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Propyl gallate, a free radical scavenger, counteracts the benefits of exogenously applied salicylic acid and aggravates the deleterious effects of the southern bean mosaic virus in *Rhizobium*-nodulated *Phaseolus vulgaris* plants

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Infection of *Rhizobium*-nodulated *Phaseolus vulgaris* by the southern bean mosaic virus (SBMV) markedly decreased the growth and nodulation of plants. Exogenous applications of salicylic acid (SA) at concentrations $\geq$10 μM further decreased growth and nodulation of virus-infected (V) plants. Only SA concentration of 5 μM in the solution improved the growth, nodulation, chlorophyll concentration and the catabolism of ureide in leaves of V plants. The spray of leaves with 2 mM propyl gallate (+Pg) decreased growth, nodulation as well as the chlorophyll and leaf ureide concentrations in V plants, regardless of the concentration of SA at which plants were grown. Ultrastructural damages in leaf cells caused by SBMV were also enhanced in V+Pg plants. The massive proliferation of virus particles and the presence of virus crystalline arrays within symbiosomes of nodules in V+Pg plants were attributed to proliferation of branched plasmodesmata in leaf vascular-parenchyma cell walls that facilitated virus movement. Virus particles were never observed in leaf and nodule tissues of V plants not sprayed with Pg. Exogenous applications of SA hindered while Pg increased the symbiotic performance of H plants, pointing out the complexity to be addressed in breeding for virus resistance in *Rhizobium*-nodulated beans.

Keywords: *Rhizobium*; southern bean mosaic virus; salicylic acid; propyl gallate; ultrastructure; *Phaseolus vulgaris*

Introduction

In plants, virus infections are know to trigger the oxidative metabolism as a defence response to pathogens (Song et al. 2008 and references therein). Concomitantly, in virus-infected (V) plants, antioxidant mechanisms are activated to protect cells from the toxic molecules produced by oxidative metabolism (Tavares et al. 2007 and references therein). Any alteration in this fragile equilibrium between the plant oxidation and detoxifying mechanisms will determine if the virus infection will become host-compatible with virus particles moving toward sink organs via plasmodesmata (Maule 2008), or the spread of the virus will be hindered by a hypersensitive reaction.

An enhancement of the oxidative metabolism and, therefore, of the resistance of plants toward virus infections, can be achieved by exposure of V plants to exogenous

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applications of salicylic acid (SA), a known primary signal inducing plant defences against pathogens (Karthikeyan et al. 2009 and references therein). The SA-induced resistance includes interference of the virus replication and the symplastic virus movement from infected leaves toward sink organs. A recent publication, however, describes an opposite effect of SA in susceptible pea cultivars infected by the clover yellow vein virus (Atsumi et al. 2009).

Reviewing the available data on legumes reveals increased systemic resistance to viruses when exposed to up to 4 mM SA (Faheed and Mahmoud 2006). All studies published were, however, conducted in legumes relying on nitrate for their nitrogen economy. In *Rhizobium*-nodulated legumes, virus infections hinder the early events of nodulation and nodule functioning (Izaguirre-Mayoral et al. 1992) due to the virus replication in cells containing the symbiosomes (Izaguirre-Mayoral et al. 1994), making nodulated legumes more sensitive to virus than those which are nitrate-fertilized (Izaguirre-Mayoral et al. 1989). To the best of our knowledge, the effects of increasing concentrations of SA in *Rhizobium*-nodulated V plants has not been investigated.

The aim of the present research was, therefore, to evaluate the effects of exogenously applied SA on several physiological parameters related to growth and symbiotic efficiency in *Rhizobium*-nodulated black beans infected by the southern bean mosaic virus (SBMV). The possibility that exposure of plants to SA suppress the establishment of the symbiosis with *Rhizobium* (Soto et al. 2009 and references therein) was also analysed. The impact of oxidative metabolism upon growth and nodulation was evaluated indirectly by spraying healthy (H) and V plants with propyl gallate (Pg). The effectiveness of Pg as a potent free radical scavenger has been reported in a number of plant species including virus-infected soybeans (Pennazio and Roggero 1992; Macri et al. 1994). The effects of the SMBV, SA and Pg on the leaf and nodule ultrastructure were also determined. Both SA and Pg treatments were applied thoughtout the whole vegetative growth of the H and V plants. The possibility of chemical interferences between compounds was eliminated by adding SA to the nutrient solution while Pg was sprayed daily on the leaves.

Materials and method

All the laboratory and glasshouse experiments were conducted at the Venezuelan Scientific Research Institute, Caracas, situated at 10° N latitude and 66° O longitude at an altitude of 1500 MSL.

Plant materials and exposure to salicylic acid

Seeds of black bean (*Phaseolus vulgaris* (L.) var. Tacarigua) were surfaced-sterilized with 95% ethanol and rinsed six times with sterile distilled water. One seed per jar was sown at a depth of 1 cm in sterilised Leonard jars containing 0.9 kg of sand and 0.8 L of a nutrient solution in the upper and lower compartments, respectively. The basic nutrient solution contained $CaCl_2$ (3.3 mM), $MgSO_4$ (2 mM), K_2SO_4 (1.3 mM), dibasic potassium phosphate buffer (2 mM, pH 6.1), Fe-EDTA (30 μM), $MnSO_4$ (20 μM), H_3BO_3 (4 μM), $ZnSO_4$ (1.6 μM), $CuSO_4$ (1.6 μM) and $NaMoO_4$ (0.1 μM).

Before sowing, 0.5 g of a commercial *Rhizobium* peat-based mixture (EMD, Crop BioScience, WI, USA) was spread as an uniform layer on the sand bed for the seeds in each jar. The jars were placed in an insect- proof glasshouse with a distance

of 20 cm between jars. Growth conditions were 30/24°C mean day/night temperatures, 70% relative humidity and natural photoperiod.

To analyse the response of beans to salicylic acid (SA), nutrient solutions were supplied with concentrations of 5, 10, 50, 100, 500 and 1000 μM SA. Nutrient solution with the prescribed SA concentrations was added daily to the lower compartment of each jar to replace transpirational losses. Solutions were completely replaced every five days during the 30 day experiment.

Southern bean mosaic virus (SBMV) culture and inoculation

A culture of virus was maintained in *P. vulgaris* (L.) var. Tacarigua plants grown in sterilised soil and under the glasshouse conditions described above. The youngest leaves showing symptoms of SBMV were macerated using a sterile pestle and mortar with chilled 100 mM phosphate buffer pH 8.5, containing 1% (W/V) magnesium trisilicate at a rate of one ml/g of leaf tissue. For inoculation, the leaf extract was rubbed on the upper surface of 600-mesh carborundum dusted primary leaves of five day old seedlings growing in each SA solution concentration. Control plants were mock-inoculated with buffer and abrasive. Excess of inoculum and abrasive were washed off using distilled water. Inoculation of five day-old seedlings with the virus ensured that initiation of nodulation took place in non-virus infected root tissues. Infectivity of virus particles in each of the virus-treatment combinations was tested by inoculation of bean seedlings. The appearance of symptoms was the indicator of the infectivity of the SBMV.

Exposure to propyl gallate

A 2 mM propyl gallate (Pg) (3,4,5-trihydroxybenzoic acid propyl ester) aqueous solution containing 0.05% Tween 20 was sprayed daily at 9:00 am and 4:00 pm on the lower surface of all leaves avoiding run-off. The first Pg spray (+Pg) took place on the day immediately after virus inoculation. Control plants were mock-sprayed with an aqueous 0.05% Tween 20 solution. Plants were exposed to Pg throughout the experimental time.

Determination of plant parameters

Six plants from each of the treatment combinations were harvested 30 days after emergence. Chlorophyll content was determined in 1 cm^2 discs removed from the central area of each of the three leaflets of the topmost fully expanded leaf (Izaguirre-Mayoral et al. 1992). The plants were then separated into leaves, shoots, roots and nodules. All components were individually oven-dried at 80°C until constant weight. Plant growth under each treatment combination was analysed, taking into account the dry weight of individual components. Dried subsamples of the leaves and nodules were used to measure ureide colorimetrically as described in Izaguirre-Mayoral et al. (1992).

Transmission electron microcopy

For electron microscopy, a 1 mm^2 area adjacent to the midvein was collected from the topmost fully expanded leaf of each plant. Two nodules of approximately 4 mm

diameter were collected from the main root of each plant. Nodules were sliced in half to expose the central infected zone, which was the focus of subsequent electron microscopic examination. Leaf and nodule tissues were fixed for 72 h with 2% v/v glutaraldehyde pH 7.3, post-fixed in 1% v/v osmium tetroxide and dehydrated through a graded ethanol series. After a change to propylene oxide, all pieces were embedded in medcast resin. Ultrathin sections were cut with a Sorvall ultramicrotome fitted with a diamond knife and stained with uranyl acetate followed by lead citrate. Ultrathin sections were observed in a CM10 Philips electron microscope. Six leaf and nodule tissue samples from each treatment combination were randomly selected for observation in the electron microscope. The presence of virus particles in developing leaves was ascertained by observations in the electron microscope of negative stained sap.

Statistical analyses

Data was analysed by one-way analysis of variance at $p \leq 0.05$. The statistical error of the mean of six replicates of each treatment combination was also calculated.

Results

Virus infection

The inoculation of bean plants with SBMV did not trigger a hypersensitive response as the infection was systemic. The V plants developed a systemic chlorotic mosaic, whereas a more general spread of chlorosis was observed in leaves of V+Pg plants. In all cases, symptoms were already evident in the first trifoliolate leaf. Electron microscopic observations of sap from all V plants revealed abundant isometric virus particles of 26 ± 2 nm on diameter. Exogenously applications of SA or exposure to Pg did not affect the infectivity of the virus as indicated by the appearance of symptoms in 100% of beans used as test plants.

Plant growth

In the absence of exogenously applied SA and without Pg, infection by SBMV reduced by 55%, 37% and 38% the aerial, root and nodule masses, respectively compared to values in H plants (Figure 1(A), (B) and (C)). Increasing concentrations of SA caused a progressive decrease in growth and nodulation of both H and V plants. The exception was the 5 μM SA concentration that promoted the aerial mass growth and nodulation of V plants compared to V plants in the absence of SA.

Exposure to Pg increased the aerial, root and nodule mass of H plants at all SA concentrations (Figure 1(A), (B) and (C)). In contrast, exposure of V+Pg plants caused a drastic reduction in growth and nodulation when compared to V plants without Pg. At SA concentration $\geq$500 μM, the growth and nodulation of V+Pg plants resembled that of H plants without Pg. At this extremely high SA concentration, only H+Pg plants displayed aerial masses close to 1 g dry wt/pl.

Chlorophyll concentrations

Patterns of chlorophyll concentrations in the leaves matched to some extent aerial plant growth (Figure 2). Within each SA concentration, lowest and highest

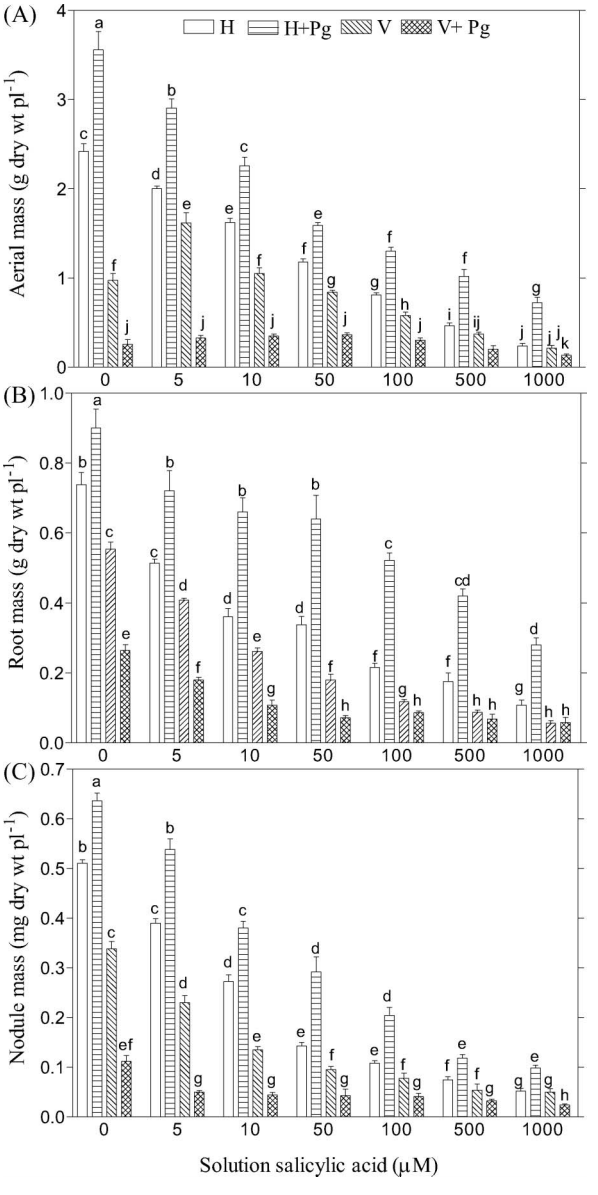

Figure 1. Aerial, root and nodule mass measured 30 days after emergence in healthy (H) and southern bean mosaic virus-infected (V) plants grown in increasing concentrations of salicylic acid in the solution culture and sprayed with 2 mM *n*-propyl gallate (+Pg). Results are the statistical mean of six replicates ± standard error of the mean. Bars with similar letters are statistically similar at $p \leq 0.05$.

chlorophyll concentrations were detected in V+Pg and H+Pg plants, respectively. Concentrations of SA $\geq$ 10 μM progressively decreased the chlorophyll concentrations of H plants. Higher chlorophyll concentrations were detected, however, in V plants that were treated with 5 and 10 μM SA compared to V plants grown in the absence of SA. Concentrations of SA $\geq$ 5 and $\leq$100 μM did not affect the

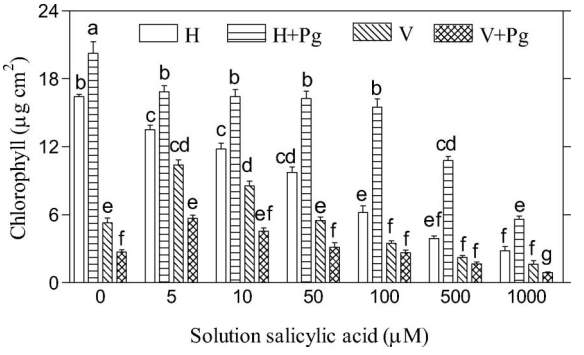

Figure 2. Chlorophyll concentration measured 30 days after emergence in mature leaves of healthy (H) and southern bean mosaic virus-infected (V) plants grown in increasing concentrations of salicylic acid in the solution culture and sprayed with 2 mM *n*-propyl gallate (+Pg). Results are the statistical mean of six replicates ± standard error of the mean. Bars with similar letters are statistically similar at $p \leq 0.05$.

chlorophyll content of H+Pg plants. Exposure to SA, Pg or infection by the SBMV did not alter the chlorophyll a/b ratio (data not shown).

Ureide concentrations in leaves and nodules

In the absence of exogenous SA, the ureide concentration in leaves of H+Pg, V and V+Pg plants was 43%, 66% and 86%, respectively lower than in H controls (Figure 3(A)). Increasing SA concentrations decreased the ureide concentrations in leaves of all H plants, the concentrations being always lower in H+Pg plants. The leaf ureide concentration in V plants was reduced to an average of 4 μmol/g dry wt. regardless of the SA concentration at which plants were grown and if sprayed or not with Pg. In contrast, the ureide concentration in nodules expressed on dry wt basis was not affected by increasing SA concentrations, virus infection or Pg treatments (Figure 3(B)).

Ultrastructural observations

In H plants growing in the absence of exogenously supplied SA, the ultrastructure of cells appeared normal without any alteration in cell organelles (Figure 4(A)). All chloroplasts were elongated, contained few starch grains and were located along the plasmalemma. Numerous mitochondria with well developed cristae were also detected in the cytoplasm. Up to four single plasmodesmata per cell showing continuity with the endoplasmic reticulum were observed in the mesophyll. Exposure of H plants to SA concentrations ≥5 and ≤100 μM did not induce detectable changes in the cell ultrastructure (image not shown). In contrast, severe alterations were detected in the cell ultrastruture of H plants grown at SA concentrations ≥500 μM. Chloroplasts showed disruption of the outer envelop, a proliferation of plastiglobuli and highly stacked grana formed by more than 10 densely arrayed thylakoids (Figure 4(B)). Concomitantly, the stromal space was hardly visible and chloroplasts were either free of or almost totally filled by large starch grains. Mitochondria were also severely distorted (image not shown).

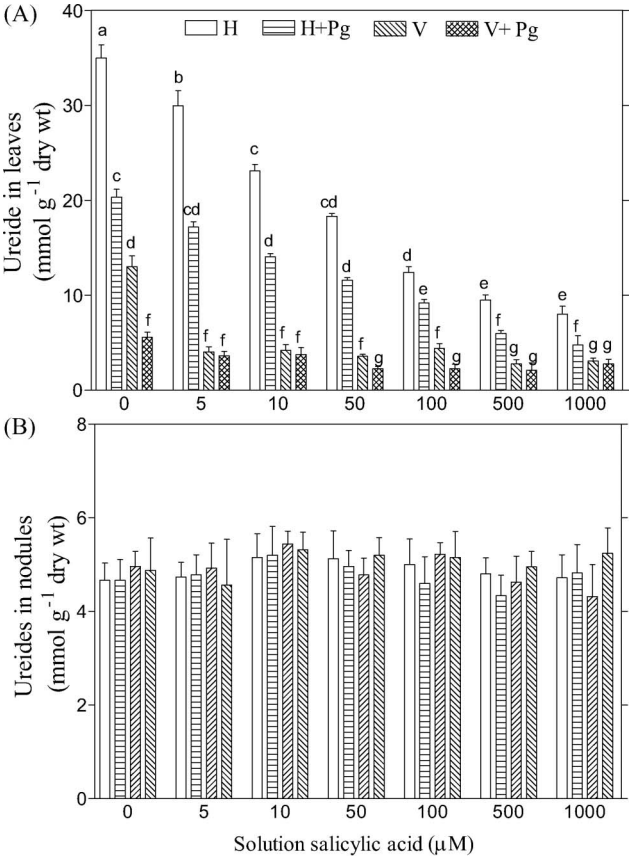

Figure 3. Ureide concentrations measured 30 days after emergence in leaves and nodules from healthy (H) and southern bean mosaic virus-infected (V) plants grown in increasing concentrations of salicylic acid in the solution culture and sprayed with 2 mM *n*-propyl gallate (+Pg). Results are the statistical mean of six replicates ± standard error of the mean. Bars with similar letters are statistically similar at $p \leq 0.05$.

Infection by SBMV of plants grown in the absence of exogenously supplied SA or supplied with $\leq$100 μM SA caused the swelling of chloroplasts, damage to the outer envelops and the distortion of grana likely due to the massive accumulation of starch grains of different sizes (Figure 4(C)). Ultrastructural observations also revealed a reduction in the number of chloroplasts per cell, with many chloroplasts found free in the cytoplasm and containing one–two vesicles. Exogenous applications of $\leq$100 μM SA concentrations did not reverse the deleterious effects of SBMV on cell ultrastructure. At SA concentrations $\geq$500 μM, chloroplasts in V plants were found disintegrated due to the fragmentation of both outer envelopes, and grana lost their arrangement with very long non-appresed membranes surrounding the starch grains (Figure 4(D)). Applications of SA and virus infection did not change the frequency of plasmodesmata in the cell walls of adjacent mesophyll cells (image not shown). It was interesting to observe cells with a normal ultrastructure in the green areas of leaves of V plants supplied with increasing SA concentrations.

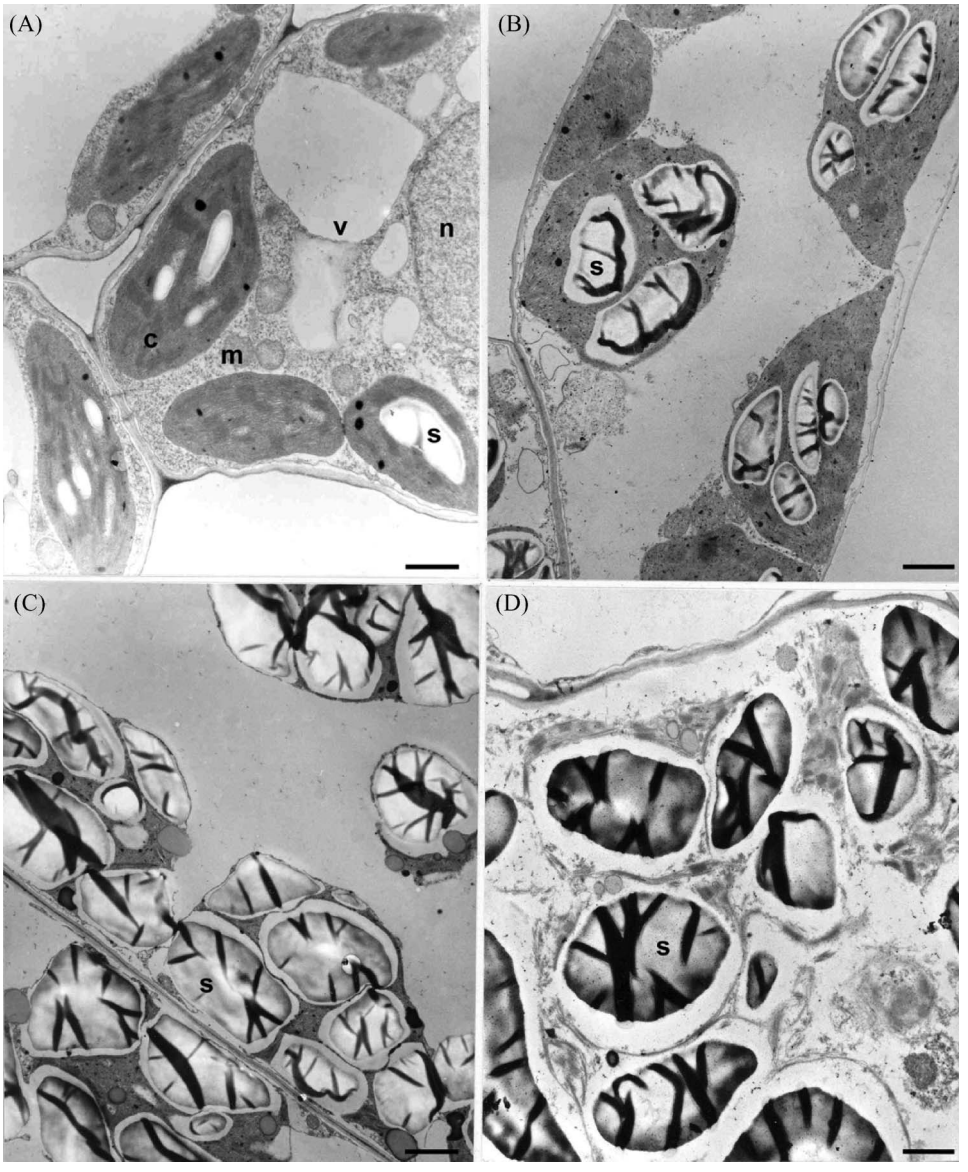

Figure 4. Electron micrograph showing the ultrastructure of mesophyll cells in (A) healthy plants grown in the absence of salicylic acid; (B) healthy plants grown in 1000 μM solution salicylic acid concentration; (C) southern bean mosaic virus-infected plants grown in the absence of salicylic acid; (D) southern bean mosaic virus-infected plants grown in 1000 μM solution salicylic acid concentration. Abbreviations in alphabetical order: c, chloroplasts; m, mitochondria; s, starch grain; v, vacuole. Scale bars: (A) = 909 nm; (B) and (D) = 11 nm; (C) = 142 nm.

Exposure of H plants growing in absence of SA or in SA concentrations $\leq$100 μM to Pg did not induce ultrastructural changes in organelles, and cells resembled those in Figure 4(A). The ultrastructure of H+Pg plants grown in SA concentrations $\geq$500 μM was similar, in turn, to those in Figure 4(B). In contrast,

the exposure of V plants to Pg elicited in chloroplasts the dilation of thylakoid membranes and the appearance of a large number of vesicles surrounded by a single membrane (Figure 5(A)). Proliferation of the endoplasmic reticulum and of mitochondria with deformed cristae was also observed (Figure 5(B)).

Ultrastucture alterations in V+Pg plants occurred at all SA concentrations tested in this investigation. Concomitantly, ultrastructural observations of vascular-parenchyma cells of minor veins in V+Pg plants revealed the de-condensation of the euchromatin within the nucleus of vascular-parenchyma cells (Figure 6(A)) and the formation of a large number of highly branched plasmodesmata in the cell walls (Figure 6(B)). Plasmodesmata formed large aggregates mainly in thickenings of the bundle sheath-vascular-parenchyma cell walls. Plasmodesmata were composed of a visible outer limiting membrane and an inner core desmotubule. Connections between endoplasmic reticulum and desmotubules were clearly observed. Virus

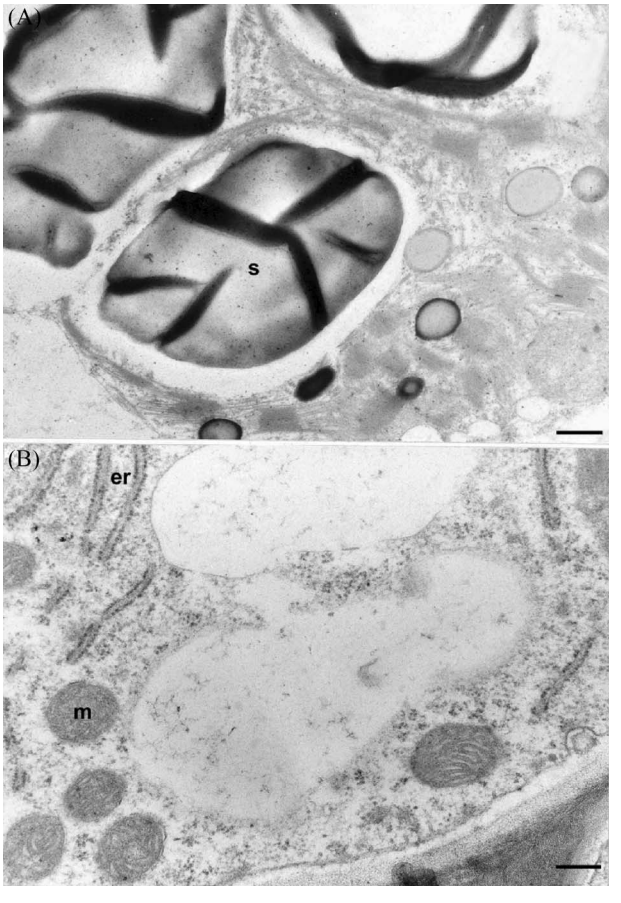

Figure 5. Electron micrograph showing the ultrastructure of mesophyll cells in southern bean mosaic virus-infected plants plants grown in 1000 μM solution salicylic acid concentration. (A) Distorted chloroplast with abundant vesicles; (B) proliferation of mitochondria with distorted crestaes and endoplasmic reticulum. Abbreviations in alphabetical order: er, endoplasmic reticulum; m, mitochondria; s, starch grain. Scale bars: (A) = 476 nm; (B) = 270 nm.

particles or virus crystalline arrays (Weintraub and Ragetli 1970) were not detected anywhere in the virus infected leaf tissues in spite of extensive search by electron microscope. Detailed electron microscopic observations of leaf tissues of V+Pg plants did not reveal the presence of cells with normal ultrastructure.

Nodules formed in V plants grown in the absence of SA or in SA concentrations $\leq$100 μM were characterised by the presence of 1–15 rod-shaped bacteroids per symbiosome (Figure 7(A)). All bacteroids contained large amounts of poly-β-hydroxybutyrate granules, the peribacteroidal space was large and symbiosomes were surrounded by an intact peribacteroidal membrane. In nodules from H plants grown in SA concentrations $\geq$500 μM, the bacterial division was constrained and the densely packed bacteroids were found free in the cytoplasm as result of the convolutions and disruptions of the peribacteroidal membrane (Figure 7(B)). A large number of small vesicles were also found scattered in the cytoplasm. The ultrastructure of nodules harvested from V plants grown in the absence of SA or in SA concentrations $\geq$500 μM resembled that in Figure 7(A) and Figure 7(B),

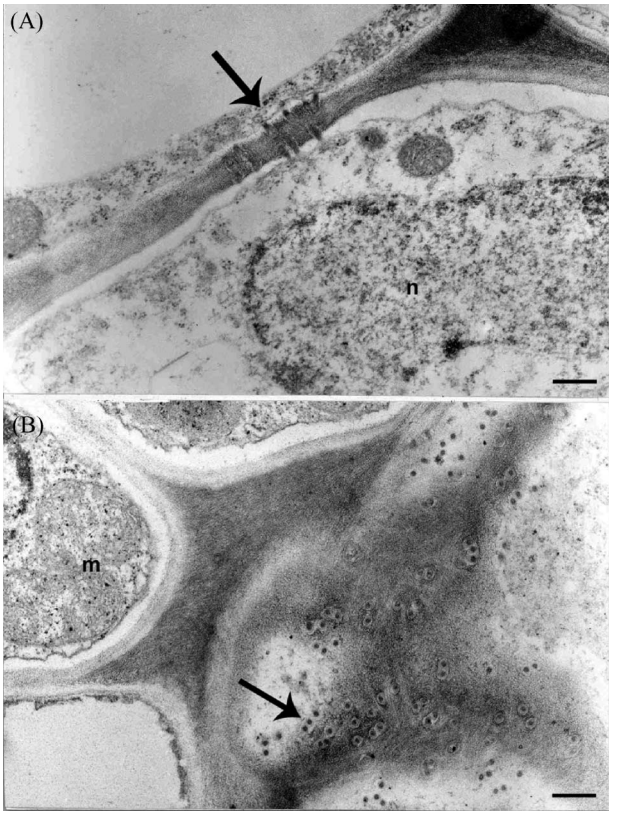

Figure 6. Electron micrograph showing the ultrastructure of leaf vascular-parenchyma cells of minor veins in southern bean mosaic virus-infected leaves of plants sprayed with propyl gallate. (A) Nucleous with dispersed heterochromatin and longitudinal sections of branched plasmodesmata in the cell wall (arrow) showing a continuity between the endoplasmic reticulum and the desmotubules; (B) transverse sections of plasmodesmata (arrow). Abbreviations in alphabetical order: m, mitochondria; n, nucleous. Scale bars: (A) and (B) = 357 nm.

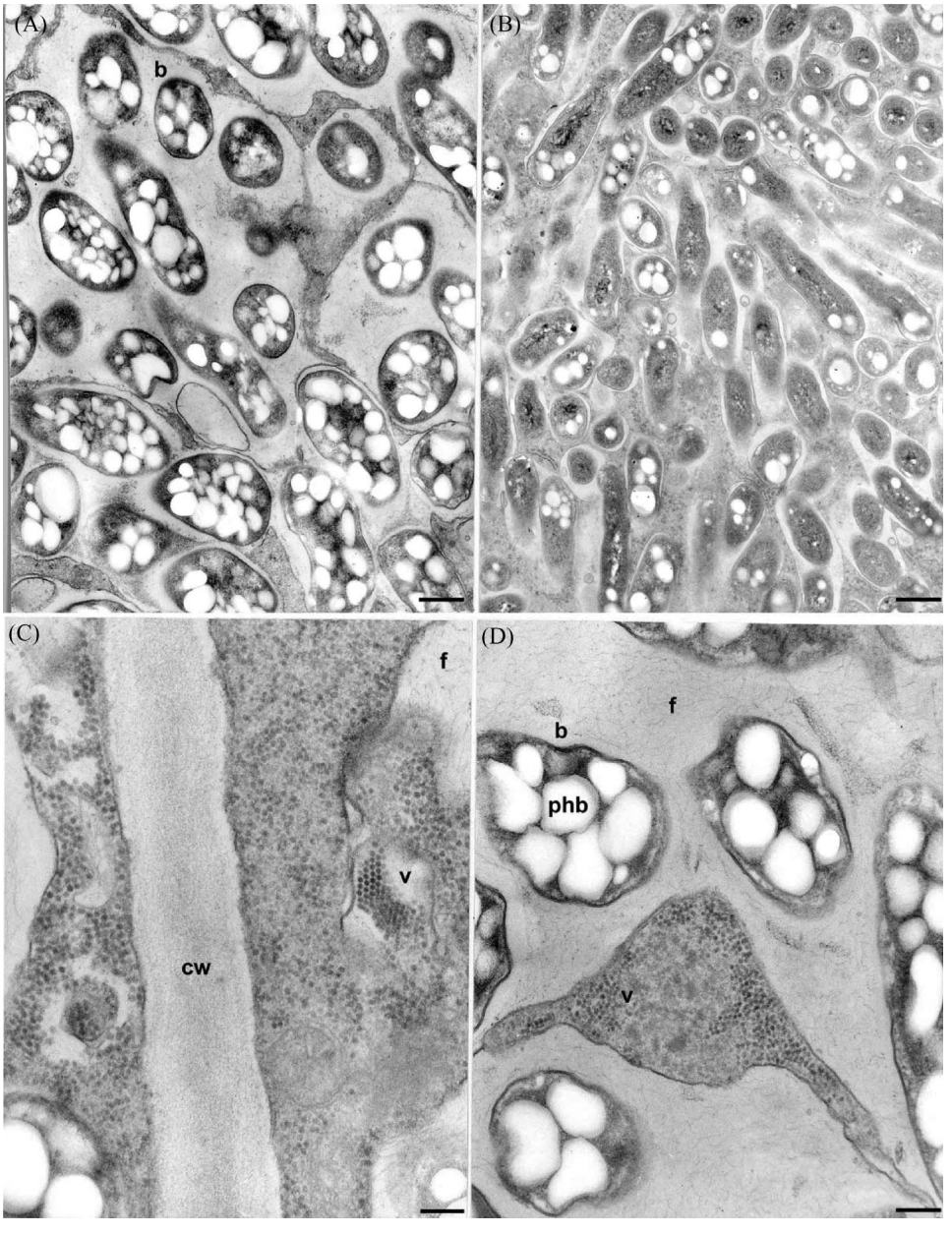

Figure 7. Electron micrograph showing the ultrastructure of nodule tissues in (A) healthy plants grown in the absence of salicylic acid; (B) healthy plants grown in 1000 μM solution salicylic acid concentration; (C) southern bean mosaic virus-infected plants grown in the absence of salicylic acid; (D) southern bean mosaic virus-infected plants grown in 1000 μM salicylic acid concentration. Abbreviations in alphabetical order: b, bacteroid; cw, cell wall; f, fiber-like structures; phb, polyhydroxybutirate granules; v, virus particles. Scale bars: (A) = 400 nm; (B) = 667 nm; (C) = 213 nm; (D) = 158 nm.

respectively. Detailed observations did not reveal virus particles within nodule tissues.

Major ultrastructural alterations were observed in nodule tissues from V+Pg plants (Figure 7(C) and (D)). The ultrastructure of the symbiosomes was totally disrupted including the lysis of bacteroids, and bacteroids were surrounded by fiber like structures. The most important observation was the presence of massive agglomerations of virus particles of the size and shape of SBMV, sometimes arranged in a cubic close packed array in the cytoplasm. Virus crystalline arrays were always located within a vacuole bounded by a monolayer membrane. In the cytoplasm the virus particles were sometimes observed freely dispersed or enclosed within a loose membrane.

Discussion

Activation of the oxidative metabolism in the V plants by exogenous application of 5 μM SA was evidenced by the marked amelioration of symptoms. The V plants exposed to 5 μM SA displayed higher chlorophyll and lower ureide concentrations in leaves as well as improved growth and nodulation when compared to H plants grown in the absence of SA. Based on previous reports, the SA-dependent defence mechanism in beans against SBMV might be mediated via expression of the phenylalanine ammonia lyase, several defence-related genes (Gális et al. 2004) and of chloroplast-targeted lipoxygenase (Porta et al. 2008).

The direct relationship existing between chlorophyll concentrations and the virus content in leaf cells (Arias et al. 2005) suggest a decline in the rate of virus replication as result of SA application. However, applications of SA did not halt the mobilisation of virus particles toward sink organs since all new leaves of all V plants had symptoms, and did not reverse the deleterious effects of SBMV on cell ultrastructure. It is of interest that applications of SA confined the virus-induced chlorosis to well defined areas in the leaves, but did not limit the infection to necrotic lesions. The overall better performance of V plants supplied with 5 μM SA was attributed to the presence of cells with a normal ultrastucture in the leaf green areas in contrast with the severe ultrastructural damaged chloroplasts and mitochondria in cells from chlorotic areas. The induction of defence responses by SA points toward a limited systemic resistance to virus in bean plants, and will explain the great susceptibility of this species to a large number of viruses (Blair and Morales 2008).

The susceptibility of bean plants to virus was further emphasised by the capability of Pg to counteract the defences elicited by exogenously applied SA (Macri et al. 1994; Xie and Chen 2000). The reduced growth, nodulation, chlorophyll and leaf ureide concentration as well as the severe ultrastructural damages in leaf and nodule tissues confirm the exacerbation of virus harmful effects by exposure of plants to Pg. These observations indicate that in this species the ratio between oxidant/antioxidant reactions is balanced toward detoxifying mechanisms, making plants highly susceptible to viruses, and ruled out the possibility of a free radical mediated photoinhibition as suggested in *Vicia faba* infected by the bean yellow mosaic virus (Radwan et al. 2008).

The larger deleterious effects of the SMBV in V+Pg plants was associated with the formation of highly branched plasmodesmata in the walls of vascular-parenchyma cells that facilitated the symplastic source to sink movement of virus particles. This high frequency of plasmodesmata explains the massive agglomeration

of virus particles in nodules which are the strongest sinks in legumes at their vegetative stages of growth (Izaguirre-Mayoral et al. 1994). Furthermore, the proliferation of plasmodesmata and the magnitude of symptoms in leaves of V+Pg plants support the suggestion that systemic infection of SBMV is mainly tubule-guided (Ritzenthaler and Hofmann 2008).

When the present results are interpreted in terms of the effect of SA upon nodulation and symbiotic performance of H and V plants, the derived conclusions are opposite to those reached in terms of virus infection. The enhanced catabolism of ureides in leaves and better growth of H+Pg plants suggest the presence of an active oxidative metabolism in the leaves resulting in a limited cell functioning. Application of Pg is known to counteract the deleterious effects of free radicals on chlorophyll (Adachi and Shimokowa 1995), chloroplastic enzymes (Ishida et al. 2002; Amane and Tadahiko 2006; Nakano et al. 2006; Quiles 2006) and on the D1 core protein of PSII (Sopory et al. 1990; Georgakopoulos and Argyroudi-Akayunoglou 1998). Exposure to Pg might also favor nodulation by counteracting the free radical induced activation of superoxide dismutases and the synthesis of jasmonic acid (Maksymiec and Krupa 2006) and of ethylene (Krens et al. 1994), limiting exacerbation of the oxidative metabolism in the mitochondria (Hoefnagel et al. 1995) in nodulating plants. There is also a possibility that application of Pg might have delayed the senescence of nodules by protecting cells from oxidative molecules. On the other hand, the progressive reduction in growth of H plants with increasing solution SA concentrations is the result of hampered symbiotic performance due to ultrastuctural alterations in nodule tissues. The SA-induced reduction in nodule mass was due to a decrease in nodule size and not in nodule number per plant (data not shown) in contrast to previous results in soybeans (Sato et al. 2002). The presence of a very large number of rhizobial cells in the commercial inoculum overrules the possibility that hampered nodulation is due to SA inhibition of bacterial growth (Stacey et al. 2006).

Under the present experimental conditions SA concentrations $\geq$10 μM proved to be toxic for the plants, and the benefits of SA on V plants did not compensate for the reduction in growth. This high sensitivity to SA might be an intrinsic condition of *P. vulgaris* var. Tacarigua, since SA concentrations, at least up to 100 μM, do not alter photosynthesis, stomatal conductance, growth, water use efficiency and the chlorophyll content in a large number of virus-infected plant species, including beans (Clarke et al. 2002; Radwan et al. 2008). It must be noted that those previous investigations were all carried out in plants subjected to SA for short period of times. Nevertheless, the existence of *P. vulgaris* hybrids with altered dosage-dependent gene system related to SA accumulation (Hannah et al. 2007), the large number of virus infecting beans and the marked genotypic differences among cultivars do not allow the extrapolation of present data to other virus-*Rhizobium*-bean varieties combinations. The observation that any attempt to alleviate the severity of the virus infection via genetic manipulations of the SA-dependent defence mechanism will negatively affect the symbiotic nitrogen fixation reveals the complexity to be addressed in breeding for virus resistance in *Rhizobium*-nodulated beans.

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