	-0.07286 to 0	No	ns	0.9917	C-F		
150mM Mannitol sip428 vs. 200mM	-0.0355	-0.1199 to 0.	No	ns	0.5881	D-E		
150mM Mannitol sip428 vs. 200mM	-0.018	-0.1024 to 0.	No	ns	0.9463	D-F		
200mM Mannitol WT vs. 200mM M	0.0175	-0.06686 to 0	No	ns	0.9518	E-F		
Test details	Mean 1	Mean 2	Mean Diff.	SE of di	n1	n2	q	DF
0mM Mannitol WT vs. 0mM Manni	0.0705	0.0655	0.005	0.0212	2	2	0.33	6
0mM Mannitol WT vs. 150mM Mar	0.0705	0.056	0.0145	0.0212	2	2	0.97	6
0mM Mannitol WT vs. 150mM Mar	0.0705	0.0265	0.044	0.0212	2	2	2.94	6
0mM Mannitol WT vs. 200mM Mar	0.0705	0.062	0.0085	0.0212	2	2	0.57	6
0mM Mannitol WT vs. 200mM Mar	0.0705	0.0445	0.026	0.0212	2	2	1.74	6
0mM Mannitol sip428 vs. 150mM M	0.0655	0.056	0.0095	0.0212	2	2	0.63	6
0mM Mannitol sip428 vs. 150mM M	0.0655	0.0265	0.039	0.0212	2	2	2.6	6
0mM Mannitol sip428 vs. 200mM M	0.0655	0.062	0.0035	0.0212	2	2	0.23	6
0mM Mannitol sip428 vs. 200mM M	0.0655	0.0445	0.021	0.0212	2	2	1.4	6
150mM Mannitol WT vs. 150mM M	0.056	0.0265	0.0295	0.0212	2	2	1.97	6
150mM Mannitol WT vs. 200mM M	0.056	0.062	-0.006	0.0212	2	2	0.4	6
150mM Mannitol WT vs. 200mM M	0.056	0.0445	0.0115	0.0212	2	2	0.77	6
150mM Mannitol sip428 vs. 200mM	0.0265	0.062	-0.0355	0.0212	2	2	2.37	6
150mM Mannitol sip428 vs. 200mM	0.0265	0.0445	-0.018	0.0212	2	2	1.2	6
200mM Mannitol WT vs. 200mM M	0.062	0.0445	0.0175	0.0212	2	2	1.17	6

One-Way ANOVA Experiment 6. Data shows the one-way analysis of variance between the catalase activity of two replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different mannitol concentrations (0mM, 150mM, and 200mM) in 7 days showing statistical significance (at p-value < 0.05).

FIG 7: CAT MANN 14DAYS				
	MANNITOL			
	0mM		150mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT
1	0.018	0.025	0.016	0.018
2	0.018	0.02	0.026	0.021
3	0.015	0.019	0.029	0.021
MEAN	**0.017**	**0.021**	**0.024**	**0.020**
SE	**0.0010**	**0.0019**	**0.0039**	**0.0010**

Experiment 7. Data shows mean and SEM of catalase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0mM, and 150mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff	95.00% C	Below thr	Summar	Adjusted P Value			
OmM Mannitol WT vs. 0mM Man	0.004333	-0.006016	No	ns	0.5651	A-B		
OmM Mannitol WT vs. 150mM M	0.001333	-0.009016	No	ns	0.9748	A-C		
OmM Mannitol WT vs. 150mM M	-0.00233	-0.01268 t	No	ns	0.8857	A-D		
0mM Mannitol sip428 vs. 150mM	-0.003	-0.01335 t	No	ns	0.7912	B-C		
0mM Mannitol sip428 vs. 150mM	-0.00667	-0.01702 t	No	ns	0.2433	B-D		
150mM Mannitol WT vs. 150mM	-0.00367	-0.01402 t	No	ns	0.6802	C-D		
Test details	Mean 1	Mean 2	Mean Dif	SE of di	n1	n2	q	DF
OmM Mannitol WT vs. 0mM Man	0.02133	0.017	0.00433	0.0032	3	3	1.9	8
OmM Mannitol WT vs. 150mM M	0.02133	0.02	0.00133	0.0032	3	3	0.58	8
OmM Mannitol WT vs. 150mM M	0.02133	0.02367	-0.0023	0.0032	3	3	1.02	8
0mM Mannitol sip428 vs. 150mM	0.017	0.02	-0.003	0.0032	3	3	1.31	8
0mM Mannitol sip428 vs. 150mM	0.017	0.02367	-0.0067	0.0032	3	3	2.92	8
150mM Mannitol WT vs. 150mM	0.02	0.02367	-0.0037	0.0032	3	3	1.61	8

One-Way ANOVA Experiment 7. Data shows the one-way analysis of variance between the catalase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different mannitol concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

FIG 8: CAT MANN 14 DAYS				
	MANNITOL			
	0mM		150mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT
1	0.0068	0.0107	0.0136	0.0093
2	0.0068	0.0107	0.0111	0.0143
3	0.0061	0.01	0.0107	0.0079
4	0.0082	0.008		
MEAN	**0.007**	**0.010**	**0.012**	**0.011**
SE	**0.0004**	**0.0006**	**0.0009**	**0.0019**

Experiment 8. Data shows mean and SEM of catalase activity in four replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under mannitol stress treatment (0mM, and 150mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Di	95.00%	Below	Summar	Adjusted P Value			
OmM Mannitol WT vs. 0mM Mani	0.0029	-0.0011	No	ns	0.1941	A-B		
OmM Mannitol WT vs. 150mM M	-0.0007	-0.0050	No	ns	0.9669	A-C		
OmM Mannitol WT vs. 150mM M	-0.002	-0.0063	No	ns	0.5443	A-D		
0mM Mannitol sip428 vs. 150mM	-0.0035	-0.0078	No	ns	0.125	B-C		
0mM Mannitol sip428 vs. 150mM	-0.0048	-0.0091	Yes	*	0.0297	B-D		
150mM Mannitol WT vs. 150mM M	-0.0013	-0.0059	No	ns	0.828	C-D		
Test details	Mean 1	Mean 2	Mean D	SE of di	n1	n2	q	DF
OmM Mannitol WT vs. 0mM Mani	0.0099	0.007	0.003	0.0013	4	4	3.1	10
OmM Mannitol WT vs. 150mM M	0.0099	0.0105	-0	0.0014	4	3	0.6	10
OmM Mannitol WT vs. 150mM M	0.0099	0.0118	-0	0.0014	4	3	1.9	10
0mM Mannitol sip428 vs. 150mM	0.007	0.0105	-0	0.0014	4	3	3.5	10
0mM Mannitol sip428 vs. 150mM	0.007	0.0118	-0	0.0014	4	3	4.8	10
150mM Mannitol WT vs. 150mM M	0.0105	0.0118	-0	0.0015	3	3	1.2	10

One-Way ANOVA Experiment 8. Data shows the one-way analysis of variance between the catalase activity of four replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different mannitol concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

SALINITY STRESS

PEROXIDASE ACTIVITY IN NACL-STRESS PLANTS FOR 7 DAYS

FIG 9: POD NACL 7 DAYS						
	NACL					
	0mM		**150mM**		**200mM**	
REPLICATES	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.433	0.413	1.56	1.363	2.139	2.233
2	0.411	0.675	1.56	1.428	2.172	2.262
3	0.505	0.735	1.65	1.65	1.962	2.367
MEAN	**0.45**	**0.61**	**1.59**	**1.48**	**2.09**	**2.29**
SE	**0.03**	**0.10**	**0.03**	**0.09**	**0.07**	**0.04**

Experiment 9. Data shows mean and SEM of peroxidase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, 150mM and 200mM) for 7 days.

Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Tukey's multiple comparisons test	Mean D	95.00%	Below	Summ	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip	0.158	-0.1482	No	ns	0.5375	A-B		
0mM NaCl WT vs. 150mM NaCl	-0.873	-1.179 t	Yes	****	<0.0001	A-C		
0mM NaCl WT vs. 150mM NaCl	-0.982	-1.289 t	Yes	****	<0.0001	A-D		
0mM NaCl WT vs. 200mM NaCl	-1.68	-1.986 t	Yes	****	<0.0001	A-E		
0mM NaCl WT vs. 200mM NaCl	-1.483	-1.790 t	Yes	****	<0.0001	A-F		
0mM NaCl sip 428 vs. 150mM N	-1.031	-1.337 t	Yes	****	<0.0001	B-C		
0mM NaCl sip 428 vs. 150mM N	-1.14	-1.447 t	Yes	****	<0.0001	B-D		
0mM NaCl sip 428 vs. 200mM N	-1.838	-2.144 t	Yes	****	<0.0001	B-E		
0mM NaCl sip 428 vs. 200mM N	-1.641	-1.948 t	Yes	****	<0.0001	B-F		
150mM NaCl WT vs. 150mM Na	-0.11	-0.4159	No	ns	0.8275	C-D		
150mM NaCl WT vs. 200mM Na	-0.807	-1.113 t	Yes	****	<0.0001	C-E		
150mM NaCl WT vs. 200mM Na	-0.611	-0.9169	Yes	***	0.0002	C-F		
150mM NaCl sip428 vs. 200mM	-0.697	-1.004 t	Yes	****	<0.0001	D-E		
150mM NaCl sip428 vs. 200mM	-0.501	-0.8072	Yes	**	0.0015	D-F		
200mM NaCl WT vs. 200mM Na	0.1963	-0.1099	No	ns	0.3245	E-F		
Test details	Mean 1	Mean 2	Mean	SE of d	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip	0.6077	0.45	0.16	0.091	3	3	2.5	12
0mM NaCl WT vs. 150mM NaCl	0.6077	1.48	-0.9	0.091	3	3	14	12
0mM NaCl WT vs. 150mM NaCl	0.6077	1.59	-1	0.091	3	3	15	12
0mM NaCl WT vs. 200mM NaCl	0.6077	2.287	-1.7	0.091	3	3	26	12
0mM NaCl WT vs. 200mM NaCl	0.6077	2.091	-1.5	0.091	3	3	23	12
0mM NaCl sip 428 vs. 150mM N	0.4497	1.48	-1	0.091	3	3	16	12
0mM NaCl sip 428 vs. 150mM N	0.4497	1.59	-1.1	0.091	3	3	18	12
0mM NaCl sip 428 vs. 200mM N	0.4497	2.287	-1.8	0.091	3	3	29	12
0mM NaCl sip 428 vs. 200mM N	0.4497	2.091	-1.6	0.091	3	3	25	12
150mM NaCl WT vs. 150mM Na	1.48	1.59	-0.1	0.091	3	3	1.7	12
150mM NaCl WT vs. 200mM Na	1.48	2.287	-0.8	0.091	3	3	13	12
150mM NaCl WT vs. 200mM Na	1.48	2.091	-0.6	0.091	3	3	9.5	12
150mM NaCl sip428 vs. 200mM	1.59	2.287	-0.7	0.091	3	3	11	12
150mM NaCl sip428 vs. 200mM	1.59	2.091	-0.5	0.091	3	3	7.8	12
200mM NaCl WT vs. 200mM Na	2.287	2.091	0.2	0.091	3	3	3	12

One-Way ANOVA Experiment 9. Data shows the one-way analysis of variance between the catalase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants)

at different NaCl concentrations (0mM, 150mM and 200mM) in 7 days showing statistical significance (at p-value < 0.05).

FIG 10: POD NACL 7 DAYS						
	NACL					
	0mM		**100mM**		**175mM**	
REPLICATES	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.811	1.046	1.432	0.617	2.299	1.379
2	0.839	1.102	1.421	0.637	1.714	1.36
3	0.845	0.79	1.477	0.638	1.452	1.343
4	0.867	0.758	1.52	0.631	1.257	1.393
MEAN	**0.84**	**0.92**	**1.46**	**0.63**	**1.68**	**1.37**
SE	**0.01**	**0.09**	**0.02**	**0.00**	**0.23**	**0.01**

Experiment 10. Data shows mean and SEM of peroxidase activity in four replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, 100mM and 175mM) for 7 days.

Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff	95.00%	Below	Summa	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip	0.0835	-0.3649	No	ns	0.9903	A-B		
0mM NaCl WT vs. 100mM NaCl V	0.2933	-0.1552	No	ns	0.3407	A-C		
0mM NaCl WT vs. 100mM NaCl s	-0.5385	-0.9869	Yes	*	0.0136	A-D		
0mM NaCl WT vs. 175mM NaCl V	-0.4448	-0.8932	No	ns	0.0526	A-E		
0mM NaCl WT vs. 175mM NaCl s	-0.7565	-1.205 t	Yes	***	0.0005	A-F		
0mM NaCl sip 428 vs. 100mM Na	0.2098	-0.2387	No	ns	0.6766	B-C		
0mM NaCl sip 428 vs. 100mM Na	-0.622	-1.070 t	Yes	**	0.0039	B-D		
0mM NaCl sip 428 vs. 175mM Na	-0.5283	-0.9767	Yes	*	0.0158	B-E		
0mM NaCl sip 428 vs. 175mM Na	-0.84	-1.288 t	Yes	***	0.0002	B-F		
100mM NaCl WT vs. 100mM NaC	-0.8318	-1.280 t	Yes	***	0.0002	C-D		
100mM NaCl WT vs. 175mM NaC	-0.738	-1.186 t	Yes	***	0.0007	C-E		
100mM NaCl WT vs. 175mM NaC	-1.05	-1.498 t	Yes	****	<0.0001	C-F		
100mM NaCl sip428 vs. 175mM N	0.09375	-0.3547	No	ns	0.9837	D-E		
100mM NaCl sip428 vs. 175mM N	-0.218	-0.6664	No	ns	0.6418	D-F		
175mM NaCl WT vs. 175mM NaC	-0.3118	-0.7602	No	ns	0.281	E-F		
Test details	Mean 1	Mean 2	Mean	SE of d	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip	0.924	0.8405	0.08	0.141	4	4	0.84	18
0mM NaCl WT vs. 100mM NaCl V	0.924	0.6308	0.29	0.141	4	4	2.94	18
0mM NaCl WT vs. 100mM NaCl s	0.924	1.463	-0.5	0.141	4	4	5.4	18
0mM NaCl WT vs. 175mM NaCl V	0.924	1.369	-0.4	0.141	4	4	4.46	18
0mM NaCl WT vs. 175mM NaCl s	0.924	1.681	-0.8	0.141	4	4	7.58	18
0mM NaCl sip 428 vs. 100mM Na	0.8405	0.6308	0.21	0.141	4	4	2.1	18
0mM NaCl sip 428 vs. 100mM Na	0.8405	1.463	-0.6	0.141	4	4	6.23	18
0mM NaCl sip 428 vs. 175mM Na	0.8405	1.369	-0.5	0.141	4	4	5.29	18
0mM NaCl sip 428 vs. 175mM Na	0.8405	1.681	-0.8	0.141	4	4	8.42	18
100mM NaCl WT vs. 100mM NaC	0.6308	1.463	-0.8	0.141	4	4	8.34	18
100mM NaCl WT vs. 175mM NaC	0.6308	1.369	-0.7	0.141	4	4	7.4	18
100mM NaCl WT vs. 175mM NaC	0.6308	1.681	-1.1	0.141	4	4	10.5	18
100mM NaCl sip428 vs. 175mM N	1.463	1.369	0.09	0.141	4	4	0.94	18
100mM NaCl sip428 vs. 175mM N	1.463	1.681	-0.2	0.141	4	4	2.19	18
175mM NaCl WT vs. 175mM NaC	1.369	1.681	-0.3	0.141	4	4	3.12	18

One-Way ANOVA Experiment 10. Data shows the one-way analysis of variance between the catalase activity of four replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at different NaCl concentrations (0mM, 100mM and 175mM) in 7 days showing statistical significance (at p-value < 0.05).

PEROXIDASE ACTIVITY IN NACL-STRESS PLANTS FOR 14 DAYS

FIG 11:POD NACL 14 DAYS				
	NACL			
	0mM		**200mM**	
REPLICAT	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.807	0.641	2.523	1.823
2	0.803	0.66	2.539	1.851
3	0.81	0.699	1.825	1.61
4	1.115	1.022	1.746	1.35
MEAN	**0.88**	**0.76**	**2.16**	**1.66**
SE	**0.08**	**0.09**	**0.22**	**0.12**

Experiment 11. Data shows mean and SEM of peroxidase activity in four replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 200mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean D	95.00	Belov	Sum	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip 4	0.034	-0.704	No	ns	0.9973	A-B		
0mM NaCl WT vs. 200mM NaCl W	-0.397	-1.135	No	ns	0.2691	A-C		
0mM NaCl WT vs. 200mM NaCl si	-1.723	-2.462	Yes	**	0.0024	A-D		
0mM NaCl sip 428 vs. 200mM NaC	-0.431	-1.169	No	ns	0.2246	B-C		
0mM NaCl sip 428 vs. 200mM NaC	-1.757	-2.496	Yes	**	0.0022	B-D		
200mM NaCl WT vs. 200mM NaCl	-1.327	-2.065	Yes	**	0.0064	C-D		
Test details	Mean 1	Mean	Mean	SE o	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip 4	0.334	0.3	0.03	0.2	2	2	0.3	4
0mM NaCl WT vs. 200mM NaCl W	0.334	0.73	-0.4	0.2	2	2	3.1	4
0mM NaCl WT vs. 200mM NaCl si	0.334	2.06	-1.7	0.2	2	2	13	4
0mM NaCl sip 428 vs. 200mM NaC	0.3	0.73	-0.4	0.2	2	2	3.4	4
0mM NaCl sip 428 vs. 200mM NaC	0.3	2.06	-1.8	0.2	2	2	14	4
200mM NaCl WT vs. 200mM NaCl	0.731	2.06	-1.3	0.2	2	2	10	4

One-Way ANOVA Experiment 11. Data shows the one-way analysis of variance between the catalase activity of four replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at

different NaCl concentrations (0mM, and 200mM) in 14 days showing statistical significance (at p-value < 0.05).

FIG 12: POD NACL 14 DAYS				
	NACL			
	0mM		**200mM**	
REPLICAT	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.21	0.366	2.232	0.569
2	0.39	0.302	1.882	0.892
MEAN	**0.30**	**0.33**	**2.06**	**0.73**
SE	**0.09**	**0.03**	**0.18**	**0.16**

Experiment 12. Data shows mean and SEM of peroxidase activity in two replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 200mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean I	95.00%	Belov	Summa	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sij	0.128	-0.4430	No	ns	0.908	A-B		
0mM NaCl WT vs. 200mM NaCl	-0.77	-1.346 t	Yes	**	0.008	A-C		
0mM NaCl WT vs. 200mM NaCl	-1.28	-1.846 t	Yes	***	1E-04	A-D		
0mM NaCl sip 428 vs. 200mM N	-0.9	-1.474 t	Yes	**	0.003	B-C		
0mM NaCl sip 428 vs. 200mM N	-1.4	-1.974 t	Yes	****	<0.0001	B-D		
200mM NaCl WT vs. 200mM Na	-0.5	-1.071 t	No	ns	0.094	C-D		
Test details	Mean 1	Mean 2	Mean	SE of d	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sij	0.884	0.756	0.13	0.192	4	4	0.9	12
0mM NaCl WT vs. 200mM NaCl	0.884	1.659	-0.8	0.192	4	4	5.7	12
0mM NaCl WT vs. 200mM NaCl	0.884	2.158	-1.3	0.192	4	4	9.4	12
0mM NaCl sip 428 vs. 200mM N	0.756	1.659	-0.9	0.192	4	4	6.6	12
0mM NaCl sip 428 vs. 200mM N	0.756	2.158	-1.4	0.192	4	4	10	12
200mM NaCl WT vs. 200mM Na	1.659	2.158	-0.5	0.192	4	4	3.7	12

One-Way ANOVA Experiment 12. Data shows the one-way analysis of variance between the catalase activity of two replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at

different NaCl concentrations (0mM, and 200mM) in 14 days showing statistical significance (at p-value < 0.05).

CATALASE ACTIVITY IN NACL-STRESSED PLANTS FOR 7 DAYS

FIG 13: CAT NACL 7 DAYS				
	NACL			
	0mM		**100mM**	
REPLICAT	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.032	0.021	0.033	0.011
2	0.032	0.022	0.037	0.013
3	0.015	0.049	0.049	0.014
MEAN	**0.026**	**0.031**	**0.040**	**0.013**
SE	**0.0057**	**0.0092**	**0.0048**	**0.0009**

Experiment 13. Data shows mean and SEM of catalase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 100mM) for 7 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Dif	95.00%	Below	Sum	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip4	0.00433	-0.022	No	ns	0.9525	A-B		
0mM NaCl WT vs. 100mM NaCl W	0.018	-0.008	No	ns	0.2167	A-C		
0mM NaCl WT vs. 100mM NaCl si	-0.009	-0.035	No	ns	0.7131	A-D		
0mM NaCl sip428 vs. 100mM NaC	0.01367	-0.013	No	ns	0.4142	B-C		
0mM NaCl sip428 vs. 100mM NaC	-0.0133	-0.040	No	ns	0.4333	B-D		
100mM NaCl WT vs. 100mM NaC	-0.027	-0.053	Yes	*	0.0484	C-D		
Test details	Mean 1	Mean 2	Mean	SE o	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip4	0.03067	0.026	0.004	0	3	3	0.7	8
0mM NaCl WT vs. 100mM NaCl W	0.03067	0.013	0.018	0	3	3	3	8
0mM NaCl WT vs. 100mM NaCl si	0.03067	0.04	-0.01	0	3	3	1.5	8
0mM NaCl sip428 vs. 100mM NaC	0.02633	0.013	0.014	0	3	3	2.3	8
0mM NaCl sip428 vs. 100mM NaC	0.02633	0.04	-0.01	0	3	3	2.3	8
100mM NaCl WT vs. 100mM NaC	0.01267	0.04	-0.03	0	3	3	4.6	8

One-Way ANOVA Experiment 13. Data shows the one-way analysis of variance between the catalase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants)

at different NaCl concentrations (0mM, and 100mM) in 7 days showing statistical significance (at p-value < 0.05).

FIG 14: CAT NACL 7 DAYS				
	NACL			
	0mM		200mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT
1	0.029	0.018	0.055	0.093
2	0.025	0.017	0.063	0.097
MEAN	**0.027**	**0.018**	**0.059**	**0.095**
SE	**0.0020**	**0.0005**	**0.0040**	**0.0020**

Experiment 14. Data shows mean and SEM of catalase activity in two replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 200mM) for 7 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean	95.00	Below	Summa	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip42	-0.01	-0.02	No	ns	0.1601	A-B		
0mM NaCl WT vs. 200mM NaCl WT	-0.08	-0.09	Yes	***	0.0001	A-C		
0mM NaCl WT vs. 200mM NaCl sip	-0.04	-0.05	Yes	***	0.001	A-D		
0mM NaCl sip428 vs. 200mM NaCl '	-0.07	-0.08	Yes	***	0.0002	B-C		
0mM NaCl sip428 vs. 200mM NaCl s	-0.03	-0.04	Yes	**	0.0027	B-D		
200mM NaCl WT vs. 200mM NaCl s	0.04	0.021	Yes	**	0.0017	C-D		
Test details	Mean	Mean	Mean	SE of d	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip42	0.02	0	-0.01	0.003	2	2	4	4
0mM NaCl WT vs. 200mM NaCl WT	0.02	0.1	-0.08	0.003	2	2	31	4
0mM NaCl WT vs. 200mM NaCl sip	0.02	0.1	-0.04	0.003	2	2	17	4
0mM NaCl sip428 vs. 200mM NaCl '	0.03	0.1	-0.07	0.003	2	2	28	4
0mM NaCl sip428 vs. 200mM NaCl s	0.03	0.1	-0.03	0.003	2	2	13	4
200mM NaCl WT vs. 200mM NaCl s	0.1	0.1	0.04	0.003	2	2	15	4

One-Way ANOVA Experiment 14. Data shows the one-way analysis of variance between the catalase activity of two replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants) at

different NaCl concentrations (0mM, and 200mM) in 7 days showing statistical significance (at p-value < 0.05).

CATALASE ACTIVITY IN NACL-STRESSED PLANTS FOR 14 DAYS

FIG 15: CAT NACL 14 DAYS				
	NACL			
	0mM		**150mM**	
REPLICAT	**MSI-2-7**	**WT**	**MSI-2-7**	**WT**
1	0.097	0.201	0.158	0.171
2	0.095	0.128	0.177	0.198
3	0.147	0.119	0.28	0.146
MEAN	**0.11**	**0.15**	**0.21**	**0.17**
SE	**0.02**	**0.03**	**0.04**	**0.02**

Experiment 15. Data shows mean and SEM of catalase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 150mM) for 14 days.

Number of families	1							
Number of comparisons per famil	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean D	95.00	Belov	Sumn	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl si	0.0363	-0.07	No	ns	0.7523	A-B		
0mM NaCl WT vs. 150mM NaC	-0.022	-0.13	No	ns	0.924	A-C		
0mM NaCl WT vs. 150mM NaC	-0.056	-0.17	No	ns	0.4614	A-D		
0mM NaCl sip428 vs. 150mM N	-0.059	-0.17	No	ns	0.4206	B-C		
0mM NaCl sip428 vs. 150mM N	-0.092	-0.20	No	ns	0.1272	B-D		
150mM NaCl WT vs. 150mM Na	-0.033	-0.14	No	ns	0.7954	C-D		
Test details	Mean 1	Mean	Mean	SE of	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl si	0.1493	0.11	0.04	0.04	3	3	1.42	8
0mM NaCl WT vs. 150mM NaC	0.1493	0.17	-0	0.04	3	3	0.87	8
0mM NaCl WT vs. 150mM NaC	0.1493	0.21	-0.1	0.04	3	3	2.17	8
0mM NaCl sip428 vs. 150mM N	0.113	0.17	-0.1	0.04	3	3	2.29	8
0mM NaCl sip428 vs. 150mM N	0.113	0.21	-0.1	0.04	3	3	3.59	8
150mM NaCl WT vs. 150mM Na	0.1717	0.21	-0	0.04	3	3	1.3	8

One-Way ANOVA Experiment 15. Data shows the one-way analysis of variance between the catalase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants)

at different NaCl concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

FIG 16:CAT NACL 14 DAYS				
	NACL			
	0mM		150mM	
REPLICAT	MSI-2-7	WT	MSI-2-7	WT
1	0.14	0.128	0.429	0.256
2	0.104	0.104	0.583	0.286
3	0.14	0.131	0.182	0.393
MEAN	**0.13**	**0.12**	**0.40**	**0.31**
SE	**0.01**	**0.01**	**0.12**	**0.04**

Experiment 16. Data shows mean and SEM of catalase activity in three replicates of control (WT) and MSI-2-7 (SIP-428 silenced plants) under NaCl stress treatment (0mM, and 150mM) for 14 days.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean D	95.00	Below	Sumr	Adjusted P Value			
0mM NaCl WT vs. 0mM NaCl sip428	-0.007	-0.289	No	ns	1	A-B		
0mM NaCl WT vs. 150mM NaCl WT	-0.191	-0.473	No	ns	0.21	A-C		
0mM NaCl WT vs. 150mM NaCl sip4	-0.277	-0.559	No	ns	0.05	A-D		
0mM NaCl sip428 vs. 150mM NaCl V	-0.184	-0.466	No	ns	0.24	B-C		
0mM NaCl sip428 vs. 150mM NaCl s	-0.27	-0.552	No	ns	0.06	B-D		
150mM NaCl WT vs. 150mM NaCl si	-0.086	-0.369	No	ns	0.77	C-D		
Test details	Mean 1	Mean	Mean	SE of	n1	n2	q	DF
0mM NaCl WT vs. 0mM NaCl sip428	0.121	0.13	-0.01	0.09	3	3	0.11	8
0mM NaCl WT vs. 150mM NaCl WT	0.121	0.31	-0.19	0.09	3	3	3.05	8
0mM NaCl WT vs. 150mM NaCl sip4	0.121	0.4	-0.28	0.09	3	3	4.44	8
0mM NaCl sip428 vs. 150mM NaCl V	0.128	0.31	-0.18	0.09	3	3	2.94	8
0mM NaCl sip428 vs. 150mM NaCl s	0.128	0.4	-0.27	0.09	3	3	4.33	8
150mM NaCl WT vs. 150mM NaCl si	0.312	0.4	-0.09	0.09	3	3	1.38	8

One-Way ANOVA Experiment 16. Data shows the one-way analysis of variance between the catalase activity of three replicates each of control (WT) and MSI-2-7 (SIP-428 silenced plants)

at different NaCl concentrations (0mM, and 150mM) in 14 days showing statistical significance (at p-value < 0.05).

IN-SILICO ANALYSIS OF THE SUBCELLULAR LOCALIZATION OF SIP428

SIP428 Sequence

>SIP428

MSMSLRLCCEPSISGLKNKRDLLGLDLAANHLNIPMRKWFSGVKKFIPFEGYVKFVQTT
ARITFPKISSDCKDNSPSNFLSHKKKVPYSDPPSMKDVDSLYEFFDRSTKLVVLTGAGMS
TESGIPDYRSPNGAYSTGFKPITHQEFLRSSKARRRYWTRSYAGWRRFTAAQPSTGHIAL
SSLEKAGHISFMITQNVDRLHHRAGSNPLELHGTVYIVACTNCGFTLPRELFQDQVKAQ
NPKWAAAIESLDYDSRSDESFGMKQRPDGDIEIDEKFWEEDFYIPDCERCQGVLKPDVV
FFGDNVPKARADVAMEAAKGCDAFLVLGSSMMTMSAFRLIKAAHEAGAATAIVNIGV
TRADDLVPLKINARVGEILPRLLNVGSLSIPAL

The full-length sequence was used to predict its subcellular localization. Seven different freely available prediction software was used. The table below shows the prediction.

S/N	PREDICTION TOOL	LOCALIZATION
1	WoLF PSORT	CHLOROPLAST
2	CELLO	Chloroplast and Mitochondria
3	Plant- mPLoc	Chloroplast
4	Plant-mSubP	Plastid
5	Busca	Plasma membrane
6	MultiLoc2	Cytoplasm
7	DeepLoc1-2	Plastid

VITA

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Presentations:

Onabanjo Mariam (2022), The Role of SIP428 in Abiotic Stress Signaling through Biochemical Analysis and Subcellular Localization, Department of Biological and Health Sciences Graduate Seminar, East Tennessee State University.

Onabanjo Mariam and Dhirendra Kumar (2022), Understanding the roles of SIP428, an NAD+ dependent deacetylase enzyme in mediating stress signaling in *Nicotiana tabacum*, Phytochemical Society of North America (PSNA), Poster Presentation

Onabanjo Mariam, Thakuri B.K. Chand, and Dhirendra Kumar (2022), Understanding the roles of SIP428 in mediating abiotic stress signaling in *Nicotiana tabacum*, Kentucky-Tennessee branch of American Society of Microbiology, Poster Presentation

Onabanjo Mariam and Dhirendra Kumar (2023), Understanding the roles of SIP428, an NAD+ dependent deacetylase enzyme in mediating stress signaling in *Nicotiana tabacum*, Appalachian Student Research Forum (ASRF), Oral Presentation

Honors and Awards:

Graduate School Research Grant, East Tennessee State University, 2022

William F. and Nina H. Fraley Award, Department of Biological Sciences, East Tennessee State University, 2022.

The Phytochemical Society of North America -NSF Travel Award, 2022