Am. J. Pot Res (2009) 86:218–226
DOI 10.1007/s12230-009-9075-1

Ameliorative Effects of Hydrogen Peroxide, Ascorbate and Dehydroascorbate in *Solanum Tuberosum* Infected by Phytoplasma

M. T. Romero-Romero · H. A. López-Delgado

Published online: 25 March 2009

Abstract Phytoplasmal infections cause loss in yield, quality and germination of tubers. Hydrogen peroxide and antioxidants such as ascorbic acid and dehydroascorbate are implicated in signaling against stress. The effects of these chemicals on minituber yield, sprouting and starch content were evaluated in plants testing positive for phytoplasma. Without chemical treatment, positive plants showed significant reductions in leaf pigments, tuber weights and starch contents, compared to uninfected controls, and had more minitubers though fewer sprouted. Hydrogen peroxide and antioxidant treatments of positive plants significantly reduced the number of minitubers, while enhancing their weights and starch contents, and increased the percentage of sprouting minitubers, while leaf pigment content also increased. This research demonstrates potential benefits of hydrogen peroxide and antioxidants in enhancing the yield and quality of tubers not destined for seed in phytoplasma positive plants.

Resumen Las infecciones por fitoplasma causan perdida en el rendimiento, calidad y germinación de los tubérculos. El peróxido de hidrógeno y antioxidantes tales como el ácido ascórbico y dehidroascorbato están implicados en el señalamiento contra estrés. Los efectos de estos químicos en el rendimiento del minitubérculo, brotación y contenido de almidón fueron evaluados en plantas positivas a fitoplasma. Las plantas positivas sin tratamiento químico mostraron una significativa reducción en los pigmentos de las hojas, peso en los minitubérculos y contenido de almidón, comparados con testigos no infectados; además, se tuvieron más minitubérculos con menos brotación.Los tratamientos de peróxido de hidrógeno y antioxidantes en plantas positivas redujeron significativamente el numero de minitubérculos, aumentando su peso, contenido de almidón y porcentaje de brotación en éstos. El contenido de pigmentos en las hojas fue mayor. Esta investigación demuestra el potencial benéfico del peróxido de hidrógeno y antioxidantes en aumentar el rendimiento y calidad de tubérculos no destinados para semilla en plantas positivas con fitoplasma.

Keywords Carotenoids · Chlorophyll · Minitubers sprouted · Starch content

Abbreviations

AA	ascorbic acid
DHA	dehydroascorbate
MS	Murashige-Skoog
ROS	reactive oxygen species

M. T. Romero-Romero · H. A. López-Delgado (✉)
Programa Nacional de Papa, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP),
Metepec 52140, México
e-mail: lopez.humberto@inifap.gob.mx

M. T. Romero-Romero
Facultad de Ciencias, UNAM, Av. Universidad 3000,
Cto. Ext. S/N C.P.,
04510 Ciudad Universitaria, D. F., México

Introduction

Phytoplasmas are minute (200 to 800 μm) pleiomorphic or filamentous bacteria, bound by triple-layered membranes without cell walls, which are parasites of phloem sieve elements (Doi et al. 1967; Lee et al. 2000). They are transmitted by specific phloem-sucking leafhoppers or psyllid species, and have not been cultured in *vitro* (Lee

et al. 2000). Phytoplasma genomes are very small (Neimark and Kirkpatrick 1993; Marcone et al. 1999), and lack genes for the pentose phosphate cycle and ATP-synthase subunits, making them dependent on their hosts (Oshima et al. 2004).

Phytoplasmal infections are the primary limiting factors for production of many important crops all over the world (Lee and Davis 1992). In Mexico, potato purple top disease is associated with phytoplasmas and is present in the main potato-producing areas in the country. The disease causes loss in yield and quality of the tubers from 30 to 95% (Cadena 1993, 2000). Symptoms in infected shoots are upward leaf rolling, chlorosis, purple discoloration of new leaves, short internodes, proliferation of axillary shoots with basal swelling, and formation of aerial tubers. Infected tubers can fail to germinate or produce hair sprouts, so that viability of tubers used as seed is diminished. Other damage in the tuber includes internal browning, which affects the commercial value of the harvest (Cadena and Galindo-Alonso 1986; Cadena et al. 2003; Leyva-López et al. 2002; Lee et al. 2004; Secor et al. 2006).

The control of phytoplasmas is very difficult as there are no curative methods, and resistance or tolerance to these organisms is rare. Classic control strategies include eradication of infected plants to decrease the amount of inoculum, use of healthy plant material, and insecticide treatments against the vectors (Garnier et al. 2001; Cadena 1996; 2000).

Plant cells have defensive responses to pathogen attack associated with changes in oxidative metabolism (Hammerschmidt 2005). One of the consequences of stress is an increase in the cellular concentration of reactive oxygen species (ROS), which are subsequently converted to hydrogen peroxide (H_2O_2). These ROS, particularly H_2O_2, play versatile roles in normal plant physiological processes and in resistance to stresses. H_2O_2 produced in excess is harmful, but lower concentrations are beneficial (Quan et al. 2008). H_2O_2 is believed to play two distinct roles in pathogenesis. One involves the oxidative burst in the hypersensitive response, which restricts pathogen growth (Low and Merida 1996; Wojtaszek 1997), and the other activates plant defense responses, including induction of phytoalexins (Apostol et al. 1989), second messengers or signaling intermediates, PR proteins, antioxidant enzymes (Low and Merida 1996, Desikan et al. 1998; 2000; 2001; Gechev et al. 2002) and cell wall reinforcement (Dempsey and Klessig 1995). For example, exogenous application of H_2O_2 induced tolerance to high temperature (López-Delgado et al. 1998) and to chilling (Mora-Herrera et al. 2005) in microplants of *Solanum tuberosum*. In transgenic tobacco deficient in the H_2O_2 removing enzyme catalase, sublethal levels of H_2O_2 activated expression of acidic and basic pathogenesis-related (PR) proteins and led to enhanced pathogen tolerance (Chamnongpol et al. 1998). Desikan et al. (2001), using cDNA microarrays, showed that in *Arabidopsis* H_2O_2 induced transcripts for proteins with functions in metabolism, energy, transport, cellular organization and biogenesis, cell rescue, defense and transcription. Genetic and physiological evidence suggests that H_2O_2 acts as a signaling second messenger, mediating the acquisition of tolerance to both biotic and abiotic stresses and providing information about changes in the external environment (Desikan et al. 2001; Bhattacharjee 2005; Quan et al. 2008).

Another molecule that participates in response to both biotic and abiotic stresses is ascorbic acid (AA), which acts as an antioxidant, protecting the cell against oxidative stress caused by environmental factors and pathogens. As a direct scavenger of ROS, protecting or regenerating carotenoids or tocopherols, AA is the major redox buffer in plants, and is present at high concentrations in most plant cell compartments, including the apoplast (Noctor and Foyer 1998). AA is a cofactor of many enzymes, such as ascorbate peroxidase, which converts H_2O_2 to water, and violaxanthin de-epoxidase, which is required for dissipation of excess excitation energy during nonphotochemical quenching of chlorophyll a fluorescence, (Eskling et al. 1997; Smirnoff 2000). AA is oxidized in many of its functions, producing the monodehydroascorbate radical, which can be reduced to ascorbate by monodehydroascorbate reductase or undergo dismutation to produce dehydroascorbate (DHA), which can be reduced back to AA by DHA reductase with glutathione as the reducing substrate (Noctor and Foyer 1998; Gillespie and Ainsworth 2007).

Changes in AA content can modulate PR gene expression and systemic acquired resistance, acting as a signal transducing molecule (Pastori et al. 2003; Foyer and Noctor 2005). In microarray analysis comparing leaf transcript abundance in the mutant *vtc1* (vitamin C-deficient *Arabidopsis*) and the corresponding wild type Col-0, a total of 171 transcripts were modified, encoding proteins implicated in DNA-binding, cell cycle control, signaling and developmental processes, carbon, cell wall and lipid metabolism, and anthocyanin synthesis. The most striking changes in transcript abundance were observed for genes involved in responses to biotic stress, in particular transcripts involved with PR proteins, translocation and protein synthesis (Pastori et al. 2003). Moreover, AA is also a regulator of cell division, cell elongation and growth (Kerk and Feldman 1995).

Considering that H_2O_2 and AA have been implicated in signaling gene expression against biotic and abiotic stresses (Mittler et al. 2004; Scandalios 2005; Foyer and Noctor 2005; Noctor 2006), the objectives of this work were to evaluate the effects of hydrogen peroxide, AA and DHA on the tuber yield, sprouting and starch content, and on photosynthetic pigments in plants positive to phytoplasma.

Materials and Methods

Plant Material

Solanum tuberosum L. microplants cv Alpha, testing virus-free, were obtained from the Germplasm Bank of the National Potato Program of the National Institute for Forestry Agriculture and Livestock Research (INIFAP) in Metepec, Mexico. They were previously obtained from the field selecting plants with symptoms of phytoplasma naturally infected. Single node cuttings were propagated in test tubes on Murashige and Skoog (1962) medium, at 20±1°C under a 16 h photoperiod (fluorescent lights, 35 μmol m^2 sec^{-1}, 400–700 nm), in sterile conditions (Espinoza et al. 1986). They were tested for the presence of phytoplasmas using nested PCRs with the phytoplasma-universal 16S rDNA-based primers P3/P7 and R16F2n/R16R2 of Smart et al. (1996a, b). Phytoplasma-tested microplants were transferred to greenhouse conditions 30 days after the single-node subculture step.

Chemical Treatments

In the greenhouse, microplants were transplanted to pots containing peat moss substrate. From 20 days later, they were sprayed twice weekly for the next 2 months with 10 mL per plant of either 1 mM H_2O_2, 3.4 mM AA, or 3.4 mM DHA at pH 5.7. Controls were sprayed with distilled water. Eight phytoplasma-positive and –negative plants were sprayed in randomized arrays for each chemical or control treatment, and each treatment was performed in three to five independent experiments. Shoot dry weight, number and weight of tubers per plant, were recorded 60 and 90 days after transplanting.

Pigment Analysis

Measurements were performed for each experiment on plants 80 days after transplanting. Five leaf discs (1.5 cm diameter) per plant were taken from mid-shoot leaves of three plants per treatment. Samples for each assay comprised 15 discs, homogenized in 4 mL of 80% acetone at 4°C. Insoluble materials were removed by centrifugation at 3 500 *g* for 10 min. Chlorophylls a and b, and carotenoids, were analyzed spectrophotometrically according to the method of Lichtenthaler and Wellburn (1983).

Starch Analysis

Tuber starch content was determined by two methods. Spectrophotometric assay by reaction with anthrone (Sigma Chemical Co.) was performed on 1 g fresh weight of tissue, by a similar method to Peña-Valdivia and Ortega-Delgado (1991). For each of the eight treatments, a composite sample of three minitubers was harvested. The 1 g sample for each anthrone essay was composed of tissue of these three minitubers. Measurements were performed for each independent experiment 3 days after harvesting.

The percentage content of starch in tubers was also determined using the relationship of specific gravity to dry matter. Minitubers were weighed at 20°C in air and then in water. Specific gravity was calculated as (weight in air)/([weight in air] -[weight in water]), and correlated with the dry matter and starch content using the values of Gould and Plimpton (1985). Specific gravity analysis was conducted on all minitubers harvested from 8 plants per treatment.

Percentage of Minitubers Sprouted

Sprouting was induced by treatment of minitubers with gibberellic acid (14.4 μM, pH 5.7) for 30 min, followed by storage in diffused light. Three months later the percentage of minitubers sprouted was evaluated for each treatment.

Statistical Analysis

Data were analyzed in Statistica 6 software (StatSoft, Inc., Tulsa, OK) by ANOVA and Duncan's Multiple Range Test (Duncan 1955), and scored as significant if $P<0.05$.

Results

Effects of H_2O_2, AA and DHA were compared on shoot growth, pigment contents, and tuber harvest, starch, and sprouting parameters of both healthy and phytoplasma-infected cv Alpha plants.

Shoot Growth

Sixty days after transplanting *in vitro* microplants to greenhouse pots, dry weights of phytoplasma-positive shoots were significantly lower (43%) than uninfected ones, in the absence of chemical treatments (Fig. 1A). Spraying H_2O_2, AA or DHA significantly increased shoot dry weight (by 87%, 86% and 127% respectively) of positive plants to values similar to uninfected plants. The H_2O_2, AA or DHA treatments did not produce any significant increments in the dry weight of uninfected shoots at 60 days (Fig. 1A). By contrast, 90 days after transplanting, each treatment had significantly increased the weight of uninfected shoots, by 37 to 46%, relative to uninfected control (Fig. 1B). In positive plants at 90 days, each treatment again increased shoot weight, though only the AA effect (42%) was statistically significant (Fig. 1B).

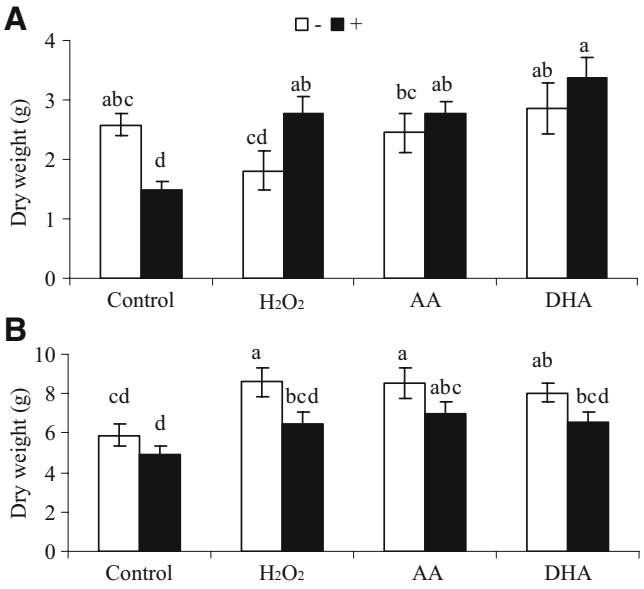

Fig. 1 Dry weight of shoots of potato plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. Data are means ± SE of three to five experiments (n=8). **A**) 60 and **B**) 90 days after transplanting. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

Pigment Analysis

Changes in photosynthetic pigment contents were evaluated 80 days after transplanting (Fig. 2A, B, C and D). Without chemical treatments, phytoplasma-positive leaves showed significant reductions, compared to uninfected leaves, in chlorophyll a (by 29%), chlorophyll b (44%), total chlorophyll (30%), and xanthophylls /carotenoids (57%). Treatments with H_2O_2, AA or DHA significantly increased pigment contents of positive plant leaves to levels similar to uninfected plants (with the exception of DHA on Chl a and AA effects on carotenoids). No significant differences were induced by these treatments in the uninfected plants (Fig. 2A, B, C and D).

Tuber Harvests

Final harvests were carried out at 60 or 90 days after transplanting. At 60 days no significant differences were observed in the number of tubers in phytoplasma-positive or uninfected control-treatments (Fig. 3A). However, at the same date, positive plants sprayed with DHA produced significantly more tubers (by 47%) than the positive controls. None of the treatments induced significant differences in the number of tubers in negative plants (Fig. 3A).

At 90 days after transplanting, the number of tubers produced by positive control plants was significantly higher than the uninfected control (by 65%) (Fig. 3B). In uninfected plants no significant differences were obtained by the treatments relative to their controls (Fig. 3B). However, all the H_2O_2, AA and DHA treatments significantly reduced the number of tubers produced per plant (by 29, 22 and 25% respectively) in the positive plants compared to their control (Fig. 3B). Interestingly, this reduced number of tubers was similar to that produced by uninfected plants subjected to any of the treatments (Fig. 3B).

Tuber weights of the uninfected control plants were significantly higher (by 80 and 64%) than the positive control by 60 and 90 days respectively (Fig. 4A and B). However, H_2O_2 and DHA treatments significantly enhanced the weight of tubers at 60 days (by 95% and 116% respectively) in the

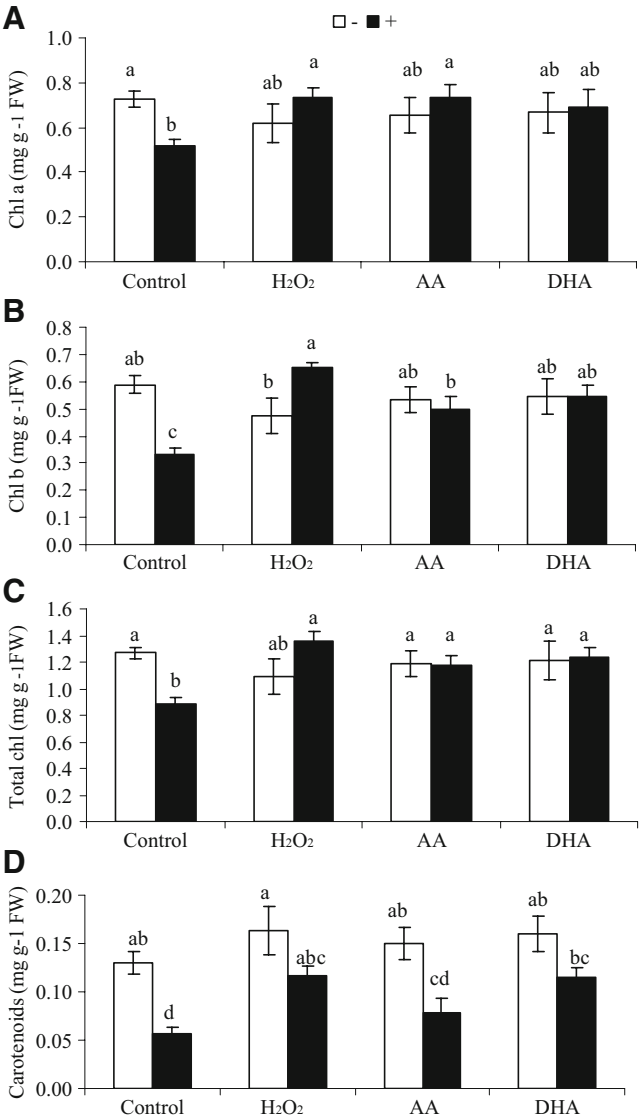

Fig. 2 Photosynthetic pigments. **A**) chlorophyll a, **B**) chlorophyll b, **C**) total chlorophyll, and **D**) carotenoids of leaves of plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. Data are means ± SE of three to five experiments (n=3) 80 days after transplanting. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

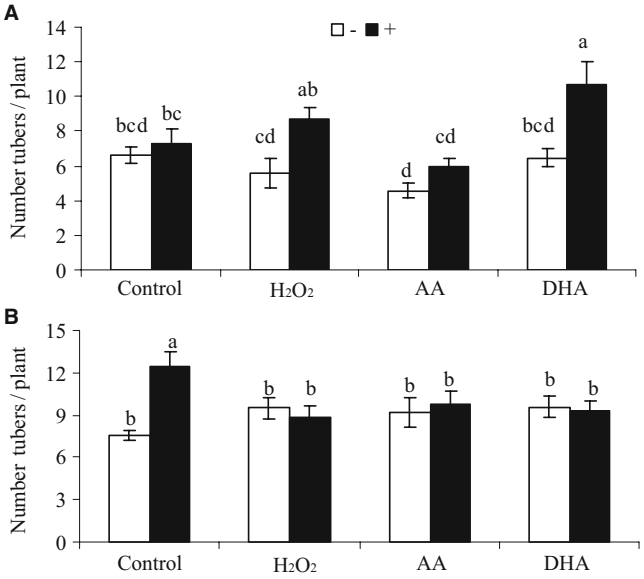

Fig. 3 Number of tubers produced by plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. Data are means ± SE of three to five experiments (n=8), at **A**) 60 days, and **B**) 90 days after transplanting. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

positive plants compared to their control (Fig. 4A). Furthermore, this response was maintained at 90 days after transplanting (107% and 78% respectively), when the AA treatment also registered a significant (47%) increase (Fig. 4B). The chemical treatments of positive plants resulted in tuber weights that were either not significantly different to, or greater than (in the H_2O_2 treatment at 90 days), those of uninfected controls (Fig. 4A and B).

Significant reduction by the chemical treatments of the weight of tubers harvested was observed in the uninfected plants compared with their control at 60 days, this effect remaining significant at 90 days for the DHA treatment (Fig. 4).

Starch Content

Starch content, determined by anthrone reaction, significantly decreased in positive control tubers (by 21%) compared to uninfected controls (Fig. 5A). However, H_2O_2 and antioxidant treatments of phytoplasma-positive plants significantly increased their tuber starch contents (H_2O_2, 61%; AA, 30%; DHA, 40%) compared with their control (Fig. 5A). Minitubers of uninfected plants following DHA treatment had significantly decreased (16%) starch content relative to uninfected controls, but treatments by H_2O_2 and AA did not show significant differences (Fig. 5A).

Starch content and dry matter, determined by the gravimetric method, were significantly decreased in tubers obtained from positive controls (67 and 56% respectively), in contrast to the negative controls. In positive plants, H_2O_2 and both antioxidants significantly increased the tuber starch content (H_2O_2, 179%; AA, 154%; DHA, 133%) (Fig. 5B), and dry matter (H_2O_2, 112%; AA, 96%; DHA, 83%) (Fig. 5C), compared to the positive controls. In negative plants treated with H_2O_2, AA or DHA, significant differences were not observed for these parameters (Fig. 5B and C).

Sprouting

The percentage of sprouted minitubers 128 days after harvesting was significantly lower (26%) for those produced by the positive control plants compared to the uninfected controls (Fig. 6). For minitubers produced by positive plants, H_2O_2, AA or DHA induced significantly higher percentages of sprouting (20, 17 and 18% respectively), relative to the positive controls. Minitubers borne by uninfected plants did not show significant differences in sprouting after H_2O_2 or DHA treatments, while the AA treatment caused significantly lower sprouting percentages than the negative controls.

Discussion

H_2O_2 is a diffusible signal-transducing molecule and its accumulation is perceived by the plant as a signal of

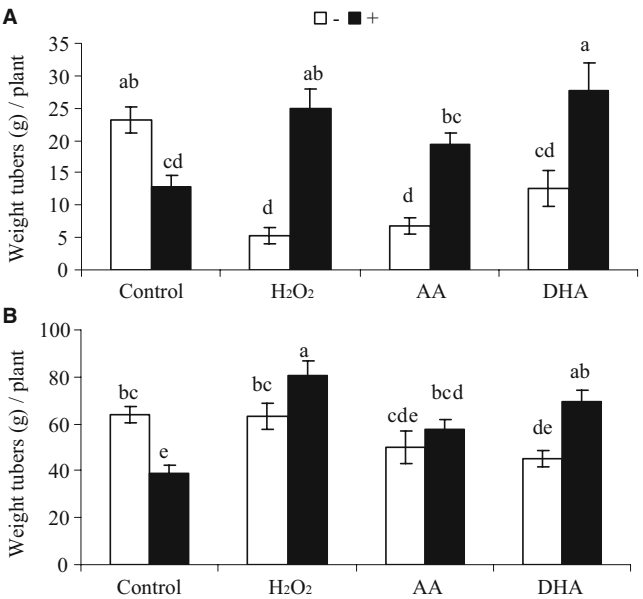

Fig. 4 Weight of tubers produced by plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. Data are means ± SE of three to five experiments (n=8) at **A**) 60 days, and **B**) 90 days after transplanting. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

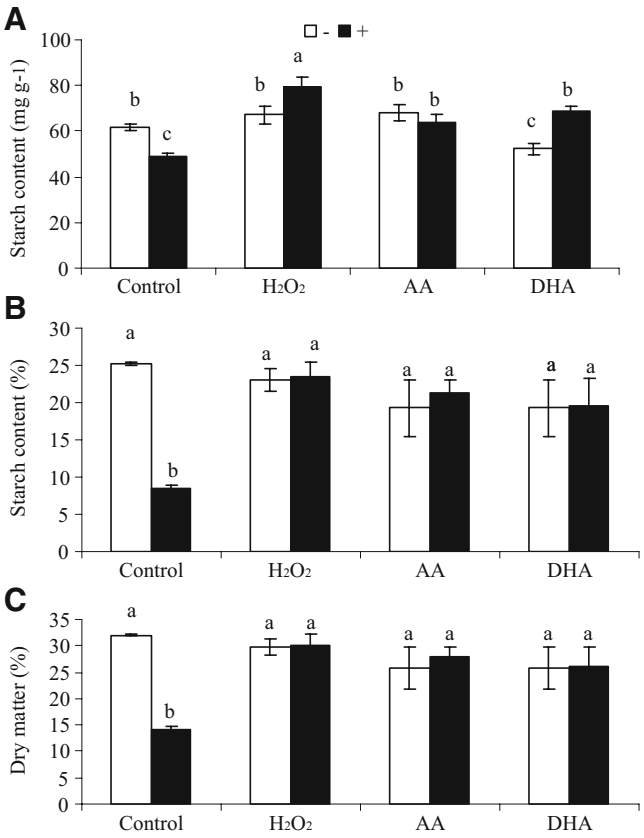

Fig. 5 Starch content of tubers produced by plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. **A**) Starch content by the anthrone method. **B**) Starch content and **C**) Dry matter by the specific gravity method. Data are means ± SE of three to five experiments (n=3) 3 days after harvest. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

environmental change, alerting the cell to both biotic and biotic threats (Noctor and Foyer 1998). It also alters the concentrations and redox status of intracellular antioxidants, such as ascorbate (Foyer and Noctor 2005), thereby stimulating induction of defense genes (Wu et al. 1997). The role of H_2O_2 in the induction of tolerance to stresses in potato plants has been demonstrated. López-Delgado et al. (1998) and Mora-Herrera et al. (2005) showed that exogenous H_2O_2 induced tolerance to high temperature and freezing in potato plants. Wu et al. (1995) observed that transgenic potato plants expressing a fungal gene encoding glucose oxidase, which generates H_2O_2 when glucose is oxidized, exhibited strong resistance to *Erwinia carotovora* subsp *carotovora*, and to *Phytophthora infestans*. This resistance to soft rot and to potato late blight was apparently mediated by elevated levels of H_2O_2. The results of the present study demonstrated that phytoplasma infected plants suffered significantly harmful effects on shoot dry weights and pigment contents, and on the number, weight, starch content, and sprouting rate of tubers. In general, these effects were reduced by spraying H_2O_2, AA or DHA.

Concerning the changes in the leaves pigment contents, foliar chlorosis is a common symptom of phytoplasmas (Lee et al. 2000). Pigment alterations may be involved in pathogenesis (Chang 1998). Our results show that the presence of phytoplasma in potato plants significantly reduced the content of chlorophyll a, chlorophyll b, total chlorophyll, and xanthophylls/carotenoids. Similar effects have been observed in periwinkles (*Catharanthus roseus*) infected by aster yellows phytoplasma or *Spiroplasma citri*, where chlorophyll content was reduced (Chang 1998). Similarly in apple (*Malus pumila*), grapevine (*Vitis vinifera* L.cv Chardonnay) and corn plants infected by phytoplasma, levels of chlorophylls and carotenoids were reduced (Bertamini et al. 2002a, b, Junqueira et al. 2004). Our results confirm that phytoplasmas can interfere with photosynthetic pigments in potato. Leaf pigments of phytoplasma-infected plants were reduced without yellowing symptoms, an effect also observed by Chang (1998) in *S. citri*-infected periwinkle leaves, where the reduction of chlorophyll occurred before yellowing symptoms became visible. H_2O_2, AA and DHA treatments of phytoplasma-positive plants significantly increased the levels of chlorophylls compared with positive control plants, while similarly treated uninfected plants sprayed with did not show significant differences in these pigments.

Under greenhouse conditions, the phytoplasma-positive controls did not show characteristic symptoms of potato purple top, but 90 days after transplanting, they produced a higher number of tubers than the uninfected controls, with a decrease in the weight and starch content of tubers relative to uninfected controls. Increased number and reduced weight of tubers is a characteristic response to stress in potato. Phytoplasmas also cause an array of symptoms suggestive of disturbances in the normal balance of plant

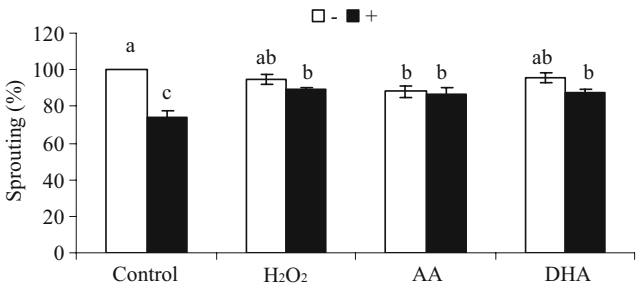

Fig. 6 Percentage of sprouting potato tubers produced by plants testing negative (□) or positive (■) to phytoplasma, following spray treatments with H_2O_2 (1 mM), AA (3.4 mM) or DHA (3.4 mM) or water (controls), twice weekly for 60 days. Data are means ± SE of three to five experiments (n=3 –5), at 128 days after harvest. Bars with different letters differ significantly by ANOVA and Duncan's test (P<0.05)

hormones such as cytokinins and auxins (Chang 1998, Lee et al. 2000). Increased number of tubers could be due to disturbance of plant hormones involved in tuber formation (Fernie and Willmitzer, 2001).

There is no information about how phytoplasmas affect sucrose and starch metabolism in potato. Negative effects of phytoplasmas on photosynthesis have been reported, and differential display studies in periwinkle infected with stolbur phytoplasma indicated down-regulation of genes involved in photosynthesis (Jagoueix-Eveillard et al. 2001). In transgenic potato plants, deficiency in starch biosynthesis might have caused an increased number and reduced size of tubers, these responses being paralleled by a decrease in starch content, and positively correlated with soluble sugar accumulating in tubers (Müller-Röber et al. 1992). In the present work, similar results were observed in tubers of phytoplasma-positive controls, both starch content and dry matter being decreased relative to uninfected controls. H_2O_2, AA or DHA treatments reversed these symptoms, along with reduction and enhancement in the number and weight of tubers respectively. In general, these treatments did not induce significant differences in tubers from negative plants.

It has been suggested that a physiological balance of antioxidant components is necessary in order to obtain protection to generalized stress; however, antioxidants are not always accessible to some of the sites where they are most needed in times of stress (Foyer et al. 1994). Our results agree with this statement since the AA and DHA treatments induced significant anti-stress effects only in the tubers from positive plants. Similar affirmation could apply for H_2O_2. Previous reports demonstrated starch accumulation in tubers and stems as effects of H_2O_2. López-Delgado et al. (2005) observed under field conditions that H_2O_2 (5 mM) treatment enhanced tuber starch accumulation. Image analysis confirmed that stems of H_2O_2 treated plants contained more starch and lignin. The present work demonstrates that at a lower concentration (1 mM) under glasshouse conditions, H_2O_2 induced starch accumulation only in tubers from phytoplasma-positive plants, suggesting that H_2O_2-induced changes in gene expression caused the increased starch content.

The percentage of post-harvest sprouting was lower in tubers from positive controls than tubers from the uninfected controls. Experimental evidences suggest that phytoplasmas might alter sucrose metabolism (Oshima et al. 2004). The possible regulatory role of sucrose in tuber sprouting has been investigated. Effects on tuber sprouting due to altered sucrose metabolism were reported by Hajirezaei et al. (2003). In transgenic potatoes, where phloem-specific expression of cytosolic invertase blocked the phloem transport of sucrose, they observed that tuber sprouting was strongly impaired with absence of visible sprout growth. In the present research, the detrimental effects of phytoplasma on tuber sprouting were reduced by the H_2O_2, AA and DHA treatments.

This work presents a novel potential approach for overcoming the most common damage in tubers of phytoplasma-infected non-seed potatoes, using natural compounds that offer the possibility of reduction of biocide usage. The elucidation of the precise mechanism of H_2O_2, AA and DHA on phytoplasmas awaits further investigation.

Acknowledgements This research was supported by CONACYT proyect SEP/CONACYT/2003/CO2/45016. The first author thanks postgraduate CONACYT scholarship. We extend our sincere thanks to Dr. Ian M Scott, University of Wales, UK, for editing the manuscript.

References

Apostol, I., P.F. Heinstein, and P.S. Low. 1989. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. *Plant Physiology* 90: 109–116.

Bertamini, M., M.S. Grando, K. Muthuchelian, and N. Nedunchezhian. 2002a. Effect of phytoplasmal infection on photosystem II efficiency and thylakoid membrane protein changes in field grown apple (*Malus pumila*) leaves. *Physiological and Molecular Plant Pathology* 61: 349–356.

Bertamini, M., N. Nedunchezhian, F. Tomasi, and M.S. Grando. 2002b. Phytoplasma [Stolbur-subgroup (Bois Noir-BN)] infection inhibits photosynthetic pigments, ribulose-1,5,-bisphosphate carboxylase and photosynthetic activities in field grown grapevine (*Vitis vinifera* L.cv Chardonnay) leaves. *Physiological and Molecular Plant Pathology* 61: 357–366.

Bhattacharjee, S. 2005. Reactive oxygen species and oxidative Burst: Roles in stress, senescence and signal transduction in plants. *Current Science* 89: 1113–1121.

Cadena-Hinojosa, M.A., and J. Galindo-Alonso. 1986. Reducción de la incidencia de la "punta morada de la papa" por medio de fechas de siembra, genotipo de planta y aplicación de insecticidas. *Revista Mexicana de Fitopatología* 3: 100–104.

Cadena-Hinojosa, M.A. 1993. La punta morada de la papa en México: I incidencia y búsqueda de resistencia. *Agrociencia* 4: 247–256.

Cadena-Hinojosa, M.A. 1996. La punta morada de la papa en México: efecto de cubiertas flotantes, genotipos y productos químicos. *Revista Mexicana de Fitopatología* 14: 20–24.

Cadena-Hinojosa, M.A. 2000. Potato purple top in México: III. Effects of plant spacing and insecticide application. *Revista Mexicana de Fitopatología* 17: 91–96.

Cadena-Hinojosa, M.A., R. Guzmán-Plazola, M. Díaz-Valasis, T.E. Zavala-Quintana, O.S. Magaña-Torres, I.H. Almeida-León, H. López-Delgado, A. Rivera-Peña, and O. Rubio-Covarrubias. 2003. Distribución, incidencia y severidad del pardeamiento y la brotación anormal en los tubérculos de papa (Solanum tuberosum L.) en valles altos y sierras de los estados de México, Tlaxcala y el Distrito Federal, México. *Revista Mexicana de Fitopatología* 21: 248–259.

Chamnongpol, S., H. Willekens, W. Moeder, C. Langebartels, H. Sandermann, M.V. Montagu, D. Inze, and W.V. Camp. 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. *Proceedings of the National Academy of Science* 95: 5818–5823.

Chang, C.J. 1998. Pathogenicity of aster yellows phytoplasma and *Spiroplasma citri* on periwinkle. *Phytopathology* 88: 1347–1350.

Dempsey, D.A., and D.F. Klessig. 1995. Signals in plant disease resistance. *Bull Inst Pasteur* 93: 167–186.

Desikan, R., A. Reynolds, J.T. Hancock, and S.J. Neill. 1998. Harpin and Hydrogen peroxide both initiate programmed cell death but have differential effects on defense gene expression in *Arabidopsis* suspension cultures. *Biochemical Journal* 330: 115–120.

Desikan, R., S.J. Neill, and J.T. Hancock. 2000. Hydrogen peroxide-induced gene expression in *Arabidopsis thaliana*. *Free Radical Biology & Medicine* 28: 773–778.

Desikan, R., S.A.H. Mackerness, J.T. Hancock, and S.J. Neill. 2001. Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiology* 127: 159–172.

Doi, Y.M., M. Teranaka, K. Yora, and H. Asuyama. 1967. Mycoplasma or PLT-group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows or paulownia witches' broom. *Ann Phytopathol Soc Jpn* 33: 259–266.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics* 11: 1–42.

Espinoza, N.O., R. Estrada, D. Silva-Rodríguez, P. Tovar, R. Lizarraga, and J.H. Dodds. 1986. The potato: A model crop plant for tissue culture. *Outlook on Agriculture* 15: 21–26.

Eskling, M., P.O. Arvidsson, and H.E. Akerlund. 1997. The xanthophyll cycle, its regulation and components. *Physiologia Plantarum* 100: 806–816.

Foyer, C.H., P. Descourvieres, and K.J. Kunert. 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant Cell and Environment* 17: 507–523.

Foyer, C.H., and G. Noctor. 2005. Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell and Environment* 28: 1056–1071.

Fernie, A.R., and L. Willmitzer. 2001. Molecular and biochemical triggers of potato tuber development. *Plant Physiology* 127: 1459–1465.

Garnier, M., X. Foissac, P. Gaurivaud, F. Laigret, J. Renaudin, C. Saillard, and J.M. Bové. 2001. Mycoplasmas, plants, insect vectors: a matrimonial triangle. *Life Sciences* 324: 923–928.

Gechev, T., I. Gadjev, F.V. Breusegem, D. Inze, S. Dukiandjiev, V. Toneva, and I. Minkov. 2002. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cellular and Molecular Life Sciences* 59: 708–714.

Gillespie, K.M., and E.A. Ainsworth. 2007. Measurement of reduced, oxidized and total ascorbate content in plants. *Nature Protocols* 2: 871–874.

Gould, W.A., and S. Plimpton. 1985. Quality evaluation of potato cultivars for processing. *Ohio Agricultural Research and Development Center Research Bulletin* 1172: 1–24.

Jagoueix-Eveillard, S., F. Tarendeau, K. Guolter, J.L. Danet, J.M. Bové, and M. Garnier. 2001. *Catharanthus roseus* genes regulated differentially by Mollicute infections. *Molecular Plant-Microbe Interactions* 14: 225–233.

Junqueira, A., I. Bedendo, and S. Pascholati. 2004. Biochemical changes in corn plants infected by the maize bushy stunt phytoplasma. *Physiological and Molecular Plant Pathology* 65: 181–185.

Hajirezaei, M.R., F. Börnke, M. Peisker, Y. Takahata, J. Lerchl, A. Kirakosyan, and U. Sonnewald. 2003. Decreased sucrose content triggers starch breakdown and respiration in stored potato tubers (*Solanum tuberosum*). *Journal of Experimental Botany* 54: 477–488.

Hammerschmidt, R. 2005. Antioxidants and the regulation of defense. *Physiological and Molecular Plant Pathology* 66: 211–212.

Kerk, N.M., and L.F. Feldman. 1995. A biochemical model for the initiation and maintenance of the quiescent center: implications for organization of root meristems. *Development* 121: 2825–2833.

Lee, I.M., and R.E. Davis. 1992. Mycoplasmas which infect plants and insects. In *Mycoplasmas: Molecular Biology and Phatogenesis*, eds. J Maniloff, RN McElhansey, LR Finch, and JB Baseman, 379–390. Washington, DC: Am.Soc. Microbiol.

Lee, I.M., R.E. Davis, and D.E. Gundersen-Rindal. 2000. Phytoplasma: Phytopathogenic mollicutes. *Annual Review Microbiology* 54: 221–255.

Lee, I.M., K.D. Bottner, J.E. Munyaneza, G.A. Secor, and N.C. Gudmestad. 2004. Clover proliferation group (16SrVI), subgroup A (16SrVI-A) phytoplasma is probable causal agent of potato purple top disease in Washington and Oregon. *Plant Disease* 88: 429.

Leyva-López, N.E., J.C. Ochoa-Sánchez, D.S. Leal-Klevezas, and J.P. Martínez-Soriano. 2002. Multiple phytoplasmas associated with potato diseases in Mexico. *Canadian Microbiology* 48: 1062–1068.

Lichtenthaler, H.K., and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11: 591–592.

López-Delgado, H., J.F. Dat, C.H. Foyer, and I.M. Scott. 1998. Induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2. *Journal of Experimental Botany* 49: 713–720.

López-Delgado, H., H.A. Zavaleta-Mancera, M.E. Mora-Herrera, M. Vázquez-Rivera, F.X. Flores-Gutiérrez, and I.M. Scott. 2005. Hydrogen peroxide increases potato tuber and stem starch content, stem diameter and stem lignin content. *American Journal of Potato Research* 82: 279–285.

Low, P.S., and J.R. Merida. 1996. The oxidative burst in plant defense: Function and signal transduction. *Physiologia Plantarum* 96: 533–542.

Marcone, C., H. Neimark, A. Ragozzino, U. Lauer, and E. Seemüller. 1999. Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups. *Phytopathology* 89: 805–810.

Mittler, R., S. Vanderauwera, M. Gollery, and F.V. Breusegem. 2004. Reactive oxygen gene network of plant. *TRENDS in Plant Science* 9: 490–498.

Mora-Herrera, M.E., H. López-Delgado, A. Castillo-Morales, and C. H. Foyer. 2005. Salicylic acid and H_2O_2 function by independent pathways in the induction of freezing tolerance in potato. *Physiologia Plantarum* 125: 430–440.

Müller-Röber, B., U. Sonnewald, and L. Willmitzer. 1992. Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. *The EMBO Journal* 11: 1229–1238.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Neimark, H., and B.C. Kirkpatrick. 1993. Isolation and characterization of full-length chromosomes from non-culturable plant pathogenic mycoplasma-like organisms. *Molecular Microbiology* 7: 21–28.

Noctor, G., and C.H. Foyer. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review Plant Physiology Plant Molecular Biology* 49: 249–279.

Noctor, G. 2006. Metabolic signaling in defense and stress: the central roles of soluble redox couples. *Plant, Cell and Environment* 29: 409–425.

Oshima, K., S. Kakizawa, H. Nishigawa, H.Y. Jung, W. Wei, S. Suzuki, R. Arashida, D. Nakata, S. Miyata, M. Ugaki, and S. Namba. 2004. Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. *Nature Genetics* 36: 27–29.

Peña-Valdivia, C.B., and M.L. Ortega-Delgado. 1991. Non-structural carbohydrate partitioning in *Phaseolus vulgaris* after vegetative growth. *Journal of the Science of Food and Agriculture* 55: 563–577.

Pastori, G.M., G. Kiddle, J. Antoniw, S. Bernard, S. Veljovic-Jovanovic, P.J. Verrier, G. Noctor, and C.H. Foyer. 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. *The Plant Cell* 15: 939–951.

Quan, L.J., B. Zhang, W.W. Shi, and H.Y. Li. 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology* 50: 2–18.

Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazilian Journal of Medical and Biological Research* 38: 995–1014.

Secor, G.A., I.M. Lee, K.D. Bottner, V. Rivera-Varas, and N.C. Gudmestad. 2006. First report of a defect of processing potatoes in Texas and Nebraska associated with a new phytoplasma. *Plant Disease* 90: 377.

Smirnoff, N. 2000. Ascorbate biosynthesis and function in photoprotection. *Philosophical Transactions of the Royal Society of London B* 355: 1455–1464.

Smart, C.D., B. Schneider, C.L. Blomquist, L.J. Guerra, N.A. Harrison, U. Ahrens, K.H. Lorenz, E. Seemüler, and B.C. Kirkpatrick. 1996a. Phytoplasma-specific PCR primers based on sequences of the 16S–23S rRNA spacer region. *Applied and Environmental Microbiology* 62: 2988–2993.

Smart, C.D., and B.C. Kirkpatrick. 1996b. Identification of host plant genes whose expression is altered upon aster yellows phytoplasma infection. *IOM Letters* 4: 274.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. *Biochemistry Journal* 322: 681–692.

Wu, G., B.J. Shortt, E.B. Lawrence, E.B. Levine, K.C. Fitzssimmons, and D.M. Shah. 1995. Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. *The Plant Cell* 7: 1357–1368.

Wu, G., B.J. Shortt, E.B. Lawrence, J. León, K.C. Fitzsimmons, E.B. Levine, I. Raskin, and D.M. Shah. 1997. Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. *Plant Physiology* 115: 427–435.