REDUCTION OF ZEBRA CHIP INFECTION SYMPTOMS IN POTATO †

[REDUCCIÓN DE LOS SINTOMAS DE LA INFECCIÓN DE ZEBRA CHIP EN PAPA]

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SUMMARY

Background: There have been few studies on the long-term effects of hydrogen peroxide and dehydroascorbate (H_2O_2 and DHA) on biotic stress, especially in potato plants infected with *Candidatus* Liberibacter solanacearum (Lso) that cause the Zebra chip disease, characterized by browning of the tubers, reduced productivity and anomalies in the morphology of the plant. **Objective**: To evaluate the long-term effects of DHA and H_2O_2 treatments on the reduction of symptoms in potato plants infected with Lso. This research was realized at the INIFAP facilities at Experimental Site Metepec, Mexico State. **Methodology**: Single nodes were waterlogged in DHA, H_2O_2, or distilled water, followed by culture in Murashige and Skoog medium (MS), and microplants obtained were transplanted in greenhouse conditions. Seventy-three days after transplanting, leaf water potential (Ψw), chlorophyll, H_2O_2 content, and catalase activity (CAT) were determined. **Results**: DHA significantly increased minituber weight and chlorophyll content in the long term. It also, reduced H_2O_2 content and increased CAT in infected plants, resulting in increased minituber weight. A long-term effect of H_2O_2 was observed, as it induced significant increases in stem height, shoot fresh and dry weight, relative chlorophyll content, and minituber weight. **Implications:** DHA and H_2O_2 participated as signal molecules, triggering physiological changes promoting increases in productivity in plants infected with Lso, justifying the importance of the study of these molecules under biotic stress conditions. **Conclusion**: Long-term effects of H_2O_2 and DHA on the amelioration of symptoms of potato Zebra chip associated with Lso solanacearum were demonstrated.
Key words: catalase; oxidative stress; hydrogen peroxide; dehydroascobate.

RESUMEN

Antecedentes: Existen pocos estudios sobre los efectos a largo plazo del H_2O_2 y el DHA sobre el estrés biótico, en plantas de papa infectadas con la bacteria *Candidatus* Liberibacter solanacearum (Lso) que promueve la enfermedad de "Zebra chip" caracterizada por un pardeamiento en los tubérculos, reducción de productividad y anomalías en la morfología de la planta. **Objetivo**: El objetivo de este estudio fue evaluar los efectos a largo plazo de los tratamientos con DHA y H_2O_2 en la reducción de síntomas en plantas de papa infectadas con Lso. **Metodología**: Esta investigación se realizó en las instalaciones del INIFAP, Sitio Experimental Metepec, Edo. de México, utilizando la siguiente metodología. Esquejes nodales de vitroplantas de papa se sumergieron en DHA, H_2O_2 o agua destilada, seguido del cultivo en medio MS, las microplantas obtenidas posteriormente se trasplantaron en condiciones de invernadero. A los 73 días después del trasplante, se determinó el potencial hídrico foliar (Ψw), contenido de clorofila, contenido de H_2O_2 y actividad de la enzima catalasa (CAT). **Resultados**: El DHA aumentó significativamente el peso de los minitubérculos y el contenido de clorofila a largo plazo. También redujo el contenido de H_2O_2 y aumentó la CAT en

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las plantas infectadas, resultando en un aumento del peso de los minitubérculos. Se observó un efecto a largo plazo del H_2O_2, induciendo aumentos significativos en la altura del tallo, peso fresco y seco de los tallos, contenido de clorofila y peso fresco de los minitubérculos. **Implicaciones:** El DHA y el H_2O_2 participaron como moléculas señal, desencadenando cambios fisiológicos promoviendo incrementos en la productividad en plantas infectadas con Lso, justificando la importancia del estudio de estas moléculas en condiciones de estrés biótico. **Conclusión**: Se demostraron los efectos a largo plazo del H_2O_2 y el DHA en la reducción de los síntomas de "Zebra chip" en la papa asociados con Lso.

Palabras clave: catalasa; estrés-oxidativo; peróxido de hidrógeno; dehidroascorbato.

INTRODUCTION

Zebra chip disease is related to *Candidatus* Liberibacter solanacearum (Lso) as an agent of this disease; it is known that these bacteria are phloem-limited and are not cultivable in artificial media. The vector is an insect (psyllid) that spreads the bacteria. The tomato and potato psyllid *Bactericera cockerelli* has been proposed as the principal vector of Lso, which induces Zebra chip disease in potato (Munyaneza *et al.,* 2007; Secor *et al.,* 2009; Munyaneza *et al.,* 2011). This polyphagous bacterium is an economically important pathogen for various host plants in the Americas, New Zealand, and Europe (Mirmajlessi *et al.,* 2019)

Potato plants infected with Lso have different physiological symptoms. Some studies have shown that Zebra chip disease causes alterations in cell membrane integrity, which implies a loss in membrane permeability and, therefore, loss of electrolytes in contrast with healthy tubers, leading to cell death (Miles *et al.,* 2010; Hao *et al.,* 2014).

Potato plants infected with Lso present several morphological and physiological alterations such as dwarfing, shortening of axillary branches, fruit set disruption, production of small, deformed, and deficient quality fruits, aerial tuber formation, chlorosis, leaf scorch, and vine collapse (Secor *et al.,* 2009; Munyaneza *et al.,* 2011). The vine collapse might be associated with a proliferation in the bacterial concentration in vessels (xylem) and perhaps is associated with wilting, since bacteria promote vessel blockage, resulting in embolism (McElrone *et al.,* 2003) or changes in shoot water relations. Phytoplasmas were reported to induce in *Solanum tuberosum*, a reduction in shot water potential, chlorophyll content, and yield (Romero-Romero and López-Delgado, 2009; Martínez-Gutiérrez *et al.,* 2012). 'Lso is responsible for severe damage to the crops from the Solanaceae family in North America and New Zealand and is a causative agent of "zebra chip" in potato (Harrison *et al.,* 2019)

Plants are continuously subjected to abiotic and biotic stresses; these stresses trigger similar responses, such as the production of reactive oxygen species (ROS), including superoxide (O^{2-}), singlet oxygen (O^2), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2). These ROS can induce deleterious effects in biological macromolecules (Smirnoff, 1993). In plant-pathogen relationships, ROS induce defense genes, antioxidant enzymes such as catalase (CAT) and guaiacol peroxidase (POX), and the accumulation of secondary metabolites (Pellinen *et al.,* 2002). H_2O_2 functions as a signaling second messenger, enabling tolerance to both biotic and abiotic stresses (Bhattacharjee, 2005; Quan *et al.,* 2008). H_2O_2 adjusts antioxidant enzymes as a response to all stresses such as heat (Gao *et al.,* 2010) and drought (Martínez-Gutiérrez *et al.,* 2012), among others, suggesting that H_2O_2 increases the antioxidant system since it is accepted as the first mechanism involved in the protection against environmental stress (Bian and Jian, 2009). It has also been found that H_2O_2 mitigates potato purple top symptoms, increasing minituber starch content, chlorophyll, CAT activity, and minituber production (Romero-Romero and López-Delgado, 2009; Martínez-Gutiérrez *et al.,* 2012) in potato plants infected with *Candidatus* phytoplasma.

Another signal molecule in plants is ascorbic acid (AsA), it is involved in the detoxification of ROS, enhancing biotic and abiotic stress tolerance as well as plant development (Pastori *et al.,* 2003; Romero-Romero and López-Delgado, 2009). AsA is oxidized during many of its functions, generating a free radical monodehydroascorbate (MDA), which might be reduced by the monodehydroascorbate reductase to generate dehydroascorbate (DHA), DHA can be reduced back to AsA by DHA reductase, using glutathione as the reducing substrate (Gillespie and Ainsworth, 2007). During this process, AsA content can modulate the expression of proteins in response to pathogenesis (PRPs) or systemic acquired resistance, acting as a signal transduction molecule (Pastori *et al.,* 2003). AsA is also a regulator of cell division, cell elongation, and growth (Kerk and Feldman, 1995).

Several studies have reported the long-term effect of H_2O_2 on tolerance to different stresses, such as lead stress in *Trapa bicornis* seedlings (Li and Yuan, 2016), freezing in potato microplants (Mora-Herrera *et al.,* 2005), salt stress in maize seedlings (Neto *et al.,* 2005) and potato virus X elimination (Aguilar-Camacho *et al.,* 2016). AsA also induces long-term tolerance to different stresses, such as water stress in *Brassica oleracea* (Latif *et al.,* 2016) and salinity in *Solanum tuberosum* (Sajid and Aftab, 2009).

There are few studies on the long-term effects of H_2O_2 and DHA on biotic stress, especially in potato plants infected with Lso; therefore, this study aimed to evaluate the long-term effects of DHA and H_2O_2 treatments on the reduction of symptoms in potato plants infected with Lso.

MATERIALS AND METHODS

Plant material

In vitro virus-free *Solanum tuberosum* L. clone 981819 plants infected in field conditions with Lso were obtained from the *in vitro* Germplasm Bank of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias at Metepec, México. These plants had been previously selected from field conditions after showing Zebra chip symptoms. Single node cuttings from microplants tested for Lso positive and negative were individually propagated *in vitro* in Murashige and Skoog medium (MS) (Murashige and Skoog, 1962), at 20±1 °C under a 16 h photoperiod (fluorescent lights: 35 µmol m^{-2} s^{-1}, 400–700 nm) and sterile conditions (Espinoza *et al.,* 1986).

DNA extraction by CETAB

For the determination of Ca. Liberibacter, DNA extraction was performed using the hexadecyltrimethylammonium bromide (CTAB) technique. Approximately 250 mg of tissue (leaves and stems from in vitro plants) were ground with 1 mL of extraction buffer. Samples were incubated at 65 °C for 1.30 h. 0.75 mL Chloroform-isoamyl alcohol (24:1) was added to the samples and they were shaken by immersion. Samples were centrifuged for 5 minutes at 10,000 g. 0.650 mL was taken from the supernatant and the same amount of isoamyl alcohol chloroform (24:1) was added, in this case 0.650 mL, and shaken by immersion. It was centrifuged for 5 min at 10,000 g. To 0.5 mL of supernatant, 166.6 µL of 7.5 M ammonium acetate was added. The samples were incubated for 10 minutes on ice, then centrifuged for 10 minutes at 14,000 g. The supernatant was transferred by inversion and 0.6 volumes of cold isopropanol were added. For each 0.4 ml of supernatant, 0.240 mL of isopropanol was added. It was shaken by inversion and left overnight at 4 °C. It was centrifuged at 10,000 g for 20 minutes. The supernatant was removed and the pellet was washed with 0.7 mL of 70% ethanol and gently shaken by inversion. It was centrifuged for 10 minutes at 10,000 g and the supernatant was discarded by emptying. The pellet was washed again by adding 0.700 mL of 70% ethanol and gently shaken by inversion, then centrifuged at 10,000 g. The supernatant was removed and the pellet was dried by inverting on blotting paper (approximately 30 min). The pellet was resuspended in 50 µL of MilliQ water.

Lso detection in microplates

The DNA obtained was amplified in the Polymerase Chain Reaction (PCR) in the sequential PCR modality to detect the presence of Lso, and the primers Lp Frag 1-(25 F)/Lp Frag 1-(427R) were used. and Lp Frag 2-(266 F)/LP Frag 2-(1138R) according to Hansen et al. (2008).

PCR reactions were performed in 0.5 mL tubes. The total reaction volume was 25 µL [50 ng of DNA, 1X buffer, 12.5 picomoles of each primer, 200 µM of each dNTP (A, T, C, G), 2.0 mM of MgCl2 and 1.5 Units of the enzyme Taq DNA polymerase (Gibco brand)]. The PCRs were carried out in an MJ Research thermocycler with the program: 1 cycle of denaturation at 94 ºC for 2 min and 35 additional cycles with 1 min of denaturation at 94 ºC; 30 s mating at 50 °C; 1.5 min of polymerization at 72 ºC; and a final extension of 72 ºC for 10 min. PCR products were fractionated on 1.5% agarose gels in 0.05% TAE buffer, stained with ethidium bromide (0.5 µg/ml), and visualized under ultraviolet light.

DHA and H_2O_2 treatments

Single nodes were placed in a jar containing MS propagation medium to avoid dehydration and then waterlogged for 1 h under sterile conditions in pH 5.6 solutions of 6 µM DHA or 5 mM H_2O_2 or distilled H_2O (control) as described by Mora-Herrera *et al.* (2005). Explants were subsequently rinsed with sterile distilled H_2O (pH 5.6) and cultured in tubes in MS propagation medium as described above for 30 days. According to our objective, only positive plants to Lso were treated with DHA or H_2O_2.

Treated and non-treated (control) vitroplants were transplanted to pots (17×14 cm length and width) containing peat moss under greenhouse conditions, 30 days after single-node subculture, fertilized (170 N, 230 P, 170 K, 90 S, 20 Ca, 20 Mg) every fifteen days and watered to soil holding waters capacity twice a week. The plants were maintained under these conditions for 120 days after transplanting (DAT).

H_2O_2 content, CAT activity, relative chlorophyll content, and water potential were evaluated at 73 DAT by sampling the 6th basipetal growing, fully expanded terminal leaflets. Tissue samples were washed with distilled water before extraction to remove surface residue. Stem length and fresh and dry weight were also measured at this date. Minituber number and weight were determined at 120 DAT as a yield estimation.

Relative chlorophyll content and leaf water potential (Ψ_w)

Relative chlorophyll content was measured in the same leaf with a SPAD-meter (502 Plus, Konica Minolta). Leaf water potential (Ψ_w) was measured with a Scholander pressure chamber (Soil Moisture Equipment Corp). The youngest full expanded leaf was placed into the chamber, and part of the petiole was exposed. The pressure in the chamber was increased slowly with nitrogen gas to obtain a pressure balance with the water column of the xylem, which was achieved when a small drop of sap appeared in the exposed petiole area (Turner, 1988).

H_2O_2 content and CAT activity

H_2O_2 content was determined by luminol-dependent chemiluminescence, as described by Mora-Herrera *et al.* (2005). Tissue samples (0.2 g) from the youngest fully expanded leaf were extracted in 1.2 mL of ice-cold 5 % (w/v) trichloroacetic acid (TCA) and centrifuged (10 min, 10,000 *g*). Samples (0.5 mL) of the supernatant fractions were passed through Dowex-1 resin (0.5 g, Fluka), followed by 3.5 mL of 5 % TCA. H_2O_2 was measured in the eluates using the luminol-dependent chemiluminescence method: 0.5 mL of the eluate was added to 0.5 mL of 0.5 mM luminol (Sigma), the volume was brought up to 4.5 mL with 0.2 M NH_4OH (pH 9), and 0.452 mL of the mixture was transferred to a polystyrene tube (12 x 75 mm, Fisher) and analyzed using an Optocomp P luminometer (MGM Instruments). Chemiluminescence was initiated by injecting 50 µL of 0.5 mM potassium ferricyanide in 0.2 M NH_4OH, and emitted photons were counted over 5 s. A parallel sample of each initial extract was processed after the addition of a known concentration of H_2O_2 to provide a recovery correlation factor in three experiments with three samples per treatment, and each assay was replicated six times.

CAT (EC 1.11.1.6) activity was determined, according to Aebi (1984). The total reaction mixture (3 mL) contained 50 mM potassium and sodium phosphate buffer (pH 7.0) and 20 µL of enzyme extract (Enzyme extraction was carried out with 50 mM potassium phosphate buffer at pH 7.2, containing 5 mM DTT, 1 mM EDTA, and 1% (w/v) PVP). The extract was centrifuged at 11,000 *g* for 15 min at 4 °C. The supernatant was used to measure CAT activity. Protein content was determined directly using a Nanodrop 1000 spectrophotometer (Thermo Scientific). The reaction was initiated by adding 30 mM H_2O_2. Decomposition was followed immediately by a decrease in absorbance at 240 nm every 20 s for 3 min at 26 °C.

Experimental design

Lso-positive and Lso-negative plants for each treatment were arranged in a completely randomized design; each plant was considered an experimental unit with 15 plants per treatment. The experiments were repeated three times. The data were analyzed using a one-way analysis of variance and Duncan´s multiple range test ($p < 0.05$) (Statgraphics Plus 5.1).

RESULTS

Stem height, fresh and dry weight, minituber number, and fresh weight

Lso-infected plants were characterized by a significant reduction ($p < 0.05$; p-value 0.0172) in stem height (Figure 1A), reduced ($p < 0.05$; p-value 0.0375) minituber number (Figure 2A), diminished ($p < 0.05$; p-value 0.0018) minituber fresh weight (Figure 2B) compared with healthy plants. The shoot dry weight of infected plants and non-infected plants were not different (Figure 1C).

DHA-treated plants were not statistically different compared with infected control plants for stem height (Figure 1A), shoot fresh weight ($p < 0.05$; Figure 1B) shoot dry weight ($p < 0.05$; Figure 1C), and minituber number/plant ($p < 0.05$; Figure 2A). DHA treatment induced significant differences compared ($p < 0.05$) with infected control plants in a higher minituber fresh weight (Figure 2B). H_2O_2 treatment promoted a significant ($p < 0.05$; p-value 0.0172) increase in stem height compared with infected control plants (Figure 1A).

H_2O_2 treatment significantly increased ($p < 0.05$; p-value 0.0172) stem height by 15 % and shoot fresh weight by 34.16 % compared with infected control plants (Figures 1A, 1B). Similar results were obtained for shoot dry weight, which was significantly increased by 46.55 % compared with infected control plants (Figure 1C). Stem height (Figure 1A) and minitubers fresh weight (Figure 2B) increased by the effect of H_2O_2 in contrast with infected control plants.

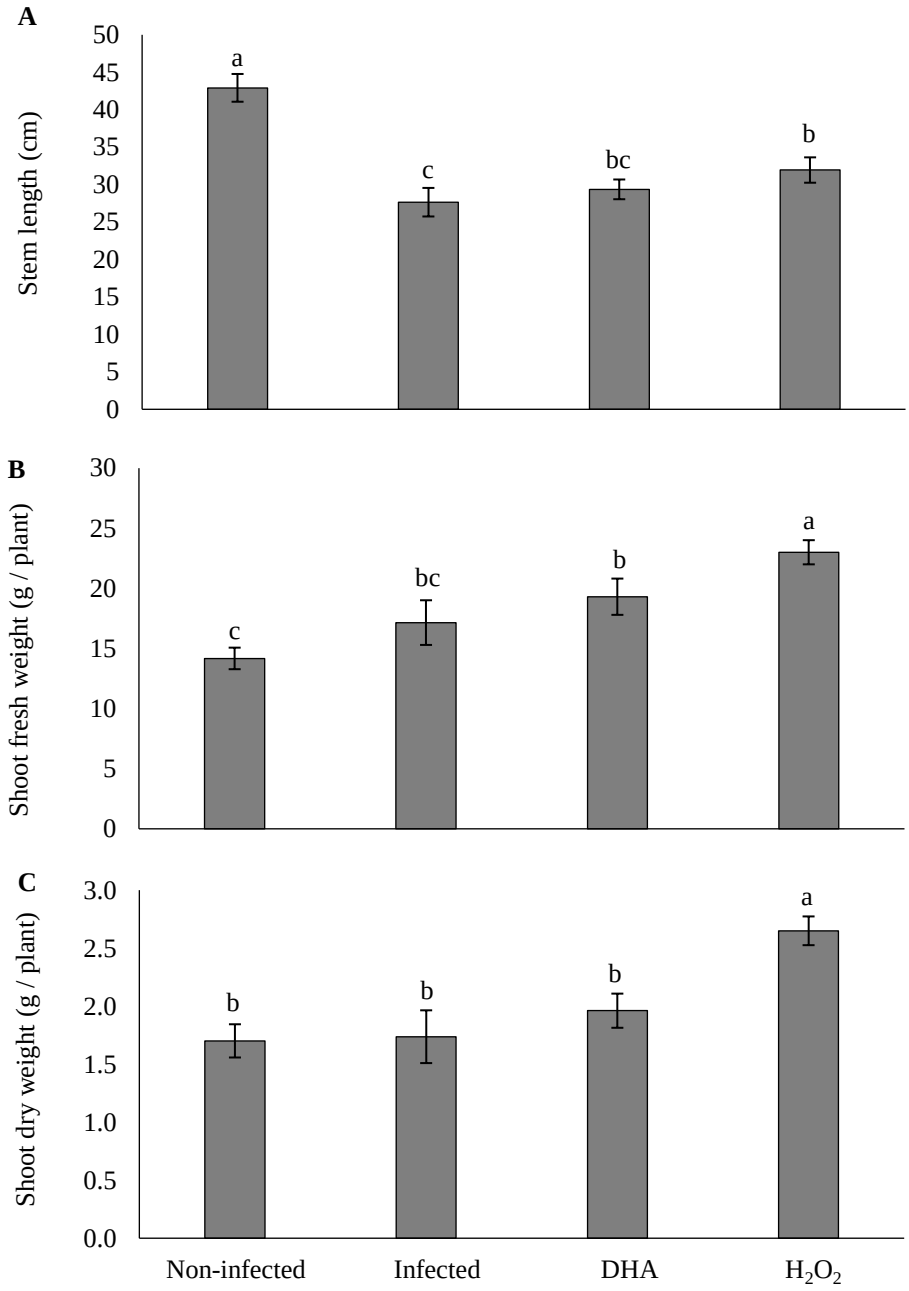

Figure 1. Long-term effects of H_2O_2 and DHA treatments on plant length (A), fresh weight (B), and dry weight (C) in potato plants infected by Lso at 73 days after transplanting. Data are means ± SE of three experiments. Means labeled with different letters differ significantly by ANOVA and Duncan's test ($p < 0.05$).

The DHA and H_2O_2 treatments did not increase the minituber number in infected plants compared with infected control plants (Figure 2A). The weight of minitubers from infected plants was significantly ($p < 0.05$; p-value 0.0018) reduced compared with those of healthy plants (Figure 2B). However, the minituber weight in infected plants significantly ($p < 0.05$; p-value 0.0018) increased by 23.23 and 18 % after treatment with DHA and H_2O_2, respectively, compared with infected control plants (Figure 2B).

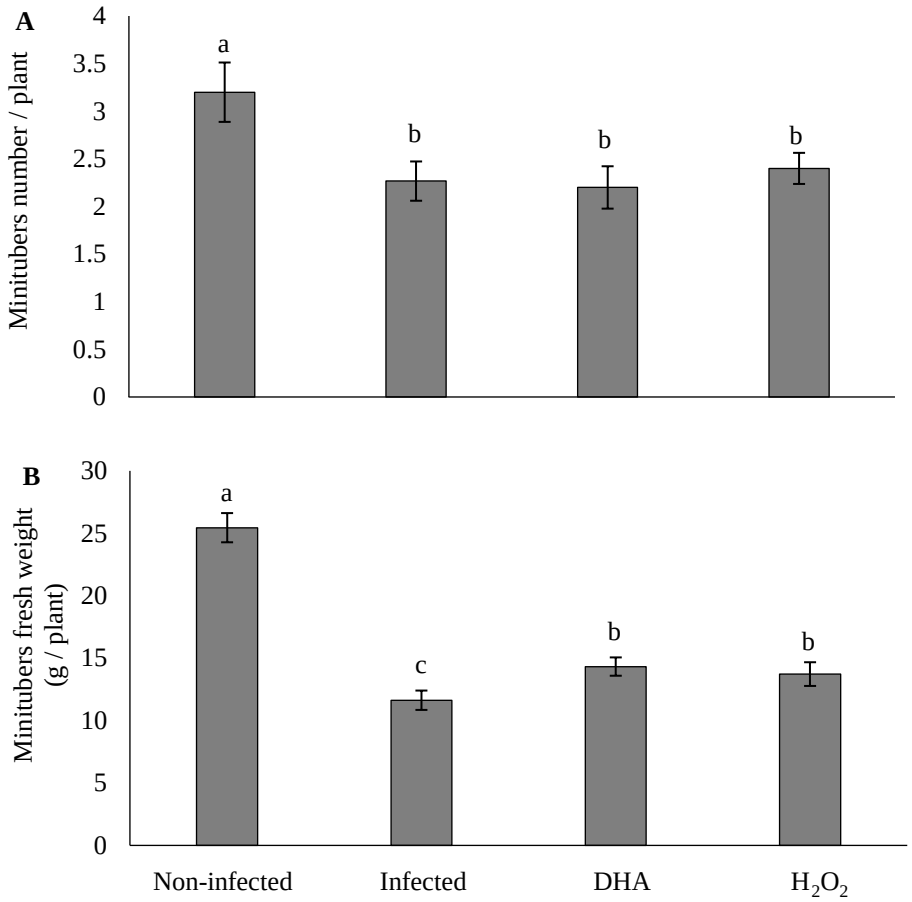

Figure 2. Long-term effects of H_2O_2 and DHA treatments on minitubers number (A), minitubers fresh weight (B) in potato plants infected by Lso at 73 days after transplanting. Data are means ± SE of three experiments. Means labeled with different letters differ significantly by ANOVA and Duncan's test *(*$p < 0.05$*).*

Leaf water potential (Ψ_w) and relative chlorophyll content

Plants infected with Lso or infected and pretreated with DHA and H_2O_2 showed higher Ψ_w than healthy plants, on average, it was 0.38 MPa higher (Table 1).

Infected control plants had significantly ($p < 0.05$; p-value 0.0420) lower concentrations of chlorophyll (35 %) than healthy control plants (Table 1). Treatments with DHA and H_2O_2 significantly ($p < 0.05$; p-value 0.0245) increased chlorophyll by 14 and 13 % respectively in infected plants, compared with infected control plants.

H_2O_2 content and CAT activity

Lso induces a significant ($p < 0.05$; p-value 0.0082) accumulation of H_2O_2 content in potato plants (Figure 3A) compared with non-infected control plants. Plants pretreated with H_2O_2 and DHA maintained higher levels of H_2O_2 compared with non-infected control plants. DHA-treated plants significantly reduced the H_2O_2 content in comparison to the infected control plants (Figure 3A). Interestingly, there were no significant ($p > 0.05$) differences in CAT activity between infected and non-infected plants (Figure 3B). Treatment with DHA and H_2O_2 significantly increased the enzyme activity by 43 and 63 %, respectively, compared with infected and non-infected control plants.

Similarly, to the H_2O_2 treatment, a long-term antioxidant effect of DHA was also observed on a significant ($p < 0.05$; p-value 0.0022) enhancement of antioxidant CAT activity (Figure 3B) and reduced H_2O_2 content (Figure 3A). These responses were also associated with a higher minituber fresh weight (Figure 2B), higher chlorophyll content, high water potential, and stomatal conductance (Table 1), compared with infected control plants.

Table 1. Long-term effects of H_2O_2 and DHA treatments on Ψ_w and relative chlorophyll content in potato plants infected by Lso at 73 days after transplanting.

Treatments	Ψ_w (MPa)	Relative content of chlorophyll (SPAD)
Non-infected plants	-0.72 ± 0.08**a**	40.08 ± 1.70**a**
Infected plants	-0.33 ± 0.04**b**	25.97 ± 0.58**c**
Infected plants + DHA	-0.43 ± 0.07**b**	29.60 ± 1.84**b**
Infected plants + H_2O_2	-0.39 ± 0.03**b**	29.28 ± 0.43**b**

Data are means ± SE of three experiments. Means labeled with different letters differ significantly by ANOVA and Duncan's test ($p < 0.05$).

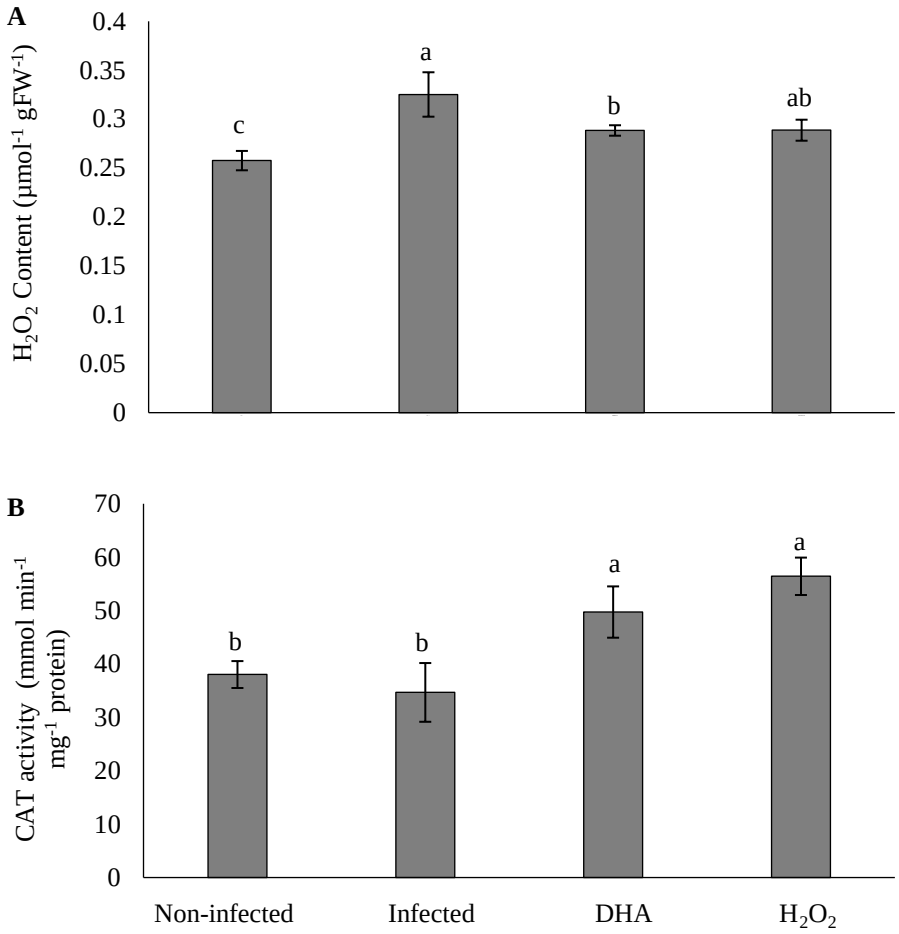

Figure 3. Long-term effects of H_2O_2 and DHA treatments on hydrogen peroxide content (A), catalase activity (CAT) activity (B) in potato plants infected by Lso at 73 days after transplanting. FW= fresh weight**.** Data are means ± SE of three experiments. Means labeled with different letters differ significantly by ANOVA and Duncan's test *(*$p < 0.05$*).*

DISCUSSION

Lso causes Zebra chip, a severe disease in México, and many other countries, and it generates productivity and economic losses in potatoes (Munyaneza *et al.,* 2007; Secor *et al.,* 2009). It is essential to seek new integrated crop management strategies to reduce the environmental impact of pesticide application and improve productivity. In this study, we observed the long-term effects of the signal molecules H_2O_2 and DHA on symptom reduction of Zebra chip associated with Lso, which were mediated by changes in CAT activity and H_2O_2 content.

A significant reduction in shoot length and augmented thickening are characteristic symptoms of Zebra chip (Munyaneza *et al.*, 2007; Secor *et al.*, 2009). This study confirmed this symptom since a significant reduction in shoot length was observed in infected control plants (Figure 1A).

Lso-infected plants exhibited a significant reduction in minituber number and weight (Figure 2A, B). These responses agree with previous reports of Munyaneza *et al.* (2007) and Rashed *et al.* (2014) in minituber number and fresh weight reduction, respectively, in Lso-infected plants. Interestingly, it has been established that Lso, has a pathogenicity gene that encodes a NahG-salicylate hydroxylase enzyme, which inhibits salicylic acid (SA) accumulation in plants. SA is reduced to catechol, preventing systemic acquired resistance and increasing susceptibility to viruses, bacteria, and fungi in potato plants, as suggested by Lin and Gudmestad (2013). Most likely, a similar effect on SA accumulation is induced in phytoplasma-infected potato plants, since attenuation of symptoms has been observed in potato plants treated with exogenous application of AsA, SA, and H_2O_2 (Romero-Romero and López-Delgado 2009; Sánchez-Rojo *et al.,* 2011; Martínez-Gutiérrez *et al.,* 2012).

H_2O_2 treatment significantly increased shoot length (Figure 1A), shoot fresh weight (Figure 1B), shoot dry weight (Figure 1C), and minituber fresh weight (Figure 2B) in the long term compared with infected control plants. These responses showed a compensation effect stimulated on these parameters, due to the stress induced by Lso. A physiological response like a higher minituber fresh weight induced by H_2O_2 relating to the infected control is potentially important, especially for tuber production, fresh consumption, and chips purposes rather than seed tuber use. Long-term physiological effects mediated by H_2O_2 treatment in potatoes have been reported, including freezing tolerance (Mora-Herrera *et al.,* 2005), micro-tuberization induction (López-Delgado *et al.,* 2012), and thermotolerance (López-Delgado *et al.,* 1998; Aguilar-Camacho *et al.,* 2016). H_2O_2 treatment increases shoot fresh and dry weight in the long-term; these results could be associated with an increase in the activity of carbonic anhydrase, which supplies carbon to ribulose bisphosphate carboxylase (Hasan *et al.,* 2016), increasing carbon fixation. In this study, we observed that H_2O_2 treatment significantly ($p < 0.05$) enhanced chlorophyll content in infected plants, promoting an increase in minituber fresh weight (Fig. 2B), similar to previous studies in phytoplasma-infected plants by Romero-Romero and López-Delgado (2009), and Martínez-Gutiérrez et al (2012).

Previous studies demonstrated that in the short-term, H_2O_2, and DHA reduced potato purple top symptoms associated with phytoplasma (Romero-Romero and López-Delgado, 2009; Martínez-Gutiérrez *et al.,* 2012). Physiological effects have also been induced in the long-term by H_2O_2 treatment in other species; for example, it enhanced salt tolerance in wheat seedlings, reducing oxidative damage (Wuahid *et al.,* 2007). Like this study, Ahmad *et al.* (2012) demonstrated that shoot fresh and dry weight in maize increased because of the H_2O_2 application.

The results obtained in this work demonstrated that DHA and H_2O_2 significantly enhanced the minituber fresh weight, as long-term effects of these molecules on stress reduction induced by the pathogen. H_2O_2 treatment promoted an increase in minituber weight associated with an increase in chlorophyll content compared to the infected control plants (Table 1).

By contrast, the reduction in minituber weight and number in infected plants may be associated with a significant reduction (35.2 %) in chlorophyll content in diseased plants compared to the non-infected control (Table 1). Furthermore, this reduction in minituber weight could be associated with an obstruction of the sieve elements (Christensen *et al.,* 2004; 2005), taking into account that pathogens interfere with carbohydrate metabolism (Junqueira *et al.,* 2004; Hren *et al.,* 2009) and therefore, with translocation to the tubers.

Lso and *Ca.* phytoplasma could affect differentially CAT activity since Martínez-Gutiérrez *et al.* (2012) reported that *Ca.* phytoplasma inhibited CAT and increased H_2O_2 content in infected potato plants, and we found that Lso did not induce changes in CAT activity and increased H_2O_2. However, both DHA and H_2O_2 induced higher CAT activity in comparison to the non-infected and infected control plants (Fig 3 B), this fact represents an enhancement of antioxidant capacity against reactive oxygen species, which are produced during pathogenesis stress, this effect on CAT activity could be also associated with the higher minituber fresh weight observed comparing with the infected control plants (Fig 2B).

The enhancement of antioxidant CAT activity as an effect of DHA observed in this work (Fig. 3 B), agrees with reports where AsA, the reduced form of DHA, promoted the alleviation to biotic (Wang *et al.,* 2011) and abiotic stress (Latif *et al.,* 2016), these responses were associated with a reduction of H_2O_2 content as observed in this work (Fig 3A) and enhancement of antioxidant enzyme activities.

Unlike the effects induced by DHA, H_2O_2 induced not only a higher minituber fresh weight and CAT activity but also greater values of shoot fresh weight (Figure 1B), shoot dry weight (Figure 1C) and chlorophyll content (Table 1), confirming the signaling role of H_2O_2 in the protection against environmental stress (Bhattacharjee, 2005; Bian and Jian, 2009, Quan *et al.,* 2008) respecting the infected control plants.

A similar effect of H_2O_2 on minituber fresh weight was reported in phytoplasma-infected potato plants by Romero-Romero and López-Delgado (2009) and Martínez-Gutiérrez *et al.* (2012). The increase in tuber

fresh weight in infected plants induced by H_2O_2 could be associated with starch accumulation, this has been previously demonstrated (López-Delgado *et al.*, 2005; Romero-Romero and López-Delgado, 2009; Martínez-Gutiérrez *et al.*, 2012) via exogenous application.

Enhanced starch accumulation mediated by H_2O_2 has been reported in other species such as melon (Ozaki *et al.*, 2009), where an increase in photosynthates was observed due to exposure of plants to H_2O_2, triggering an increase in shoot dry weight and fruit number.

It is worth emphasizing the long-term effect on tuber weight observed in this work, since single nodes from microplants were waterlogged for only 1 h in H_2O_2, rather than an exogenous application under greenhouse conditions.

Further research is needed to elucidate the potential long-term effects of H_2O_2 and DHA on the control of Zebra chip symptoms. A better understanding of the physiology of these molecules will help to reduce the use of pesticides in integrated crop management strategies.

CONCLUSIONS

In this study, H_2O_2 and DHA treatments had long-term effects on the ameliorative symptoms of Zebra chip associated with Lso. Further research is necessary to elucidate the potential long- and short-term effects of H_2O_2 and DHA on the control of Zebra chip symptoms to reduce the use of pesticides in integrated crop management strategies.

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