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Chitosan-PVA and Copper Nanoparticles Improve Growth and Overexpress the SOD and JA Genes in Tomato Plants under Salt Stress

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Received: 3 August 2018; Accepted: 6 September 2018; Published: 8 September 2018

Abstract: Saline stress severely affects the growth and productivity of plants. The activation of hormonal signaling cascades and reactive oxygen species (ROS) in response to salt stress are important for cellular detoxification. Jasmonic acid (JA) and the enzyme SOD (superoxide dismutase), are well recognized markers of salt stress in plants. In this study, the application of chitosan-polyvinyl alcohol hydrogels (Cs-PVA) and copper nanoparticles (Cu NPs) on the growth and expression of defense genes in tomato plants under salt stress was evaluated. Our results demonstrate that Cs-PVA and Cs-PVA + Cu NPs enhance plant growth and also promote the expression of JA and SOD genes in tomato (*Solanum lycopersicum* L.), under salt stress. We propose that Cs-PVA and Cs-PVA + Cu NPs mitigate saline stress through the regulation of oxidative and ionic stress.

Keywords: gene expression; crop growth; oxidative stress; ionic stress; SOD gene; JA gene

1. Introduction

Plants have developed and evolved a spectrum of defense machinery to adapt to an array of environmental stresses. Salinity is one of the main types of abiotic stress that affects plants, decreasing their capacity for growth and productivity [1]. Plants suffer saline stress when sodium ions (Na) encounter the roots and are transported through non-selective cation channels. Calcium, reactive oxygen species (ROS), and hormone signaling cascades are then activated, resulting in the expression and activation of cellular detoxification mechanisms such as the salt overly sensitive (SOS) signaling pathway and the Na^+/H^+ exchanger (NHX) responses [2]. The jasmonic acid (JA) pathway is one of the key hormonal signaling pathways that mitigates the effect of salt stress on plants. Jasmonic acid enhances tolerance to both osmotic and oxidative stress, via a systemic physiological alteration in the plant instead of simply controlling the ionic homeostasis of the plant [3]. Another mechanism a plant employs for enhanced tolerance to salt stress is through antioxidant enzyme activation. The enzyme SOD, forms the first line of defense against ROS, converting highly toxic ($O_2^{\bullet}$) into less toxic H_2O_2,

whit the resulting H_2O_2 subsequently scavenged by enzymes such as, catalase (CAT), peroxidase ascorbate (APX), and peroxidase glutathione (GPX) [4].

Currently, nanotechnology is a science that is being widely explored in plants due to the unique properties of nanoparticles. Copper nanoparticles are the most investigated nanoparticles in plants. The chitosan + Cu NPs (copper nanoparticles) are less toxic to plants than $CuSO_4$ and free Cu NPs [5]. Previously, some studies have been conducted on the use of Cu NPs in combination with chitosan that have shown positive results in the growth and activation of bioactive compounds in plants [6–10]. Chitosan is a multifunctional natural polymer, due to its ability to crosslink with, and to exchange, cations in acid solutions as well as to show high affinity to metal ions [11]. In agriculture, chitosan is used as a growth biostimulant and as an inducer of defense responses in plants under abiotic and biotic stress [12,13]. Nanomaterials have shown positive results as inducers of tolerance to abiotic stress [14]. Previously, we demonstrated that chitosan-polyvinyl alcohol hydrogels (Cs-PVA) + Cu NPs improved the tolerance to salinity in tomato plants by increasing the content of phenols, β-carotene, vitamin C, and lycopene in stressed plants [6,8]. Cs-PVA + CuNPs were also demonstrated to increase the enzyme activity of SOD, CAT, APX, GPX, and phenylalanine ammonia lyase (PAL) [6,8]. Therefore, the objective of this study was to evaluate the response of Cs-PVA and Cu NPs in the growth and expression of defense genes in tomato plants under salt stress.

2. Materials and Methods

2.1. Synthesis of Cs-PVA and Cu NPs Absorption

The synthesis and characterization of the Cs-PVA and Cu NPs used in this experiment are described in Pinedo-Guerrero et al. [10] The synthesis was carried out in the pilot plant of the Research Center for Applied Chemistry (Saltillo, México).

2.2. Plant Growth Conditions and Sampling

Tomato (*Solanum lycopersicum* L.) plants (hybrid var. "Huno F1", Harris Moran Seed Company, Modesto, CA, USA; saladette type and indeterminate growth) were established in a polyethylene covered greenhouse at 21 °C at the Department of Horticulture of the Autonomous University of Agriculture Antonio Narro. The average light intensity was 565 μmol m^2 s^{-1}, and the average relative humidity was 51%. Specifically, seeds were planted in a standard soil mixture in polystyrene trays, and were cultivated for 30 days until the plants had developed four true leaves. A density of three plants per square meter was used. A mixture of peat moss (pH = 5.5–6.5, electric conductivity = 0.6–0.8 mmhos/cm, moisture content = 45–50%, organic matter = 68–82%) and perlite (50:50 *v/v*) placed into black polyethylene 12 L bags was used.

The treatment regimens that were applied prior to plant transplantation, consisted of 1 g of chitosan-PVA hydrogel distributed in the low, medium, and high part of the soil mixture to have a better dispersion of the Cu NPs in the root area. The experiment was carried out under two conditions: (1) 10 mg of Cu NPs were absorbed in 1 g of chitosan-PVA hydrogel, chitosan-PVA (1 g) hydrogel treatment, and a control; and (2) the same treatments were evaluated, however a treatment of 100 mM NaCl delivered in nutrient solution was applied 21 days after transplantation (DAT). A directed irrigation system was used to water the plants. The Steiner nutrient solution [15] at 50% was used with the following micronutrients in chelated form including EDTA (2,2′,2″,2‴-[Ethane-1,2-diyldinitrilo] tetraacetic acid)/Fe EDTA = 3.75 ppm; Mn EDTA = 1.85 ppm; B = 0.35 ppm; Zn EDTA = 0.30 ppm; Cu EDTA = 0.15 ppm; and Mo = 0.10 ppm.

Two leaves were sampled for the assessment of gene expression. The first sampling was performed at 20 DAT (one day before the application of saline stress), while the second sampling was performed at 23 DAT (48 h after the application of saline stress). Samples consisted of five randomly selected plants for each treatment. The youngest leaf was completely expanded and placed in an aluminum bag, immediately frozen with liquid nitrogen, then stored in an ultra-freezer at −80 °C. Plant growth

was measured at 28 DAT (one week after the application of saline stress) and 42 DAT (three weeks after the application of saline stress). Plant height was measured with a flexometer from the base of the soil surface to the growth apex. The stem diameter was measured with a digital caliper between the first and second leaves from the base of the plant, representing internode 2, a fully expanded internode. The number of leaves and the number of nodes (third week after the application of salt stress) were also counted.

2.3. Real-Time Reverse Transcriptase PCR

RNA was extracted using TRIzol reagent, purified with chloroform, and precipitated with isopropanol, as outlined in Cui et al. [16]. RNA quantification was determined using a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, USA), including determining the 260/280 nm ratio of each sample, and RNA quality was visually determined by denaturing electrophoresis. The synthesis of cDNA was performed using a commercial kit, and according to the manufacturer© instructions (Promega, Madison, WI, USA). The primers corresponded to an endogenous internal control gene (actin) and five study genes (SOD, CAT, GPX, PR1, and JA), which were designed using the software Amplifix 1.7.0 (CNRS by Nicolas Jullien, Marseille, France), Oligoanalizer 3.1 (Integrated DNA Technologies IDT, Coralville, USA), and Primers BLAST (National Center for Biotechnology Information NCBI, Bethesda, USA) as described in Table 1.

Table 1. Primers sequence of analyzed genes.

Title 1	Forward Primer 5′-3′	Reverse Primer 5′-3′
ACT	CCCAGGCACACAGGTGTTAT	CAGGAGCAACTCGAAGCTCA
PR1	AAGTAGTCTGGCGCAACTCA	GTCCGATCCAGTTGCCTACA
JA	TGGTTCGTCGACTTCGTCAT	CTCGGCCTTGAGAGAGTTCA
SOD	TGATGGGCCAACTACGGTTAA	AAAATGGGCTCCTGTAGACATACAT
GPX	AGGAGCCTGGAAACATTGAAGA	CCATTCACGTCAACCTTGTCA
CAT	CCCTCTAAGTATCGCCCATCAA	TTGTACACAGGACCACCAGCAT

Real-time PCR reactions were analyzed in an Applied Biosystems StepOne™ Equipment version 2.3 (Thermo Fisher Scientific, Waltham, MA, USA) by the standard relative curve method, measuring the fluorescence intensity of Sybr Green. The PCR reaction for all genes was performed in a total volume of 20 µL. Regarding the Actin gene, 10 µL of Master Mix (Applied Biosystems, Foster City, CA, USA) was added along with 0.10 µL of first forward (72 nM), 0.08 µL of first reverse (60 nM), 2 µL of cDNA, and 7.82 µL of nuclease-free water. Regarding the PR1 gene, 10 µL of Master Mix was added along with 0.03 µL of first forward (20 nM), 0.05 µL of first reverse (40 nM), 2 µL of cDNA, and 7.92 µL of nuclease-free water. Regarding the JA gene, 10 µL of Master Mix, 0.05 µL of first forward (40 nM), 0.08 µL of first reverse (60 nM), 2 µL of cDNA, and 7.87 µL of nuclease-free water were added. As for the SOD, GPX, and CAT genes, 10 µL of Master Mix, 0.13 µL of first forward (100 nM), 0.13 µL of first reverse (100 nM), 2 µL of cDNA, and 7.73 µL of nuclease-free water were added. The qPCR was run with the following program in the thermal cycler: Hot Start, 10 min at 95 °C and PCR (40 cycles), 15 s at 95 °C and 1 min at 60 °C.

2.4. Data Analysis

The expression of genes of interest, namely SOD, CAT, GPX, PR1, and JA, was normalized via comparison to the expression of the internal reference gene, ACTIN. The standard error was calculated from the standard deviation and the variation coefficient of the reference gene and of the genes under assessment. For the growth variables, a completely randomized design with 16 replicates per treatment was used, with one plant considered as an experimental unit. The statistical language R CRAN was used, in which an analysis of variance and Fisher Least Significant Difference test ($\alpha \leq 0.05$) was conducted.

3. Results and Discussion

3.1. Cs-PVA and Cu NPs Improve the Growth of Plants under Salt Stress

The Cs-PVA and Cu NPs showed statistical differences in the growth parameters of the tomato plants evaluated (Table 2). Without stress, the Cu NPs increased the stem diameter by 4% when compared to the absolute control in the two measurements made (one and three weeks after the application of saline stress). Plant height, number of leaves, and number of nodes were not affected by the treatments. Under saline stress conditions, Cs-PVA and Cu NPs increased plant height (9% and 6%, respectively) and stem diameter (8% and 5%, respectively) when compared to the NaCl treatment in both of the measurements made. The Cu NPs significantly increased the number of leaves (5%) when compared to the NaCl treatment in the first measurement.

Table 2. Growth parameters of tomato plants.

Measuring	Stress	Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Leaves	Number of Clusters
28 DAT	Without Stress	T0	49.19 ± 0.61 a	7.51 ± 0.11 b	10.13 ± 0.13 a	nd
		Cs	48.38 ± 0.79 a	7.68 ± 0.14 ab	10.13 ± 0.15 a	nd
		nCu-Cs	47.69 ± 0.38 a	7.87 ± 0.11 a	10.44 ± 0.13 a	nd
	NaCl	NaCl	38.16 ± 0.79 b	6.31 ± 0.12 b	9.44 ± 0.13 b	nd
		Cs NaCl	42.19 ± 0.85 a	6.87 ± 0.15 a	9.69 ± 0.15 ab	nd
		nCu-Cs NaCl	41.44 ± 0.83 a	6.66 ± 0.12 ab	9.94 ± 0.14 a	nd
42 DAT	Without Stress	T0	96.75 ± 1.46 a	11.42 ± 0.20 ab	15.06 ± 0.14 a	2.63 ± 0.13 a
		Cs	95.56 ± 1.41 a	10.94 ± 0.20 b	14.81 ± 0.19 a	2.69 ± 0.12 a
		nCu-Cs	95.44 ± 1.21 a	11.74 ± 0.26 a	15.13 ± 0.13 a	2.75 ± 0.11 a
	NaCl	NaCl	76.50 ± 1.27 b	9.27 ± 0.15 b	13.56 ± 0.16 a	2.56 ± 0.13 a
		Cs NaCl	81.81 ± 0.98 a	9.79 ± 0.22 a	13.81 ± 0.16 a	2.56 ± 0.13 a
		nCu-Cs NaCl	79.00 ± 1.39 ab	9.57 ± 0.17 ab	13.75 ± 0.14 a	2.69 ± 0.12 a

DAT: Days after transplanting. T0: Absolute control. Cs = 1 g Cs-PVA hydrogel; nCu-Cs = 10 mg copper nanoparticles (Cu NPs) + Cs-PVA. NaCl: witness + 100 mM NaCl. Cs NaCl: Cs-PVA + 100 mM NaCl. nCu-Cs NaCl: 10 mg Cu NPs + Cs-PVA + 100 mM NaCl. nd: not determined. Data are the average of 16 replicas ± standard error. Means with the same letter within the same column of each treatment are not different according to the Fisher Least Significant Difference test ($\alpha \leq 0.05$).

In previous studies, we showed that Cu NPs improved the growth of tomato plants [7,8]. Similarly, in this study, we confirmed that Cu NPs increased the stem diameter of plants, so they could be used as growth stimulants. It is well known that salt stress severely affects the growth of plants [1], as was additionally demonstrated in this study. However, the Cs-PVA and the Cu NPs showed a positive effect by increasing the growth of the plants when compared to the NaCl treatment. Previously, we showed that Cs-PVA increased plant height with respect to NaCl treatment [8]. The CeO_2 NPs also showed similar effects in canola plants under salt stress [17]. This increase in growth could be due to the positive regulation of oxidative stress and ionic stress caused by salinity [18].

3.2. SOD Gene Expression Is Promoted in Response to Cs-PVA + Cu NPs and Cs-PVA

The expression of genes related to antioxidant enzymes at 20 and 23 DAT are presented in Figure 1. An alternative hypothesis was established as the fold change was greater than one (in absolute value) when compared to the control [19]. According to this hypothesis, the results of this investigation determined that Cs-PVA + Cu NPs elevated the expression of the SOD gene (7- and 4.3-fold change, respectively, Figure 1a) at 20 and 23 DAT. The expression of the CAT gene was only elevated at 20 DAT (1.1-fold change, Figure 1b). In contrast, the expression of the GPX gene was repressed at 20 and 23 DAT (0.6- and 0.7-fold change, respectively, Figure 1c) and the CAT gene at 23 DAT (0.56-fold change).

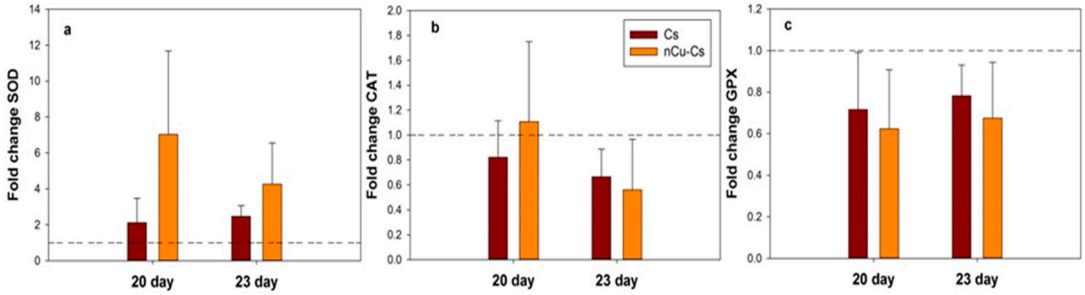

Figure 1. Expression of superoxide dismutase (SOD) (**a**), catalase (CAT) (**b**), and peroxidase glutathione (GPX) (**c**) genes in tomato leaves at 20 and 23 days after transplant. Cs = 1 g Cs-PVA hydrogel; nCu-Cs = 10 mg Cu NPs + Cs-PVA. Data represent the mean ± the standard error and the reference line represents the constant value of the absolute control.

Copper nanoparticles activate the antioxidant defense mechanism of plants. In a previous study, we showed that the Cs-PVA + Cu NPs increased the enzymatic SOD activity of tomato plants [8]. Similarly, Choudhary et al. [18] reported that chitosan + Cu NPs increased the activity of the SOD enzyme in maize leaves. This mataloenzyme converts highly toxic ROS ($O_2^{\bullet-}$) into less toxic ROS (H_2O_2), is localized to chloroplasts, and is the first line of defense against ROS [20]. In this study, we confirm that Cs-PVA + Cu NPs promote SOD gene expression. This activation of the antioxidant system in the plant could be used to protect against different types of environmental stress.

Cs-PVA also induced SOD gene expression (2.1- and 2.5-fold change, respectively) at 20 and 23 DAT. Consequently, chitosan-PVA showed between a 1- and 1.5-fold changes more than the control (Figure 1a). In contrast, Cs-PVA application repressed the expression of both the CAT (0.82- and 0.67-fold change, respectively) and GPX gene (0.6- and 0.7-fold change, respectively) at 20 and 23 DAT (Figure 1b,c). This could be due to a higher production of anion superoxide radicals and a lower production of hydrogen peroxide. Chitosan is widely known as an activator of plant defense pathways [21]. This study demonstrated that Cs-PVA activated the antioxidant defense mechanism of the plant through induction of SOD gene expression. Similarly, in *Camellia sinensis* plants, chitosan application was demonstrated to result in SOD and CAT gene expression induction [22].

3.3. Expression of PR1 and JA Genes in Response to Cs-PVA + Cu NPs and Cs-PVA

Both the Cs-PVA + Cu NPs and Cs-PVA applications repressed the expression of the PR1 gene at 20 DAT (0.2- and 0.6-fold change, respectively) and at 23 DAT (0.2- and 0.5-fold change, respectively, Figure 2a). The JA gene returned a different expression profile to that determined for PR1 post treatment, that is, JA expression was repressed with Cs-PVA and Cs-PVA + Cu NPs at 20 DAT (0.9- and 0.8-fold change, respectively) but was elevated at 23 DAT (1.2- and 1.1-fold change respectively, Figure 2b) when compared to the control.

Induced resistance is an improved condition of the defensive capacity developed by a stress exposed plant. Acquired systemic resistance (ASR) and induced systemic resistance (ISR) are two forms of resistance where the defense system of plants can be "preconditioned" [23]. The induction of ASR occurs mainly via the SA signaling pathway in addition to activation of protein resistance (PR) [24], whereas ISR is through the JA and/or ethylene signaling pathways [25]. Therefore, jasmonates and salicylic acid are considered the key regulators of plant growth as well as defense responses to biotic and abiotic stress through the interaction with hormones such as ethylene, auxins, and gibberellins [26–28]. Chitosan induces defense response genes activation through the SA pathway in plants under biotic stress [29] and through the JA pathway under conditions of abiotic stress [30]. Therefore, it is suggested that Cs-PVA + Cu NPs and Cs-PVA could be mediated by ISR through the octadecanoid pathway of the jasmonates. For example, in wheat, TiO_2 NPs were demonstrated to activate the JA pathway [31].

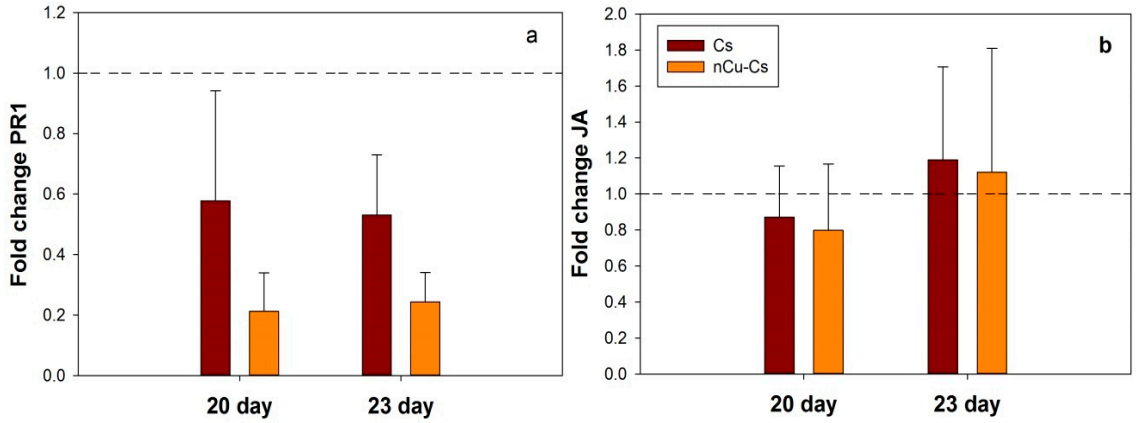

Figure 2. Expression of PR1 (**a**) and jasmonic acid (JA) (**b**) genes in tomato leaves at 20 and 23 days after transplant. Cs = 1 g Cs-PVA hydrogel; nCu-Cs = Cs-PVA + 10 mg Cu NPs. The data represent the mean ± standard error and the reference line represents the constant value of the absolute control.

3.4. Cs-PVA and Cs-PVA + Cu NPs Induce SOD Gene Expression in Plants under Salt Stress

After 48 h of saline stress application, the SOD gene was elevated (1.9-fold change) while CAT and GPX gene expression was repressed (0.8- and 0.9-fold change, respectively) (Figure 3). The combination of saline stress and Cs-PVA + Cu NPs also induced SOD gene expression (1.5-fold change) and repressed the expression of the CAT and GPX genes (0.9- and 0.5-fold change, respectively). In addition, the combination of saline stress and Cs-PVA induced a higher degree of SOD (1.9-fold change) and CAT (1.1-fold change) gene expression activation, and also repressed GPX gene expression (0.5-fold change) (Figure 3).

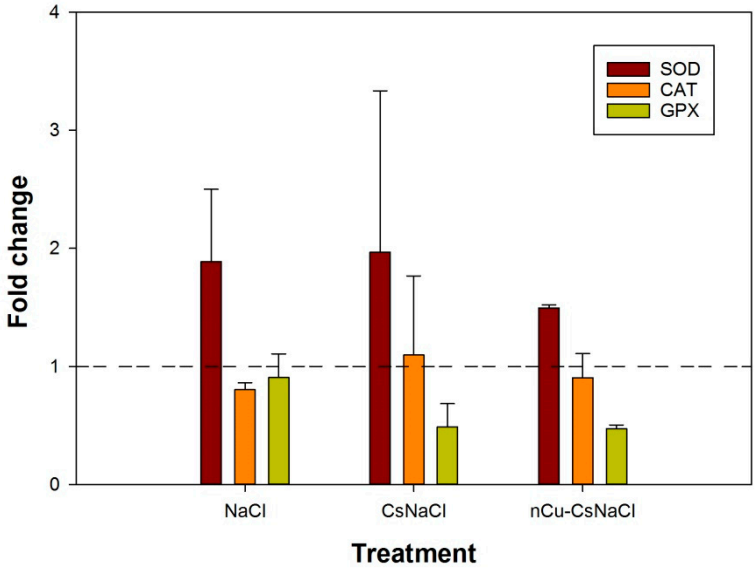

Figure 3. Expression of antioxidant genes in tomato leaves after 48 h under saline stress (23 days after transplant). NaCl: witness + 100 mM NaCl. CsNaCl: Cs-PVA + 100 mM NaCl. nCu-CsNaCl: Cs-PVA + 10 mg Cu NPs + 100 mM NaCl. The data represent the mean ± standard error and the reference line represents the constant value of the absolute control.

In this study, saline stress resulted in a 0.9-fold elevation in SOD gene expression. The antioxidant machinery of the plant was activated to scavenge the ROS caused by saline stress [32]. Chitosan could alleviate the damage of salt stress through the regulation of antioxidant enzymes [13,33]. Previously, we showed that Cs-PVA increased the activity of the SOD enzyme in tomato plants treated with salt stress [8]. Here, we show that SOD gene expression is enhanced via application of Cs-PVA. Similarly,

in wheat plants, the combination of salt stress and chitosan also induced SOD gene expression [13]. The NPs have shown positive effects to mitigate saline stress in plants. The combination of saline stress and ZnO NPs activated the expression of the SOD and GPX genes in tomato plants [34]. Cu NPs showed scavenging activity against free radicals such as 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) [35,36], nitric oxide, and superoxide anion [36]. In this study, the combination of Cs-PVA + Cu NPs and salt stress resulted in a 0.4-fold change of less activation of SOD gene expression than plants treated only with salt stress (Figure 3). This decrease in expression induction of the SOD gene could be explained by the dual effect of the Cu NPs and the SOD enzyme, since both act as scavengers of the superoxide anion. In *Arabidopsis* plants, CeO_2 NPs catalytically reduce ROS including hydroxyl radicals ($^{\bullet}OH$) that lack enzymatic scavenging pathways [37]. Latef et al. [38] showed that 0.01 and 0.02% concentrations of TiO_2 NPs decreased the hydrogen peroxide content in the leaves of bean plants under conditions of saline stress. Similarly, Farhangi-Abriz and Torabian [39] reported that concentrations of 0.5 and 1.0 mM of nano-SiO_2 decreased ROS production ($O_2^{\bullet-}$ and H_2O_2) in the roots and leaves of soybean under different levels of salt stress. Additional studies have further revealed that cerium-based metal nanoparticles act as inorganic antioxidants because they are able to capture free radicals such as $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2 in biological systems through mechanisms similar to antioxidant enzymes [40,41]. Thus, Cu NPs in chitosan–PVA hydrogels could also potentially function as free radical scavengers.

3.5. Cs-PVA and Cs-PVA + Cu NPs Induce JA Gene Expression in Plants under Salt Stress

Saline stress was determined to repress PR1 gene expression (0.3-fold change) and induce JA gene expression (5.9-fold change) when compared to the control (Figure 4). The combination of saline stress and Cs-PVA + Cu NPs, saline stress and Cs-PVA, repressed PR1 gene expression (0.3-fold change, respectively) and to induce the expression of the assessed JA gene (1.5- and 2-fold change, respectively) when compared to the control (Figure 4). Saline stress increased the expression of the JA gene 4.9-fold compared to the control.

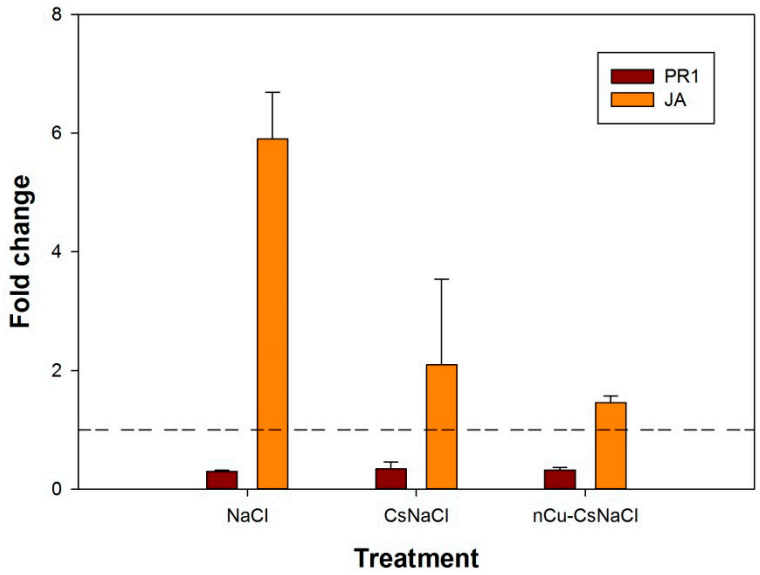

Figure 4. Expression of PR1 and JA genes in tomato leaves after 48 h under salt stress (23 days after transplant). NaCl: witness + 100 mM NaCl. CsNaCl: Cs-PVA + 100 mM NaCl. nCu-CsNaCl: Cs-PVA + 10 mg Cu NPs + 100 mM NaCl. The data represent the mean ± standard error and the reference line represents the constant value of the absolute control.

Jasmonic acid application has been demonstrated to improve a plant tolerance to both osmotic and oxidative stress in plants also experiencing salt stress, with the beneficial effect of JA achieved through a systemic physiological alteration instead of by simply controlling ionic homeostasis [3]. However, the Cs-PVA + Cu NPs and the Cs-PVA resulted in 75% and 66% less activation of expression of the

assessed JA gene than plants treated only with saline stress (Figure 4). This decrease in the expression induction of the JA gene suggests that the Cs-PVA + Cu NPs and Cs-PVA mitigate saline stress through the regulation of oxidative and ionic stress, which was reflected in an improvement in plant growth (Table 2). Chitosan activates the octadecanoid pathway of jasmonates [42] and protects plants from salt stress by regulating the concentration of cell ions [13]. Wu et al. [37] demonstrated that CeO_2 NPs enhanced salt stress tolerance in *Arabidopsis* through the long-term catalytic elimination of $^{\bullet}OH$ which in turn allows the plasma membrane channels/transporters to coordinately retain higher levels of K^+ in the leaf mesophyll. In a previous study, we showed that Cs-PVA and the Cs-PVA + Cu NPs decreased the sodium content in tomato leaves [8]. Similarly, Rossi et al. [17] reported a lower sodium content in the leaves and roots of *Brassica napus* treated with 1000 mg kg^{-1} of CeO_2 NPs and 100 mM of NaCl. Furthermore, Farhangi-Abriz and Torabian [39] also reported that concentrations of 0.5, 1.0, and 2.0 mM of nano-SiO_2 decreased the sodium content in roots and soybean leaves under different levels of salt stress. Therefore, chitosan and Cs-PVA + Cu NPs could be alternatives to mitigate saline stress.

4. Conclusions

In this study, we demonstrated that the application of Cs-PVA + Cu NPs increased the stem diameter of tomato plants cultivated under non-stressed conditions. Interestingly, and under salt stress conditions, the application of Cs-PVA and Cs-PVA + Cu NPs increased plant height and stem diameter. The application of Cs-PVA and Cs-PVA + Cu NPs was also demonstrated to induce the expression of the SOD and JA genes either in the presence or absence of salt stress. This finding strongly suggests that the application of Cs-PVA and the Cu NPs activates the antioxidant defense mechanisms of a plant and are mediated by the octadecanoid pathway of the jasmonates. Therefore, Cs-PVA and Cu NPs could potentially be used to induce the tolerance of plants to salt stress, and potentially to other forms of abiotic stress.

Author Contributions: A.J.-M. and S.G.-M. conceived and designed the experiments; H.H.-H. performed the analysis of laboratory and field experiments; A.B.-M. and D.S.-A. performed the data analysis; H.O.-O. and G.C.-P. contributed reagents and materials. All authors were responsible for manuscript writing. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Acknowledgments: Universidad Autónoma Agraria Antonio Narro (UAAAN) Proyecto interno 38111-425104001-2230: "Nanopartículas de cobre en hidrogeles de Quitosán-PVA para la inducción de capacidad antioxidante, tolerancia a estrés abiótico y expresión génica en solanáceas".

Conflicts of Interest: The authors declare no conflict of interest.

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