Effects of Exogenous Application of Methyl Jasmonate and Salicylic Acid on the Physiological and Molecular Response of 'Dusa' Avocado to *Rosellinia necatrix*

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Abstract

Methyl jasmonate (MeJA) and salicylic acid (SA) are important in mediating plant responses to abiotic and biotic stresses. MeJA and SA can act as elicitors by triggering plant defense responses similar to those induced by pathogens and may even provide long-term protection against them. Thus, exogenous application of MeJA and SA could protect susceptible avocado plants against white root rot (WRR) disease caused by the necrotrophic fungus *Rosellinia necatrix*, one of the main diseases affecting avocado orchards. This work evaluates the effects of MeJA or SA on the physiological and molecular response of susceptible 'Dusa' avocado rootstock and their ability to provide some protection against WRR. The application of MeJA and SA in avocado increased photoprotective mechanisms (nonphotochemical chlorophyll fluorescence quenching) and upregulated the *glutathione S-transferase*, suggesting the triggering of mechanisms closely related to oxidative stress relief and reactive oxygen species scavenging. In contrast to SA, MeJA's effects were more pronounced at the morphoanatomical level, including functional traits such as high leaf mass area, high stomatal density, and high root/shoot ratio, closely related to strategies to cope with water scarcity and WRR disease. Moreover, MeJA upregulated a greater number of defense-related genes than SA, including a *glu protease inhibitor*, a key gene in avocado defense against *R. necatrix*. The overall effects of MeJA increased 'Dusa' avocado tolerance to *R. necatrix* by inducing a primed state that delayed WRR disease symptoms. These findings point toward the use of MeJA application as an environmentally friendly strategy to mitigate the impact of this disease on susceptible avocado orchards.

Keywords: elicitors, gene expression, methyl jasmonate, morpho-physiological response, priming, salicylic acid, white root rot disease

Pests and diseases are among the main threats affecting crop productivity worldwide. Until now, pesticides have played a key role in overcoming sanitary problems in agricultural crops, but no effective pesticides are available against some pathogens. Moreover, the overuse of pesticides has negative economic, ecological, and environmental effects (Awang et al. 2013; Pimentel and Burgess 2014; Rani et al. 2021) and also triggers the development of resistance mechanisms in pathogens (ten Hoopen and Krauss 2006). These facts highlight the importance of finding alternatives to minimize or avoid the spraying of toxic products in the environment for controlling pest and disease incidence in field crops.

In this context, elicitors are emerging as a possible alternative (Awang et al. 2013). Elicitors are molecules and compounds such as carbohydrates, proteins, peptides, lipids, glycoproteins, plant hormones, and so on that can induce physiological changes or stimulate certain defense mechanisms in plants (Baenas et al. 2014; Jamiołkowska 2020). According to Zheng et al. (2020), the use of elicitors in crops is safe, nontoxic, and environmentally friendly, and small amounts are enough to provide the plants with long-term protection against a wide range of pathogens. Methyl jasmonate (MeJA), a derivative from jasmonic acid (JA), and salicylic acid (SA) are the most widely used elicitors for disease control (Awang et al. 2013; Thakur and Sohal 2013). To date, numerous studies have shown that exogenous application of MeJA and SA increases tolerance to pathogens by inducing plant defense-related genes (Awang et al. 2013; Cervantes-Landaverde et al. 2009; Laredo Alcalá et al. 2017), and by triggering several signaling pathways, for example, ion flux and the synthesis of signal molecules resulting in the reinforcement of cell walls, accumulation of antimicrobial compounds and synthesis of proteins that inhibit pathogen growth and proliferation (Moreno-Pérez et al. 2020; Thakur and Sohal 2013; van den Berg et al. 2018; Yu et al. 2018).

Commonly, SA is related to the activation of defense response against biotrophic and hemibiotrophic pathogens (Ali et al. 2018; Verma et al. 2016) by inducing the expression of *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1* (*NPR1*) gene, which regulates salicylic acid signaling (Ali et al. 2018; Makandar et al. 2010). On the other hand, MeJA is involved in the defense against necrotrophic pathogens and herbivorous insects (Verma et al. 2016) through the induction of defense-related genes and pathogenesis-related proteins involved in oxidative stress, phytoalexin accumulation, cell wall strengthening, stomatal closure, and photosynthetic parameters (Hanaka et al. 2015; Oliveira et al. 2015). However, SA and MeJA can have different effects depending on plant species, concentration applied, and stress situation (Ahmad et al. 2016; Hanaka et al. 2015; Khan et al. 2015; Moreno-Pérez et al. 2020). Although both SA and JA can act synergistically (i.e., enhanced volatile emissions and antiherbivore functions in tea plants; Jiao et al. 2022) or antagonistically (i.e., expression of acidic and basic pathogenesis-related proteins in tobacco leaves; Niki et al. 1998), the latter option is usually the predominant one (Pieterse et al. 2012).

One emerging disease affecting avocado productivity in the Mediterranean region is WRR (Pliego et al. 2012), caused by the pathogenic fungus *Rosellinia necatrix*. This soilborne pathogen is a necrotrophic fungus that is spread through contact of infected and healthy roots. Affected avocado trees show rotten roots and are characterized by a yellowing of the leaves, which eventually wilt, ultimately resulting in the death of the tree. Invasion of avocado roots by *R. necatrix* usually occurs by the formation of mycelial aggregates over the root

surface, which penetrate the tissues among epidermal and cortical cells, collapsing the vascular system of the plant (Pliego et al. 2009).

Management of WRR disease is difficult owing to several traits of *R. necatrix*: resistance to drought, survival in acidic soils, deep penetration into the soil, capacity to colonize more than 170 plant species, and tolerance to various common fungicides (Khan 1955; Kulshrestha et al. 2014; López-Herrera and Zea-Bonilla 2007; Pérez-Jiménez 2006; Pliego et al. 2012). Nowadays, the main avenues explored to minimize or eradicate this pathogen in avocado orchards are (i) cultural practices, such as the use of noninfected plant material, removal and burning of infected trees (Mendoza Garcia et al. 2003), and induction of plant tolerance by stimulating a drought-priming state through irrigation management ("cross-factor priming"; Martínez-Ferri et al. 2019); (ii) physical control, such as soil solarization at regular intervals (López-Herrera 1998; López-Herrera et al. 1999); (iii) chemical application, such as fluazinam (López-Herrera and Zea-Bonilla 2007; Magagula et al. 2021); (iv) biological control through the use of biological control agents (Magagula et al. 2021; Ruano-Rosa et al. 2014); and (v) the use of WRR-tolerant rootstocks. The latter approach is becoming one of the most promising alternatives (Barceló-Muñoz et al. 2007); however, although a breeding program for selecting avocado rootstocks tolerant to *R. necatrix* has been underway at IFAPA-Málaga for the last 20 years, there are still no commercial tolerant rootstocks available to growers.

Molecular approaches for assessing the response of avocado to soilborne pathogens can be a useful tool in breeding and selection of tolerant rootstocks by aiding in the assignment of resistance, tolerance, and susceptibility to genotypes from germplasm banks. In this sense, molecular studies were carried out in the avocado/*R. necatrix* pathosystem, selecting potential genes that could play a role in avocado tolerance against this pathogen (i.e., *universal stress protein, PR4*, *PR5*, *glutathione S-transferase*, *NAC domain-containing protein, endochitinase* and *protease inhibitors*) (Moreno-Pérez et al. 2023; Zumaquero et al. 2019). Protease inhibitor (*trypsin inhibitor*, *glu protease inhibitor*) proteins are the ones most likely to be related to tolerance against *R. necatrix* (Martínez-Ferri et al. 2019; Moreno-Pérez et al. 2023; Zumaquero et al. 2019).

Given that no single method is fully efficient against this pathogen (Pliego et al. 2012), and that most avocado orchards are established using nontolerant rootstocks, the use of elicitors, able to induce defense pathways and genes involved in avocado tolerance against *R. necatrix*, is proposed as a very promising approach. Therefore, the objective of this work was to assess the effect of the exogenous application of MeJA and SA elicitors on the physiological and molecular response of avocado 'Dusa' plants susceptible to *R. necatrix* and to monitor the WRR disease progression after inoculation with the fungus.

Materials and Methods

Plant material and experimental design

To study the effect of elicitors MeJA and SA on avocado plants and to test whether they can be used to induce tolerance to *R. necatrix*, an elicitation experiment was carried out at the Institute of Agricultural Research and Training (IFAPA) (Málaga, southeastern Spain, 36°40'25"N, 04°30'11"W, elevation of 18 m). Eighty-three 4-year-old clonal 'Dusa' plants

propagated by Brokaw Nursery (Brokaw España S.L., Spain) using a modified Frolich method (Frolich and Platt 1972) were grown in 16-liter pots containing a sterilized mixture of organic substrate and sand supplemented with a slow-release fertilizer (Basacote Plus 6M, Compo Expert GmbH, Spain).

Plants of 'Dusa' avocado rootstock, susceptible to *R. necatrix*, were kept in a greenhouse under daylight illumination and semicontrolled conditions of air temperature (T) and RH. Photosynthetic photon flux density (PPFD), T, and RH conditions inside the greenhouse were continuously monitored by a quantum sensor (Apogee SQ-110, U.S.A.) and by a T/RH U23-001 HOBO Pro v2 logger (Onset Computer Corporation, U.S.A.). Maximal midday values of PPFD varied between 440 and 1,012 µmol m^{-2} s^{-1}, and daily T was allowed to fluctuate according to external weather conditions, but its variation range inside the greenhouse was maintained at 23 ± 6°C by an automatic cooling system. The RH values inside the greenhouse were always >36%. Plants were watered regularly to maintain soil moisture at 80 to 90% of soil water retention at field capacity (~0.4 vol/vol), which was monitored throughout the experiment on all plants with a moisture sensor (HH2 Moisture meter, Delta-T Devices, U.K.), previously calibrated for the substrate.

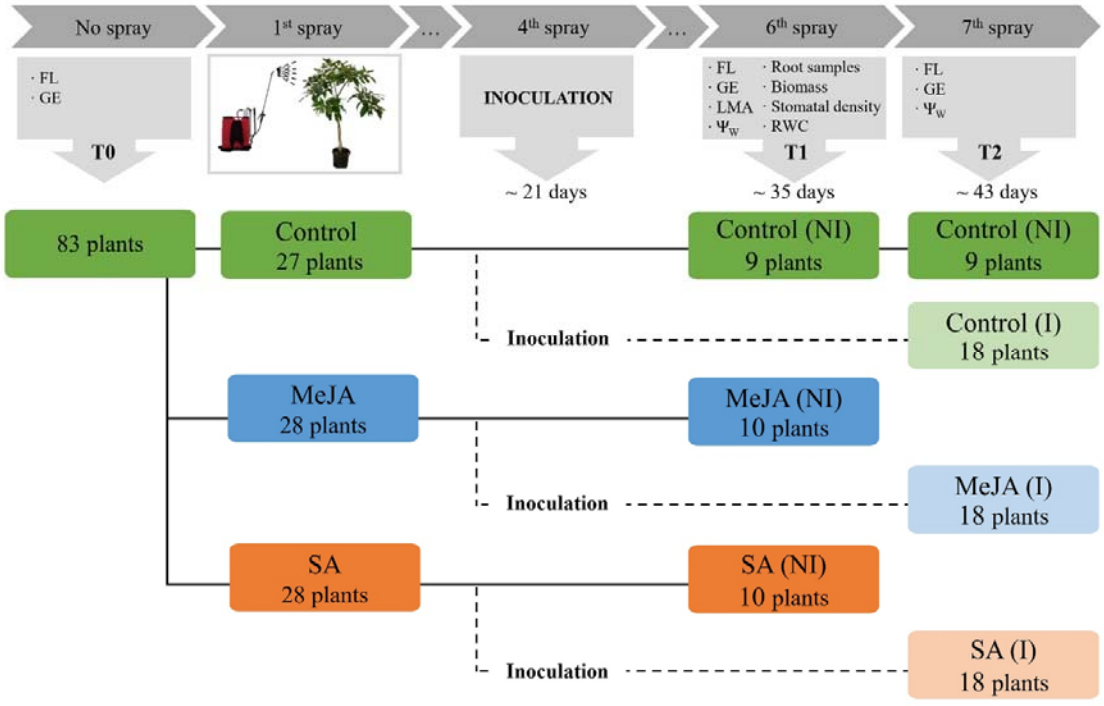

Fig. 1. Schematic illustration of the experimental design. Plants of 4-year-old 'Dusa' avocado rootstock were sprayed weekly with 5 mM methyl jasmonate (MeJA) or salicylic acid (SA) solutions. Before the first elicitor application and 12 h after the sixth and seventh applications, physiological measurements were taken (T0, T1, and T2, respectively) on noninoculated (NI) plants and inoculated (I) plants. FL = fluorescence; GE = gas exchange; LMA = leaf mass area; Ψ_w = leaf water potential; RWC = leaf relative water content.

The experimental design is depicted in Figure 1. At the beginning of the experiment (T0), the physiological status of all plants was tested nondestructively by measuring chlorophyll fluorescence at predawn and gas exchange. Plants were randomly distributed into three separate sets of 27, 28, and 28 plants and assigned to control, MeJA, and SA treatments, respectively. Plants were treated based on doses and application instructions previously set up in avocado by van den Berg et al. (2018); thus, elicited plants were sprayed until run-off with 5 mM of MeJA (Sigma-Aldrich, U.S.A.) or SA (Sigma-Aldrich, France) solutions for each treatment. Control plants were also sprayed with distilled water. Elicitors solutions were prepared following the manufacturer's instructions and supplemented with Tween 20 at 0.05%. Following three sprays, plants from each treatment were divided into two groups. Plants from the first batch (n = 9, 10, and 10 plants) were treated three more times and, after six sprays (T1), physiological measurements and root and biomass sampling were conducted. Plants of the second batch (n = 18, 18, and 18 plants) were inoculated as described below with a fungal strain of *R. necatrix* and sprayed four more times with the corresponding elicitor. After seven sprays (T2), physiological measurements were carried out on the inoculated plants. Following inoculation, the appearance of visible symptoms was monitored weekly to perform the pathogenicity test with *R. necatrix* as described below.

Physiological measurements

In vivo chlorophyll *a* fluorescence signals were measured with a portable fluorometer (PAM-2100, Heinz Walz, Germany) at predawn (T0) and midday (T0, T1, and T2) in one leaf per plant on 8 to 18 plants per treatment. Leaves were labeled for subsequent measurements throughout the experiment. The so-called saturation pulse method was used to determine all fluorescence parameters (Schreiber et al. 1995). Dark-adapted parameters (i.e., minimal fluorescence [F_0], maximal fluorescence [F_m], and maximal photochemical efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$] were determined at predawn (06:00 to 07:00 a.m.). The maximal fluorescence (F_m') and minimal fluorescence yield of a preilluminated sample (F_0') were assessed in light-acclimated leaves (~700 µmol quanta m^{-2} s^{-1}). The maximum photochemical efficiency of the open reaction centers of PSII ($F_v'/F_m' = [F_m' - F_0']/F_m'$) and the extent of "Stern-Volmer" nonphotochemical chlorophyll fluorescence quenching (NPQ = $[F_m - F_m']/[F_m']$) were calculated according to Baker et al. (2007).

Leaf gas exchange was measured at midday (11:00 a.m. to 2:00 p.m.) at T0, T1, and T2 in the same mature exposed leaves used for chlorophyll fluorescence determinations. Measurements were performed with an open portable photosynthesis system (model LI-6400, LI-COR, U.S.A.) equipped with an LED light source (6400-02B), coupled to a sensor head/IRGA, and with a CO_2 mixer (6400-01) to modify the incoming air's CO_2 concentrations. The operating flow rate was 500 ml min^{-1} and CO_2 partial pressure was 400 ppm. Settings for standardized measurements were leaf temperature ~20°C, relative humidity ~50% (vapor pressure deficit ~1.4 kPa), and saturating PPFD (1,000 µmol m^{-2} s^{-1}). Net CO_2 assimilation rates (A_N), stomatal conductance (g_s), intercellular CO_2 concentration (Ci), and transpiration rates (E) were estimated with the equations of von Caemmerer and Farquhar (1981). Intrinsic water use efficiency (A_N/g_s) and rubisco instantaneous carboxylation efficiency (A_N/Ci; Zhang et al. 2001) were also determined.

Leaf water potential was measured at midday (12:00 p.m. to 2:00 p.m.) in 8 to 10 plants of each treatment at both T1 and T2, using a Schölander-type pressure pump (model 3005; Soil Moisture Equipment Corporation, U.S.A.). Measurements were made in one mature, fully developed leaf per plant, close to the main stem. After cutting, leaves were immediately placed in the chamber following the recommendations made by Hsiao (1990).

Relative leaf water content (RWC), the specific leaf mass area (LMA), and relative chlorophyll content (SPAD index) were measured only at T1 on the same plants used for leaf water potential determinations. For RWC and LMA determinations, 2-cm^2 leaf discs were sampled at midday, weighed to obtain fresh weight (F_W), and immediately imbibed on distilled water for 24 h at 5°C in darkness for obtaining turgid weight (T_W). Afterward, samples were oven dried at 80°C for 48 h to obtain dry weight (D_W). RWC and LMA were calculated as follows:

$$\mathrm{RWC}\ (\%) = [(\mathrm{F_W} - \mathrm{D_W}) / (\mathrm{T_W} - \mathrm{D_W})] \times 100$$

$$\mathrm{LMA}\ \left(\mathrm{g\,cm^{-2}}\right) = \mathrm{D_W}/\mathrm{leaf\ disc\ area}$$

The SPAD index, which provides an estimation of leaf chlorophyll content (Uddling et al. 2007), was nondestructively measured at midday on one leaf per plant by a handheld SPAD 502 meter (Minolta, Japan). On each plant, averaged SPAD values were calculated from three readings per leaf taken along the midrib.

Stomatal density and biomass partitioning

Stomatal density (number of stomata per unit of area) was determined at T1 on healthy mature leaves from control and MeJA- and SA-treated plants (n = 8, n = 5, and n = 5, respectively). Leaf epidermal imprints were taken by a transparent adhesive tape after covering ~1 cm^2 of the abaxial leaf surface with clear nail polish, avoiding leaf ribbing, and allowing to dry for 5 min. Three leaf impressions were taken from each plant, and from each imprint, stomata of three fields of view (0.056 mm^2) were counted under an optical light microscope (Nikon ECLIPSE 50i, Tokyo, Japan) using an objective lens of 40× magnification and 10× eyepiece (Camargo and Marenco 2011; Gokbayrak et al. 2008; Peel et al. 2017).

After completing the physiological measurements at T1, three plants from each treatment were separated into leaves, stems, and roots. Dry weight of each fraction was measured after oven drying at 80°C for 48 h to estimate total plant dry weight and biomass partitioning variables, including root, stem, and leaf weight ratios (%) and the root/shoot ratio (root dry weight per shoot dry weight). Total plant leaf area (m^2) was estimated from leaf dry weight and LMA values.

RNA extraction

Roots from nine avocado plants from control, MeJA, and SA treatments were harvested after six sprays at T1. Three biological replicates were used for RNA extraction. The expression data are the mean of three biological replicates. Each replicate consisted of a bulk sample from three plants. RNA from ground root tissue was extracted using the CTAB extraction method (Chang et al. 1993), with slight modification. The chloroform:isoamyl alcohol step was

repeated three to five times, depending on the stability of the interphase and color of the sample. RNA quantity and quality were determined based on A260/280 and A260/230 wavelength ratios using a NanoDrop ND-1000 (Nanodrop Technologies, Inc., U.S.A.) spectrophotometer. RNA integrity was confirmed by the appearance of ribosomal RNA bands and lack of degradation products after separation on a 2% agarose gel and Red Safe staining. DNase treatment of RNA was performed by the addition of 1 U of RNase-free DNase (Thermo Scientific, Life Technologies Inc., U.S.A.), 1 µl of 10× reaction buffer with $MgCl_2$, 1 µg of RNA, 0.5 µl of RiboLock RNase Inhibitor (Thermo Scientific Inc., California, U.S.A.), and diethylpyrocarbonate-treated water to a final volume of 10 µl. The mixture was incubated at 37°C for 45 min followed by incubation at 65°C for 10 min.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Single-stranded cDNA was synthesized using iScript Reverse Transcription Supermix (Bio-Rad Laboratories Inc., U.S.A.) according to the manufacturer's instructions. The cDNA was analyzed for genomic DNA contamination by PCR using gene-specific primers actin-F (5'-GAATCTGGACCATCTATTG-3') and actin-R (5'-TACCAACCAAACCAAATC-3'), which flank an intron of the *actin* gene. PCR amplifications were carried out as previously described by Engelbrecht and van den Berg (2013) using first-strand cDNA as the template.

The expression of 11 avocado genes selected based on their implication in avocado defense response against fungal pathogens (Backer et al. 2015; van den Berg et al. 2018; Zumaquero et al. 2019) was investigated. The *actin* gene (Mahomed and van den Berg 2011) was used as an endogenous control for normalization. Primer sequences for the endogenous control gene and the 11 avocado genes are presented in Supplementary Table S1. Primer pairs were chosen to generate fragments between 70 and 150 bp and were designed using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/) (Koressaar and Remm 2007; Kõressaar et al. 2018; Untergasser et al. 2012). Primer specificity was tested by first performing a conventional PCR and confirmed by the presence of a single melting curve during RT-qPCR. Serial dilutions (1:10, 1:20, 1:50, and 1:200) were made from a pool of cDNA from each treatment, and time points and calibration curves were performed for each gene. For RT-qPCR, the reaction mixture consisted of cDNA first-strand template, primers (500 nmol final concentration), and SYBR Green Master Mix (SsoAdvanced Universal SYBR Green Supermix, Bio-Rad, U.S.A.) in a total volume of 20 µl. The PCR conditions were as follows: 30 s at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 60°C, 3 min at 72°C, and finally 1 min at 95°C. The reactions were performed using an iQ5 real-time PCR detection system (Bio-Rad). Relative quantification of the expression levels for the target genes were analyzed using the ΔΔCt method (Livak and Schmittgen 2001; Pfaffl 2001). All reactions were done in triplicate.

Disease development in avocado plants

To perform the pathogenicity test of the avocado plants subjected to each elicitation treatment, 12 h after the fourth elicitation, 18 'Dusa' rootstock from each treatment were inoculated with 3.75 g of *R. necatrix*–colonized wheat seeds per liter of substrate. The inoculum was prepared according to Sztejnberg and Madar (1980). Four 0.5-cm-diameter potato dextrose agar discs of a 2-week-old culture of *R. necatrix* were incubated, with 400 g of autoclave-sterilized wheat seeds in 1-liter glass bottles for 3 weeks at 24°C in the dark. To

ensure the spread of inoculum, it was placed at four scattered points around the stem (~3.5 cm apart) in each pot and introduced at two depths (~5 and ~15 cm, respectively). Disease progression was evaluated by measuring the aerial symptoms of WRR according to Martínez-Ferri et al. (2019) on a scale from 1 to 5: 1 = healthy plant; 2 = mild wilting; 3 = wilting; 4 = desiccated; and 5 = death. The disease index, which represents the cumulative percentage of plants with symptoms for each treatment, and the area under the disease progress curve (AUDPC) were calculated as previously described by Teixeira de Sousa (1985) and Campbell and Madden (1990), respectively.

Statistical analysis

Data were analyzed using the analytical software STATISTICA 7 (StatSoft, Inc., U.S.A.). For each sampling point, differences among treatments in physiological and biomass-related variables and AUDPC data were evaluated by analysis of variance (one-way ANOVA). RT-qPCR data were analyzed by a Student's *t*-test to compare the fold change (FC) of treated plants relative to control plants. Significant differences were considered at the 5% probability level unless otherwise stated. Prior to ANOVA, normality and homogeneity assumptions were tested by using the Kolmogorov-Smirnov and the Cochran's C test, respectively. When significant differences were found ($P < 0.05$), Tukey's honest significant difference (HSD) test was used to compare mean values.

Results

Physiological and molecular response of avocado 'Dusa' rootstock to MeJA and SA elicitors

To investigate the effect of exogenous application of MeJA and SA on the 'Dusa' rootstock, plants were divided into three batches: two were sprayed weekly with the elicitors, and one was used as a control (nonelicited). Physiological measurements were taken before (T0), to check that the plants were in similar physiological conditions, and after the sixth application of elicitor (T1) together with root sampling for molecular studies (Fig. 1).

At the beginning of the experiment (T0), no significant differences were found between plant groups in the photosynthesis-related parameters (i.e., photochemistry and gas exchange), showing values of maximal photochemical efficiency of photosystem II (PSII) (F_v/F_m) >0.8, indicating good plant health, and net CO_2 assimilation rates of approximately 8.07 ± 0.54 mmol CO_2 m^{-2} s^{-1}, which are within the normal range for avocado plants (Figs. 2 and 3).

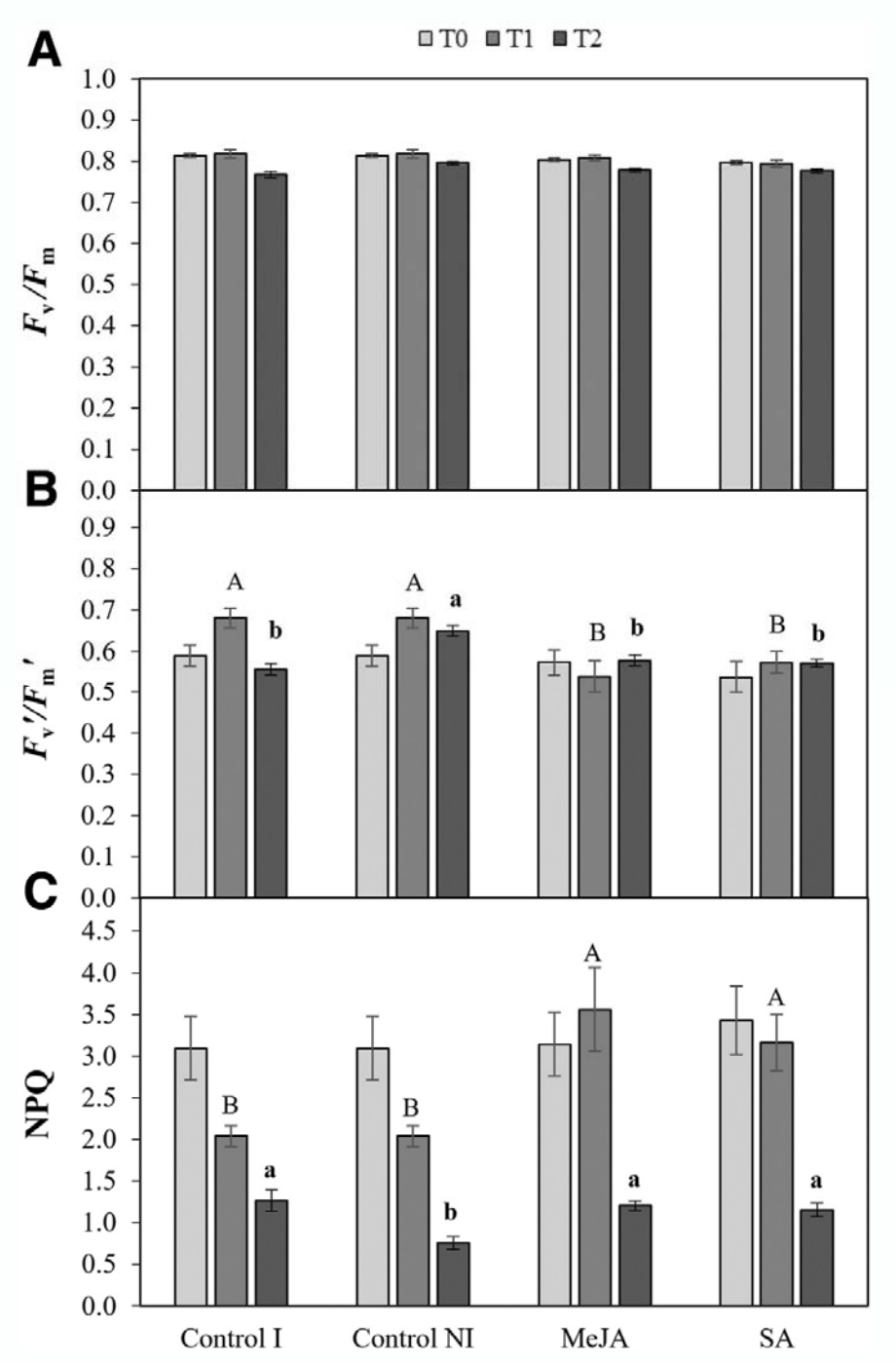

Fig. 2. Mean values (±SE) of maximal photochemical efficiency of PSII (F_v/F_m; **A**), maximum photochemical efficiency of the open reaction centers of PSII (F_v'/F_m'; **B**), and nonphotochemical chlorophyll fluorescence quenching (NPQ; **C**) measured at each time point—T0, T1, and T2—on inoculated (I) and noninoculated (NI) control plants of avocado 'Dusa' and in plants elicited with methyl jasmonate (MeJA) and salicylic acid (SA). Within each time point, different letters indicate significant differences among treatments ($P < 0.05$; one-way analysis of variance followed by HSD) (n = 11 to 13, n = 9 to 10, and n = 9 to 18 in T0, T1, and T2, respectively).

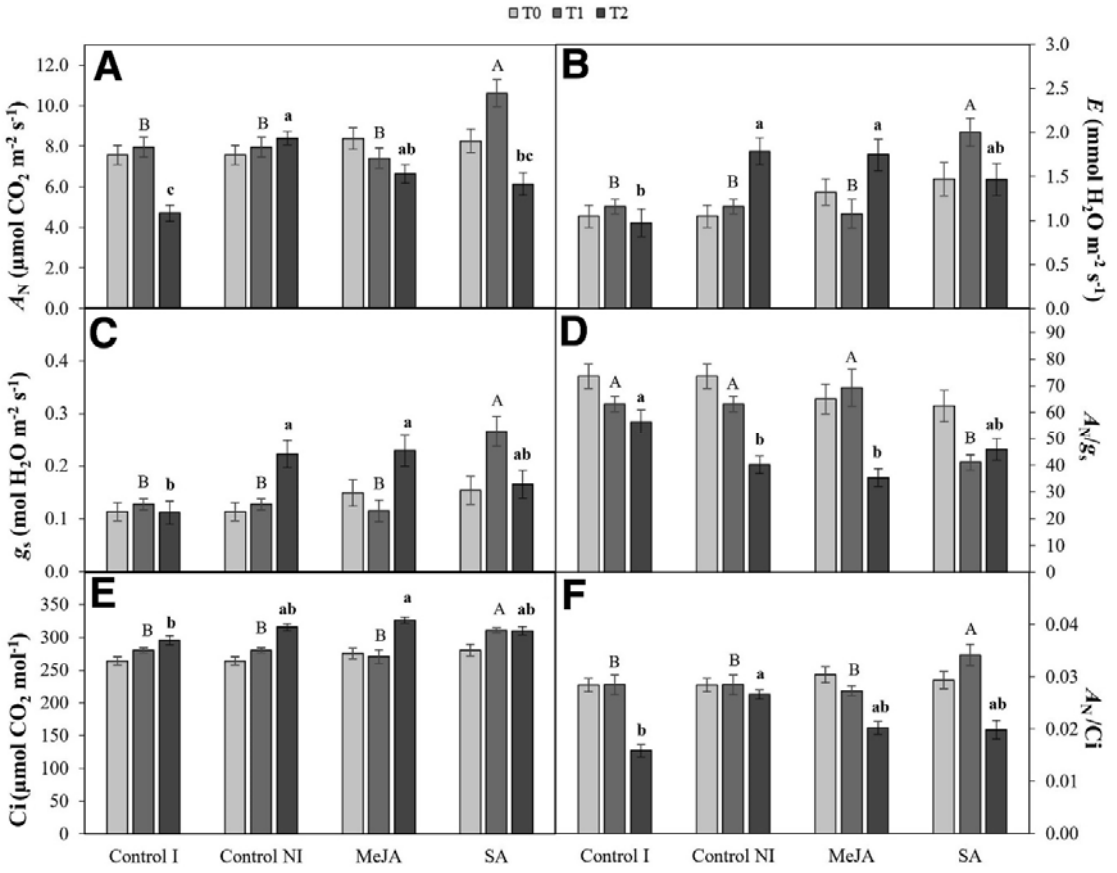

Fig. 3. Mean values (±SE) of net CO_2 assimilation rate (A_N; **A**), transpiration rate (E; **B**), stomatal conductance (g_s; **C**), intrinsic water use efficiency (A_N/g_s; **D**), intercellular CO_2 concentration (Ci; **E**), and rubisco instantaneous carboxylation efficiency (A_N/Ci; **F**) measured at each time point—T0, T1, and T2—on inoculated (I) and noninoculated (NI) control plants of avocado 'Dusa' and in noninoculated (T1) and inoculated (T2) plants elicited with methyl jasmonate (MeJA) and salicylic acid (SA). Within each time point, different letters indicate significant differences among treatments ($P < 0.05$; one-way analysis of variance followed by HSD) (n = 11 to 13, n = 9 to 10, and n = 9 to 18 in T0, T1, and T2, respectively).

After six sprays (T1), exogenous application of MeJA and SA resulted in a significant increase in the NPQ ($P < 0.05$; Fig. 2C) and a decrease in the maximum photochemical efficiency of the open reaction centers of PSII (F_v'/F_m') compared with control plants ($P < 0.05$; Fig. 2B), indicating the activation of energy-dissipating mechanisms closely related to the relief of oxidative stress (Martínez-Ferri et al. 2019).

Regarding gas exchange–related parameters, A_N, E, g_s, Ci, and A_N/Ci showed a marked and significant increase in SA-treated plants ($P < 0.05$; Fig. 3A to C, E, and F) in comparison with MeJA and control plants, which did not differ significantly. In SA-treated plants, the increase of A_N was relatively low compared with the degree of stomatal opening (g_s) resulting in lower intrinsic water use efficiency (A_N/g_s) ($P < 0.05$; Fig. 3D).

Table 1. Relative chlorophyll content (SPAD) index, leaf relative water content (RWC), stomatal density, leaf mass area (LMA), and plant dry biomass parameters in control, methyl jasmonate (MeJA), and salicylic acid (SA) elicited and noninoculated 'Dusa' plants (T1)[z]

Parameter	Control	MeJA	SA
SPAD index	50.00 ± 3.06	48.14 ± 2.69	44.88 ± 2.71
RWC (%)	93.55 ± 0.99 a	90.59 ± 0.54 ab	87.63 ± 1.47 b
Stomatal density (n° mm^{-2})	596.5 ± 56.5 b	819.9 ± 39.0 a	597.3 ± 45.0 b
LMA (g m^{-2})	65.48 ± 5.41 b	89.02 ± 3.13 a	70.86 ± 4.73 b
Plant dry weight (g)	1,014.2 ± 99.3	1,008.9 ± 140.9	978.0 ± 54.9
Leaves (%)	17.27 ± 1.48	16.27 ± 0.30	18.59 ± 0.76
Stem (%)	38.41 ± 1.21 a	33.92 ± 0.51 b	39.44 ± 0.07 a
Root (%)	44.32 ± 2.64 ab	49.82 ± 0.82 a	41.97 ± 0.83 b
Root/shoot ratio	0.80 ± 0.08 ab	0.99 ± 0.03 a	0.72 ± 0.02 b
Plant leaf area (m^2)	2.69 ± 0.39	1.86 ± 0.29	2.58 ± 0.25

[z] The table shows mean values (±SE; n = 3 to 10). Different letters indicate significant differences among treatments ($P < 0.05$; one-way analysis of variance followed by HSD).

Relative chlorophyll content (SPAD index) and leaf water potential in elicited plants did not differ significantly from control plants (Table 1 and Fig. 4), but SA-treated plants displayed significantly lower values of RWC than control and MeJA-treated plants ($P < 0.05$; Table 1). LMA and stomatal density differed among treatments (Fig. 5), with significantly higher values in leaves of MeJA-treated plants than in control and SA-treated plants ($P < 0.05$; Table 1).

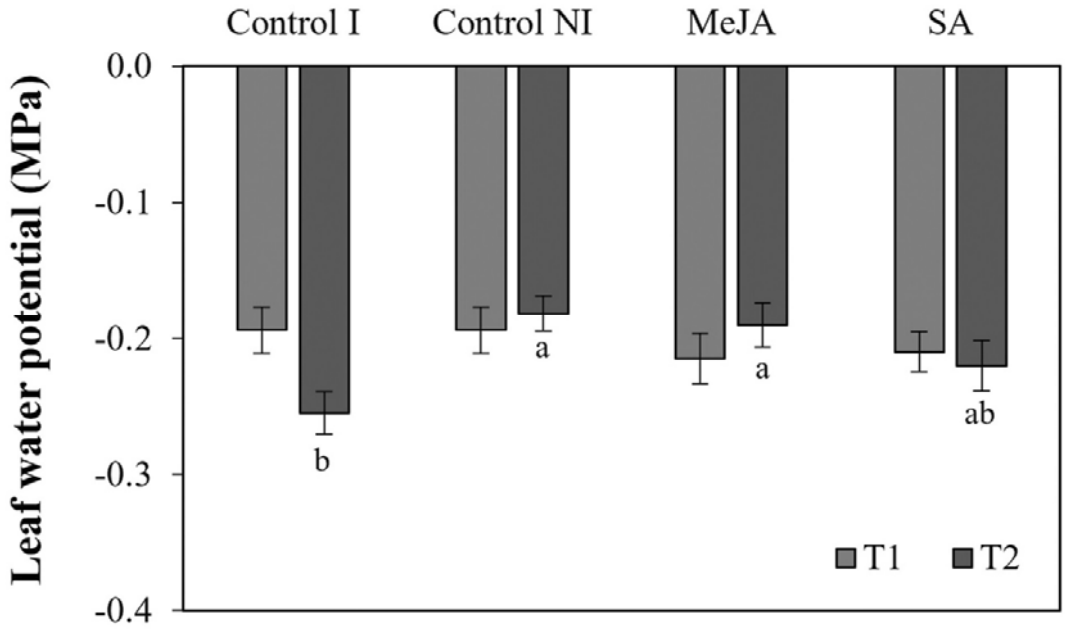

Fig. 4. Mean values (±SE) of variation in leaf water potential measured at each time point—T1 and T2 (n = 8 to 10 in T1 and T2)—in inoculated (I) and noninoculated (NI) control plants of avocado 'Dusa' and in noninoculated (T1) and inoculated (T2) plants elicited with methyl jasmonate (MeJA) and salicylic acid (SA) treatments. Within each time point, different letters indicate significant differences among treatments ($P < 0.05$; one-way analysis of variance followed by HSD).

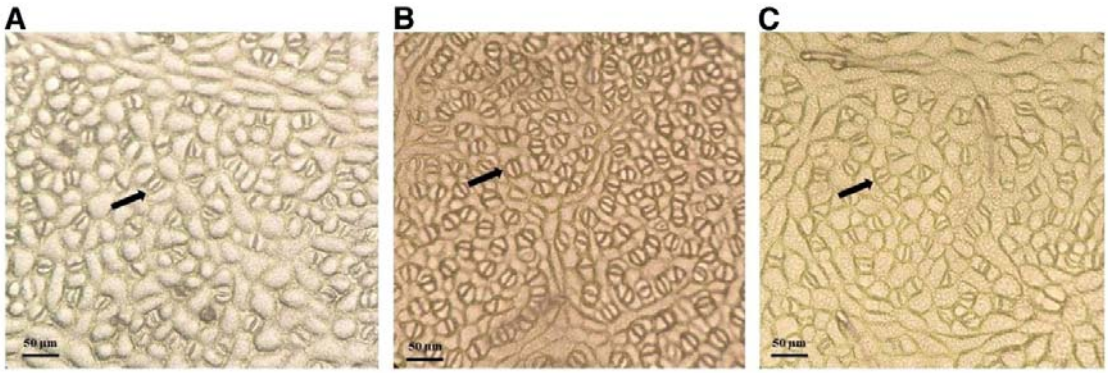

Fig. 5. Stomatal density from nail polish imprint images of the abaxial leaf surface of 'Dusa' avocado rootstock of nonelicited control plants (**A**) and after six sprays with methyl jasmonate (**B**) and salicylic acid (**C**) observed under a light microscope with 400× magnification. Scale bar = 50 µm. Arrows point to stomata.

Although most biomass-related variables did not differ significantly between control and elicited plants (Table 1), MeJA-treated plants showed a significantly higher percentage of root dry weight ($P < 0.05$) along with lower percentages of stem dry weight ($P < 0.05$), resulting in significantly higher root/shoot ratios ($P < 0.05$; Table 1). A trend toward lower total plant leaf area values was also observed in MeJA-treated plants, consistent with their higher LMA values.

Gene expression of 11 genes on roots of 'Dusa' avocado rootstock subjected to six MeJA and SA applications was analyzed by performing RT-qPCR. Genes were selected for their implication in avocado defense against soilborne pathogens (Backer et al. 2015; Moreno-Pérez et al. 2023; van den Berg et al. 2018; Zumaquero et al. 2019). Involvement of these genes in salt, oxidative, osmotic, and water stress responses was reported by Martínez-Ferri et al. (2019). The *actin* gene was used as an endogenous constitutive gene to normalize the expression results, and negative controls were used to confirm the absence of contamination. The relative quantification for the expression of the selected genes by the ΔΔCt method is shown in Table 2.

A trend toward higher expression values of defense-related genes was observed after application of MeJA relative to SA. Five genes (*kunitz trypsin inhibitor* [Contig02540], *endochitinase* [Pa_Contig00535], *glutathione S-transferase* [Pa_Contig00778], *PR4* [Pa_Contig07140], *PR5* [Pa_Contig01450]) were significantly induced after MeJA treatment, and three genes (*NPR1* [KR056089], *glutathione S-transferase* [Pa_Contig00778], and *universal stress protein* [Pa_Contig01245]) were significantly induced after SA application. The *glutathione S-transferase* (Pa_Contig00778) was the only gene induced by both MeJA and SA, displaying similar FC values after both elicitor treatments. Genes encoding the *endochitinase* (Pa_Contig00535) and the *NPR1* (KR056089) exhibited the highest expression values upon elicitation with MeJA and SA, respectively. Although no significant gene suppression was observed after MeJA treatment, SA elicitation suppressed three (*trypsin inhibitor* [Contig04097], *metallothionein-like protein* [Pa_Contig04910], and *NAC domain-containing protein 72* [Pa_Contig00313]) of the 11 defense-related genes.

Effects of MeJA and SA on the 'Dusa' avocado response to white root rot disease

To investigate the response of 'Dusa' avocado plants treated with MeJA and SA to WRR disease, a subset of plants from each treatment (i.e., control and MeJA- and SA-treated plants) was inoculated with wheat grains infected with *R. necatrix*. Physiological measurements were taken prior to the observation of any aboveground symptoms at 23 days postinoculation (dpi) (T2) on inoculated plants and on noninoculated control plants (Fig. 1). Disease progression was also assessed by monitoring the appearance of aerial visible symptoms.

At the photochemical level, and regardless of the elicitation treatment, all inoculated plants showed significantly higher values of NPQ than noninoculated control plants ($P < 0.05$; Fig. 2C), which were accompanied by significant lower F_v'/F_m' values (Fig. 2B), indicating the operation of energy-dissipating mechanisms.

Table 2. Gene expression analysis by real-time quantitative PCR assay of 11 selected genes in 'Dusa' plants treated with methyl jasmonate (MeJA) or salicylic acid (SA)[z]

Contig	Description	Function	FC MeJA vs. control	FC SA vs. control	References
Contig02540	*Kunitz trypsin inhibitor*	Protease inhibitor	1.65 ± 0.42	0.56 ± 0.36	Major and Constabel 2008
Contig04097	*Trypsin inhibitor*	Protease inhibitor	2.28 ± 0.90	**−2.04 ± 0.62**	Srinivasan et al. 2009
Contig05213	*Glu protease inhibitor*	Oxidative stress	**1.88 ± 0.05**	1.38 ± 0.48	Srinivasan and Kirti 2012
KR056089	*NPR1*	Pathogen response	−2.47 ± 1.62 b	**2.50 ± 1.07 a**	Backer et al. 2015
Pa_Contig00313	*NAC domain-containing protein 72*	Transcription factor	2.15 ± 0.94 a	**−1.28 ± 0.27 b**	Yuan et al. 2019
Pa_Contig00535	*Endochitinase*	Pathogen and salt stress response	**4.25 ± 0.39**	2.36 ± 0.61	Engelbrecht and van den Berg 2013
Pa_Contig00778	*Glutathione S-transferase*	Detoxification and oxidative stress	**1.63 ± 0.01**	**1.79 ± 0.21**	Gullner et al. 2018
Pa_Contig01245	*Universal stress protein*	Oxidative stress	3.06 ± 1.18	**2.27 ± 0.35**	Jung et al. 2015
Pa_Contig01450	*PR5*	Antifungal activity, salt and osmotic stress response	**3.37 ± 0.50**	2.51 ± 0.19	Zhang et al. 2018
Pa_Contig04910	*Metallothionein-like protein*	Reactive oxygen species scavenging and metal homeostasis	1.17 ± 0.11 a	**−1.22 ± 0.01 b**	Wong et al. 2004
Pa_Contig07140	*PR4*	Pathogen, salt, and water stress response	**3.01 ± 0.41**	1.48 ± 0.27	Ali et al. 2018

[z] Data are displayed as fold change (FC) calculated by comparing treatments with nontreated control plants. The expression data are the mean (±SE, $n = 9$) of three biological replicates with three technical replicates each. Bold indicates statistical differences to control plants (Student's t-test, $P < 0.05$), and letters indicate differences between MeJA and SA treatments ($P < 0.05$; one-way analysis of variance followed by HSD).

In contrast, at the gas exchange level, inoculated MeJA- and SA-elicited plants displayed similar values of E, g_s, and A_N/g_s (Fig. 3B to D) to those of noninoculated control plants. Although a significant decrease of A_N and A_N/Ci values was observed in all inoculated plants ($P < 0.05$; Fig. 3A and F) compared with noninoculated control plants, this decrease was less pronounced in MeJA- and SA-treated plants than in inoculated control plants, the latter displaying the lowest values of A_N, E, g_s, and Ci (Fig. 3A–C and E). The same was true for the leaf water potential ($P < 0.05$; Fig. 4).

Initial aboveground WRR symptoms appeared slightly earlier in control and SA-treated plants (48 and 49 dpi) than in MeJA-treated plants (56 dpi). Whereas at 69 dpi, 50% of control and SA-treated plants (nine plants) displayed wilting symptoms, only 10% of MeJA-treated plants (two plants) showed initial aboveground WRR symptoms. Five months postinoculation, all SA-treated and control plants were completely desiccated at stage 5 (dead), whereas 8% of MeJA-treated plants (four plants) remained alive. A significant reduction in the AUDPC values was observed in MeJA-treated plants ($P < 0.05$) (Fig. 6).

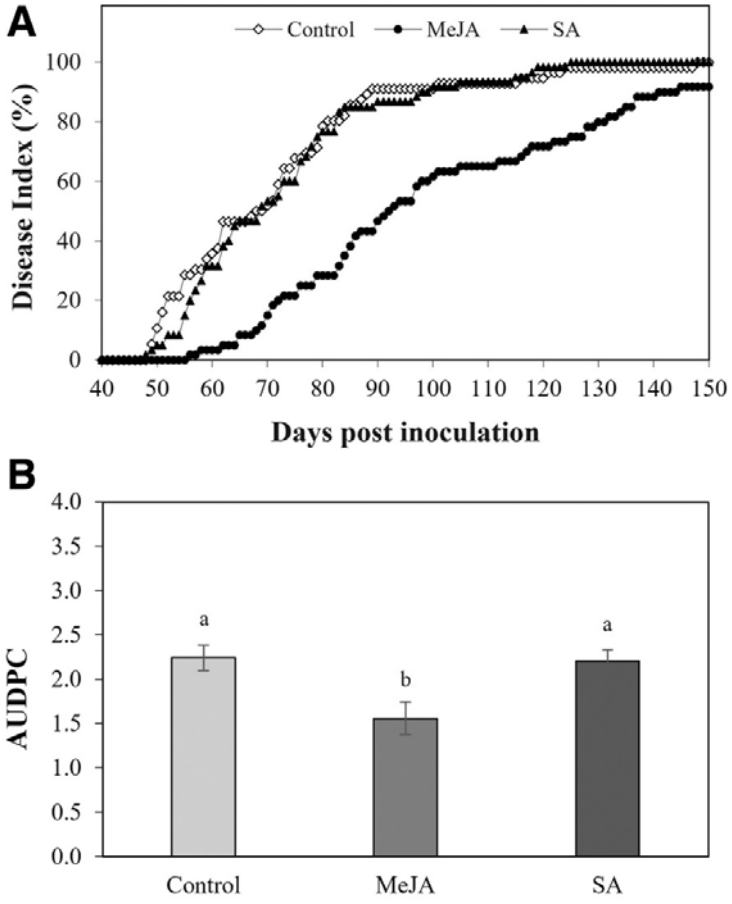

Fig. 6. Disease index calculated by evaluating aerial white root rot symptoms in control, methyl jasmonate (MeJA)–treated, and salicylic acid (SA)–treated 'Dusa' plants infected with *Rosellinia necatrix* (**A**), and mean values (±SE, $n = 18$, in all treatments) of area under disease progression curve (AUDPC) for each treatment (**B**). Different letters indicate significant differences among treatments ($P < 0.05$; one-way analysis of variance followed by HSD).

Discussion

Elicitors have been proposed as a harmless tool for integrated pest management in agriculture, among which MeJA and SA are the most widely used. In avocado, the effect of the exogenous application of MeJA and SA in the molecular response of young avocado 'Dusa' plants, tolerant to the hemibiotrophic oomycete *Phytophthora cinnamomi*, revealed similarities between biotrophic- and necrotrophic-based plant responses and the involvement of both compounds in the molecular pathways associated with a successful defense response of avocado against this pathogen (van den Berg et al. 2018). This study also revealed that, among others, plant defense–related genes were overexpressed after 6, 18, and 24 h of the application of both elicitors, suggesting the induction of a "primed state" that could confer some protection against further pathogen attacks.

Therefore, it is reasonable to suggest the use of these elicitors for reducing susceptibility of avocado 'Dusa' to the necrotrophic ascomycete *R. necatrix*, in the same way as the primed state induced by mild water stress slowed disease progression (cross-factor priming; Martínez-Ferri et al. 2019). In the present work, we have studied the effect of those elicitors on the physiological and molecular response of avocado 'Dusa' plants before and after inoculation with the necrotroph *R. necatrix*, as well as their impact on disease progression.

Physiological and molecular response of avocado 'Dusa' rootstocks to MeJA and SA application

Exogenous application of MeJA and SA (at the concentrations used in this study) induced a differential response in 'Dusa' avocado plants at the morphophysiological and molecular levels. Thus, in comparison with nonelicited control plants, MeJA- and SA-treated plants increased photoprotective mechanisms (i.e., NPQ values), consistent with the observed decrease in the intrinsic photochemical efficiency of the open reaction centers of PSII (F_v'/F_m') (Murchie and Lawson 2013). These results support the triggering of energy dissipation mechanisms related to the alleviation of oxidative stress, in line with their role in the plant defense response against biotic and abiotic stress (Ali et al. 2018; Moustaka et al. 2015; Oliveira et al. 2015). However, although both elicitors induced a similar response at the photochemical level, gas exchange photosynthetic parameters were only improved by SA, which agrees with previous investigations reporting the enhancement of A_N, E, g_s, and Ci by exogenous application of SA (Alam et al. 2023; Arif et al. 2020; Chen et al. 2020; Hayat et al. 2008; Khan et al. 2003, 2013). This enhancement of the photosynthetic capacity was not associated with higher chlorophyll content (i.e., SPAD index was similar in all treatments; Hayat et al. 2008) or with a differentially higher stomatal density (Bertolino et al. 2019; Tanaka et al. 2013; Xu and Zou 2008), but it was associated with higher A_N/Ci, suggesting that SA stimulated rubisco activity rather than an increase in pigment content (Khan et al. 2013; Khodary 2004). However, the increased stomatal opening of SA-treated plants resulted in higher transpiration rates, involving higher water loss consistent with the lower RWC values, as well as lower intrinsic water use efficiency (A_N/g_s). These results would imply a lower ability of SA-treated plants to withstand water stress (Hayat et al. 2008; Martínez-Ferri et al. 2019) the results also contrast with the involvement of SA in plant tolerance to water stress (Hayat et al. 2010; Lobato et al. 2021) and with the SA-induced stomatal closure described in previous studies (Khokon et al. 2011; Mori et al. 2001; Vlot et al. 2009). It is noteworthy that the higher

photosynthetic rates of SA-treated plants did not translate into higher biomass, contrasting with the role of SA as a growth promoter (Gonçalves et al. 2020; Nazar et al. 2015; Sánchez-Chávez et al. 2011; Zafar et al. 2023). These discrepancies may be attributed to the fact that the mode of action of exogenous SA application is highly dependent on several factors (Janda et al. 2014; Khan et al. 2015), such as plant species (Khan et al. 2003), concentration applied (Lotfi et al. 2020), environmental conditions (Khan et al. 2013), and the time elapsed after elicitor application (Gonçalves et al. 2020; Khan et al. 2003).

Conversely to SA, photosynthetic performance of avocado 'Dusa' plants was not affected by MeJA, despite the observed increase in LMA in MeJA-treated plants. This increase of LMA is consistent with the MeJA-induced enhancement in leaf thickness and/or leaf density previously reported (Havko et al. 2016; Li et al. 2018) and would entail lower water and CO_2 diffusion through mesophyll cells and lower photosynthetic rates (Kofidis et al. 2004; Muir et al. 2014). Likewise, it has also been reported that exogenous MeJA application reduced assimilation and transpiration rates and induced stomatal closure in other plant species (Huang et al. 2017; Pospíšilová 2003; Qiu et al. 2020; Rohwer and Erwin 2008), associated with a water conservative strategy under water stress (Pospíšilová 2003). Consequently, the higher stomatal density (of similar size) of the MeJA-treated avocado leaves could counteract the diffusional limitations of photosynthesis by increasing the amount of CO_2 entering the mesophyll cells and thus enhancing photosynthetic capacity (Tanaka et al. 2013). Regardless, it should be kept in mind that the effect of MeJA on photosynthesis is dose and plant species dependent (Fatma et al. 2021; Hanaka et al. 2015; Qiu et al. 2020).

At the plant level, although elicitor treatments did not significantly affect most biomass-related parameters, MeJA showed a tendency to reduce leaf dry weight and significantly increase root dry weight, resulting in a significantly higher root/shoot ratio compared with control and SA-treated plants. This result suggests that MeJA induces greater carbon partitioning to roots in avocado, in agreement with previous reports on other crops describing increased adventitious root biomass and root length by the exogenous application of MeJA (Sirhindi et al. 2020; Yoshida et al. 2020). Consistently, MeJA-treated plants showed lower total plant leaf surface than in the other treatments, suggesting that a smaller leaf size may be associated with lower leaf water losses and, consequently, lower plant transpiration (Wang et al. 2019). These characteristics, together with high root/shoot ratios, have been related to plant adaptability to water stress (Anjum et al. 2011; Kou et al. 2022; Poorter et al. 2012; Vadez et al. 2007) but also to avocado tolerance to *R. necatrix* (Magagula et al. 2021; Martínez-Ferri et al. 2016) and *P. cinnamomi* (Coffey 1987; van den Berg et al. 2021).

Likewise, a differential molecular response of 'Dusa' avocado roots was observed after treatment with MeJA or SA. The expression of 10 of the 11 defense-related genes, selected by their involvement in tolerance to soilborne pathogens (Moreno-Pérez et al. 2023; van den Berg et al. 2018; Zumaquero et al. 2019), was deregulated depending on the type of elicitor applied. Moreover, although the major effect of MeJA treatment was the upregulation of five defense-related genes (i.e., *endochitinase*, *PR5*, *PR4*, *glu protease inhibitor*, and *glutathione S-transferase*), SA treatment repressed three of the five differentially expressed genes (*trypsin inhibitor*, *metallothionein-like protein*, and *NAC domain-containing protein 72*) and upregulated three genes (NPR1, *glutathione S-transferase*, and *universal stress protein*).

MeJA treatment significantly upregulated *glu protease inhibitor*. This gene encodes proteins linked to different aspects of plant defense such as oxidative stress (Srinivasan and Kirti 2012), which may encompass an enhanced ability of plants to withstand *R. necatrix* infection, consistent with its important role in avocado tolerance to this pathogen (Moreno-Pérez et al. 2023; Zumaquero et al. 2019). Similarly, the enhanced expression of *endochitinases*, *PR4*, and *PR5* induced by MeJA could also represent a benefit to overcome WRR disease (Martínez-Ferri et al. 2019) by minimizing pathogen load or disease onset in uninfected plant organs (Ali et al. 2018).

Overall, the overexpression of the abovementioned genes (Sharma et al. 2011; Vaghela et al. 2022), has been described in the response of tolerant avocado plant material to *P. cinnamomi* and *R. necatrix* (Engelbrecht and van den Berg 2013; Moreno-Pérez et al. 2023; van den Berg et al. 2018; Zumaquero et al. 2019), as well as in susceptible rootstocks after priming with mild water stress (Martínez-Ferri et al. 2019). The common overexpression of these genes under diverse stress conditions points to their involvement in a broad range of plant responses to biotic (Dai et al. 2016; Jalil et al. 2015) and abiotic stress (Edreva 2005; Hakim et al. 2018). This agrees with the role of MeJA in triggering plant responses against necrotrophic pathogen attack and other stresses (Ali et al. 2018; Campos et al. 2014; Thomma et al. 2000; Wang et al. 2021).

Both MeJA and SA induced the upregulation of *glutathione S-transferase*, which has been associated with plant response to oxidative stress and inactivation of toxic compounds (Gullner et al. 2018; Marrs 1996; Sappl et al. 2004, 2009). This response against oxidative stress is consistent with the higher levels of NPQ observed in treated plants compared with control plants. *Glutathione S-transferase* gene is induced by several stresses (Szalai et al. 2009; Tiwari et al. 2022) and has been linked mainly to the SA regulation pathway (Sappl et al. 2004; van den Berg et al. 2018), but its overexpression in MeJA-treated plants suggests a concomitant crosstalk between both regulatory pathways despite their commonly reported antagonistic interaction (Ding et al. 2022; Verma et al. 2016). This interconnection could also explain the expression pattern of *PR5* in MeJA-treated plants (Ali et al. 2018).

Thus, our results show that the effects of MeJA treatment of avocado are evidenced by the expression of defense-related genes and through changes at the morphoanatomical level rather than by modifications at the photosynthetic level. Morphoanatomical changes include functional traits, such as high LMA, high stomatal density, high root/shoot ratios, and reactive oxygen species (ROS)–scavenging mechanisms, closely related to strategies for coping with harsh environments, such as water scarcity (Boughalleb and Hajlaoui 2011; Chartzoulakis et al. 1999, 2002; Patakas and Noitsakis 1999). These features have been also associated with resistance to soilborne pathogen infections (Magagula et al. 2021; Martínez-Ferri et al. 2016; van den Berg et al. 2021).

Effect of MeJA and SA on the response of avocado 'Dusa' rootstocks to inoculation with R. necatrix

Inoculation of 'Dusa' avocado plants with *R. necatrix* affected the physiological performance of treated and nontreated plants prior to the appearance of any visible symptoms, compared with noninoculated control plants. Thus, regardless of elicitor treatment, all inoculated plants

showed increased levels of energy-dissipating mechanisms (NPQ) and decreased efficiency of PSII open reaction centers (F_v'/F_m'), consistent with commonly observed responses following pathogen attacks (Berger et al. 2007; Martínez-Ferri et al. 2016) and with the enhanced generation of ROS by phytotoxic metabolites produced by *R. necatrix* that could disrupt photosynthetic electron transport (Grimmer et al. 2012; Martínez-Ferri et al. 2016; Selvaraj and Fofana 2012).

Conversely, *R. necatrix* inoculation induced a differential response between treated and nontreated plants in gas exchange–related parameters and water potential. Thus, A_N, E, g_s, Ci, and A_N/Ci were decreased in inoculated control plants compared with noninoculated control and MeJA- and SA-treated plants. The same applies to water potential, suggesting some degree of water stress (Martínez-Ferri et al. 2019) that could be linked to *R. necatrix* colonization of the root vascular system (Pliego et al. 2009). This response supports previous results of the effect of *R. necatrix* on avocado photosynthesis (Martínez-Ferri et al. 2016; Pliego et al. 2009; Zumaquero et al. 2019). These effects were to some extent mitigated in treated plants, which showed similar values to noninoculated control plants for most parameters (i.e., E, g_s, Ci, and A_N/g_s), except for A_N and A_N/Ci, which were lowered. These results suggest that MeJA and SA could be counteracting the stomatal and/or diffusional limitations of photosynthesis associated with the effect that *R. necatrix* infection has on water relations (Martínez-Ferri et al. 2016; Zumaquero et al. 2019); the results also point out the impairment of rubisco carboxylation activity in the early stages of disease progression.

Despite the similar effect on photosynthetic performance of both elicitors, a delay in disease progression was observed in MeJA-treated plants in comparison with control and SA treatments. This suggests that the improved ability of avocado plants to cope with *R. necatrix* infection after MeJA treatment could be associated with the MeJA-induced morphophysiological changes and with the activation of different pathways (Ali et al. 2018) involving the differential expression of specific defense-related genes mentioned above. The increase of leaf thickness/density, stomatal density, and root dry weight (%) together with the lower plant leaf surface in MeJA-treated plants could be counteracting the impairment of water relations produced by the collapse of the plant's vascular system after *R. necatrix* root invasion (Atucha et al. 2014; Martínez-Ferri et al. 2016, 2019; Pliego et al. 2009). These features in combination with the greater upregulation of key defense-related genes associated with tolerance to *R. necatrix* in MeJA-treated plants compared with SA-treated plants (i.e., *glu protease inhibitor*) might play an important role in the better performance of MeJA-treated plants against *R. necatrix*. This study suggests the use of MeJA to increase avocado tolerance to *R. necatrix*, as observed against other necrotrophic pathogenic fungi in different crops (Kępczyńska and Król 2012; Krokene et al. 2008).

This work reveals that the effect of the necrotrophic pathogen *R. necatrix* in susceptible avocado 'Dusa' plants involved the onset of ROS detoxification mechanisms and photosynthesis limitation owing to both diffusional (i.e., impaired water relations and stomatal closure) and nondiffusional limitations (i.e., reduced rubisco carboxylation capacity) in the early stages of disease progression. Furthermore, it shows that MeJA treatment can be used to improve avocado tolerance to soilborne pathogens such as *R. necatrix*. Thus, exogenous application of MeJA delayed WRR disease symptoms in susceptible avocado 'Dusa' by combining the induction of a priming state involving the activation of key defense-

response pathways related to tolerance against *R. necatrix,* with the modification of morphoanatomical characteristics related to tolerance to water stress and other biotic stresses.

In conclusion, the results of the present study postulate the use of MeJA as an environmentally friendly strategy to increase avocado tolerance to the pathogenic fungus *R. necatrix* that could mitigate the impact of this disease on susceptible rootstocks in avocado orchards. This strategy may be of particular relevance in view of the European obligation to carry out integrated pest management in an effort to gradually eliminate fungicides because of their negative environmental and climate change effects. Further studies will focus on the use of elicitors to improve avocado defense against soilborne pathogens under field conditions to ensure their validity for commercial exploitation.

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Literature Cited

Ahmad, P., Rasool, S., Gul, A., Sheikh, S. A., Akram, N. A., Ashraf, M., Kazi, A. M., and Gucel, S. 2016. Jasmonates: Multifunctional roles in stress tolerance. Front. Plant Sci. 7:813.

Alam, P., Balawi, T. A., and Faizan, M. 2023. Salicylic acid's impact on growth, Photosynthesis, and antioxidant enzyme activity of *Triticum aestivum* when exposed to salt. Molecules 28:100.

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S. T., Mushtaq, M., Yadav, P., Rawat, S., and Grover, A. 2018. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiol. Res. 212-213:29-37.

Anjum, S. A., Xie, X.-y., Wang, L.-c., Saleem, M. F., Man, C., and Lei, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6:2026-2032.

Arif, Y., Sami, F., Siddiqui, H., Bajguz, A., and Hayat, S. 2020. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. Environ. Exp. Bot. 175: 104040.

Atucha, A., Emmett, B., and Bauerle, T. L. 2014. Growth rate of fine root systems influences rootstock tolerance to replant disease. Plant Soil 376:337-346.

Awang, N. A., Islam, M. R., Ismail, M. R., Zulkarami, B., and Omar, D. 2013. Effectiveness of different elicitors in inducing resistance in chilli (*Capsicum annuum* L.) against pathogen infection. Sci. Hortic. 164:461-465.

Backer, R., Mahomed, W., Reeksting, B. J., Engelbrecht, J., Ibarra-Laclette, E., and van den Berg, N. 2015. Phylogenetic and expression analysis of the *NPR1*-like gene family from *Persea americana* (Mill.). Front. Plant Sci. 6:300.

Baenas, N., García-Viguera, C., and Moreno, D. A. 2014. Elicitation: A tool for enriching the bioactive composition of foods. Molecules 19:13541-13563.

Baker, N. R., Harbinson, J., and Kramer, D. M. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 30:1107-1125.

Barceló-Muñoz, A., Zea-Bonilla, T., Jurado Valle, I., Imbroda-Solano, I., Vidoy-Mercado, I., Pliego-Alfaro, F., and López-Herrera, C. J. 2007. Selection program for avocado rootstocks tolerant to white rot caused by *Rosellinia necatrix* in southern Spain (1995-2007). www.avocadosource.com/WAC6/en/Extenso/1a-9.pdf (accessed 20 December 2023).

Berger, S., Sinha, A. K., and Roitsch, T. 2007. Plant physiology meets phytopathology: Plant primary metabolism and plant–pathogen interactions. J. Exp. Bot. 58:4019-4026.

Bertolino, L. T., Caine, R. S., and Gray, J. E. 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. Front. Plant Sci. 10: 225.

Boughalleb, F., and Hajlaoui, H. 2011. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol. Plant. 33:53-65.

Camargo, M. A. B., and Marenco, R. A. 2011. Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. Acta Amaz. 41: 205-212.

Campbell, C. L., and Madden, L. V. 1990. Temporal analysis of epidemics. I: Descriptions and comparisons of disease progress curves. Pages 161-162 in: Introduction to Plant Disease Epidemiology. C. L. Campbell and L. V. Madden, eds. Wiley, Hoboken, NY.

Campos, M. L., Kang, J.-H., and Howe, G. A. 2014. Jasmonate-triggered plant immunity. J. Chem. Ecol. 40:657-675.

Cervantes-Landaverde, J. J., Torres-Pacheco, I., Olvera-Luna, A., Bárcena-Romero, A. R., Quijas-Martínez, J., Rosales-Cueto, L., and Gómez-Renauld, V. M. 2009. Aplicación de elicitores como medios de activación de defensa en *Capsicum annuum* contra el patógeno Phytophthora capsici. https://www.uaq.mx/investigacion/difusion/veranos/memorias-2009/OctavoVerano_38/5_Cervantes_Landaverde.pdf (accessed 21 Jul 2023).

Chang, S., Puryear, J., and Cairney, J. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. 11:113-116.

Chartzoulakis, K., Patakas, A., and Bosabalidis, A. M. 1999. Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. Environ. Exp. Bot. 42:113-120.

Chartzoulakis, K., Patakas, A., Kofidis, G., Bosabalidis, A., and Nastou, A. 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Sci. Hortic. 95:39-50.

Chen, Y.-E., Mao, H.-T., Wu, N., Mohi Ud Din, A., Khan, A., Zhang, H.-Y., and Yuan, S. 2020. Salicylic acid protects photosystem II by alleviating photoinhibition in *Arabidopsis thaliana* under high light. Int. J. Mol. Sci. 21:1229.

Coffey, M. D. 1987. Phytophthora root rot of avocado: An integrated approach to control in California. Plant Dis. 71:1046-1052.

Dai, L., Wang, D., Xie, X., Zhang, C., Wang, X., Xu, Y., Wang, Y., and Zhang, J. 2016. The novel gene VpPR4-1 from *Vitis pseudoreticulata* increases powdery mildew resistance in transgenic *Vitis vinifera* L. Front. Plant Sci. 7:695.

Ding, L.-N., Li, Y.-T., Wu, Y.-Z., Li, T., Geng, R., Cao, J., Zhang, W., and Tan, X.-L. 2022. Plant disease resistance-related signaling pathways: Recent progress and future prospects. Int. J. Mol. Sci. 23:16200.

Edreva, A. 2005. Pathogenesis-related proteins: Research progress in the last 15 years. Gen. Appl. Plant Physiol. 31:105-124.

Engelbrecht, J., and van den Berg, N. 2013. Expression of defence-related genes against *Phytophthora cinnamomi* in five avocado rootstocks. S. Afr. J. Sci. 109:2012-0058.

Fatma, M., Iqbal, N., Sehar, Z., Alyemeni, M. N., Kaushik, P., Khan, N. A., and Ahmad, P. 2021. Methyl jasmonate protects the PSII system by maintaining the stability of chloroplast D1 protein and accelerating enzymatic antioxidants in heat-stressed wheat plants. Antioxidants 10:1216.

Frolich, E. F., and Platt, R. G. 1972. Use of the etiolation technique in rooting avocado cuttings. California Avocado Society 1971-72 Yearbook 55: 97-109.

Gokbayrak, Z., Dardeniz, A., and Bal, M. 2008. Stomatal density adaptation of grapevine to windy conditions. Trakia J. Sci. 6:18-22.

Gonçalves, F. C. d. M., Parreiras, N. d. S., Campos, F. G., Mantoan, L. P. B., and Boaro, C. S. F. 2020. Exogenous salicylic acid modifies gas exchange and biomass production of Mentha × piperita L. Aust. J. Crop Sci. 14:98-107.

Grimmer, M. K., John Foulkes, M., and Paveley, N. D. 2012. Foliar pathogenesis and plant water relations: A review. J. Exp. Bot. 63:4321-4331.

Gullner, G., Komives, T., Király, L., and Schröder, P. 2018. Glutathione S-transferase enzymes in plant-pathogen interactions. Front. Plant Sci. 9:1836.

Hakim, Ullah, A., Hussain, A., Shaban, M., Khan, A. H., Alariqi, M., Gul, S., Jun, Z., Lin, S., Li, J., Jin, S., and Munis, M. F. H. 2018. Osmotin: A plant defense tool against biotic and abiotic stresses. Plant Physiol. Biochem. 123:149-159.

Hanaka, A., Maksymiec, W., and Bednarek, W. 2015. The effect of methyl jasmonate on selected physiological parameters of copper-treated *Phaseolus coccineus* plants. Plant Growth Regul. 77:167-177.

Havko, N. E., Major, I. T., Jewell, J. B., Attaran, E., Browse, J., and Howe, G. A. 2016. Control of carbon assimilation and partitioning by jasmonate: An accounting of growth-defense tradeoffs. Plants. 5:7.

Hayat, Q., Hayat, S., Irfan, M., and Ahmad, A. 2010. Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot. 68:14-25.

Hayat, S., Hasan, S. A., Fariduddin, Q., and Ahmad, A. 2008. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. J. Plant Interact. 3:297-304.

Hsiao, T. C. 1990. Measurements of plant water status. Pages 243-279 in: Irrigation of Agricultural Crops. Monograph No. 30. B. A. Stewart and D. R. Nielsen, eds. American Society of Agronomy, Madison, WI.

Huang, H., Liu, B., Liu, L., and Song, S. 2017. Jasmonate action in plant growth and development. J. Exp. Bot. 68:1349-1359.

Jalil, S. U., Mishra, M., and Ansari, M. I. 2015. Current view on chitinase for plant defence. Trends Biosci. 8:6733-6743.

Jamiołkowska, A. 2020. Natural compounds as elicitors of plant resistance against diseases and new biocontrol strategies. Agronomy 10:173.

Janda, T., Gondor, O. K., Yordanova, R., Szalai, G., and Pál, M. 2014. Salicylic acid and photosynthesis: Signalling and effects. Acta Physiol. Plant. 36: 2537-2546.

Jiao, L., Bian, L., Luo, Z., Li, Z., Xiu, C., Fu, N., Cai, X., and Chen, Z. 2022. Enhanced volatile emissions and anti-herbivore functions mediated by the synergism between jasmonic acid and salicylic acid pathways in tea plants. Hortic. Res. 9:uhac144.

Jung, Y. J., Melencion, S. M. B., Lee, E. S., Park, J. H., Alinapon, C. V., Oh, H. T., Yun, D.-J., Chi, Y. H., and Lee, S. Y. 2015. Universal stress protein exhibits a redox-dependent chaperone function in *Arabidopsis* and enhances plant tolerance to heat shock and oxidative stress. Front. Plant Sci. 6:1141.

Kępczyńska, E., and Król, P. 2012. The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porrif*. sp. solani in tomato plants. J. Plant Interact. 7:307-315.

Khan, A. H. 1955. Biology and pathogenicity of *Rosellinia necatrix* (Hart.) bel. Biologia 5:199-425.

Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., and Khan, N. A. 2015. Salicylic acid-induced abiotic stress tolerance and under lying mechanisms in plants. Front. Plant Sci. 6:462.

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S., and Khan, N. A. 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal. Behav. 8:e26374.

Khan, W., Prithiviraj, B., and Smith, D. L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. J. Plant Physiol. 160:485-492.

Khodary, S. E. A. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J. Agric. Biol. 6:5-8.

Khokon, M. A. R., Okuma, E., Hossain, M. A., Munemasa, S., Uraji, M., Nakamura, Y., Mori, I. C., and Murata, Y. 2011. Involvement of extracellular oxidative burst in salicylic acid-induced stomatal closure in *Arabidopsis*. Plant Cell Environ. 34:434-443.

Kofidis, G., Bosabalidis, A. M., and Chartzoulakis, K. 2004. Leaf anatomical alterations induced by drought stress in two avocado cultivars. J. Biol. Res. 1:115-120.

Kõressaar, T., Lepamets, M., Kaplinski, L., Raime, K., Andreson, R., and Remm, M. 2018. Primer3_masker: Integrating masking of template sequence with primer design software. Bioinformatics 34:1937-1938.

Koressaar, T., and Remm, M. 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23:1289-1291.

Kou, X., Han, W., and Kang, J. 2022. Responses of root system architecture to water stress at multiple levels: A meta-analysis of trials under controlled conditions. Front. Plant Sci. 13:1085409.

Krokene, P., Nagy, N. E., and Solheim, H. 2008. Methyl jasmonate and oxalic acid treatment of Norway spruce: Anatomically based defense responses and increased resistance against fungal infection. Tree Physiol. 28:29-35.

Kulshrestha, S., Seth, C. A., Sharma, M., Sharma, A., Mahajan, R., and Chauhan, A. 2014. Biology and control of *Rosellinia necatrix* causing white root rot disease: A review. J. Pure Appl. Microbiol. 8:1803-1814.

Laredo Alcalá , E. I., Martí nez Hernández, J. L., Iliná , A., Guillen Cisneros, L., and Herná ndez Castillo, F. D. 2017. Aplicaci´on de ´acido jasm´onico como inductor de resistencia vegetal frente a patógenos. Rev. Mex. Cienc. Agr´ıc. 8:673-683.

Li, C., Wang, P., Menzies, N. W., Lombi, E., and Kopittke, P. M. 2018. Effects of methyl jasmonate on plant growth and leaf properties. J. Plant Nutr. Soil Sci. 181:409-418.

Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−DDCT method. Methods 25: 402-408.

Lobato, A. K. d. S., Barbosa, M. A. M., Alsahli, A. A., Lima, E. J. A., and Silva, B. R. S. d. 2021. Exogenous salicylic acid alleviates the negative impacts on production components, biomass and gas exchange in tomato plants under water deficit improving redox status and anatomical responses. Physiol. Plant. 172:869-884.

López-Herrera, C. J., Pérez-Jiménez, R. M., Basallote-Ureba, M. J., Zea-Bonilla, T., and Melero-Vara, J. M. 1999. Loss of viability of *Dematophora necatrix* in solarized soils. Eur. J. Plant Pathol. 105:571-576.

López-Herrera, C. J., and Zea-Bonilla, T. 2007. Effects of benomyl, carbendazim, fluazinam and thiophanate methyl on white root rot of avocado. Crop Prot. 26: 1186-1192.

López-Herrera, C. J. 1998. Hongos de suelo en el cultivo del aguacate (*Persea americana* Mill) del litoral andaluz. V Jornadas Andaluzas de Frutos Tropicales. Congresos y Jornadas 47:139-152.

Lotfi, R., Ghassemi-Golezani, K., and Pessarakli, M. 2020. Salicylic acid regulates photosynthetic electron transfer and stomatal conductance of mung bean (*Vigna radiata* L.) under salinity stress. Biocatal. Agric. Biotechnol. 26:101635.

Magagula, P., Taylor, N., Swart, V., and van den Berg, N. 2021. Efficacy of potential control agents against *Rosellinia necatrix* and their physiological impact on avocado. Plant Dis. 105:3385-3396.

Mahomed, W., and van den Berg, N. 2011. EST sequencing and gene expression profiling of defence-related genes from *Persea americana* infected with Phytophthora cinnamomi. BMC Plant Biol. 11:167.

Major, I. T., and Constabel, C. P. 2008. Functional analysis of the Kunitz trypsin inhibitor family in poplar reveals biochemical diversity and multiplicity in defense against herbivores. Plant Physiol. 146:888-903.

Makandar, R., Nalam, V., Chaturvedi, R., Jeannotte, R., Sparks, A. A., and Shah, J. 2010. Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis* interaction with Fusarium graminearum. Mol. Plant-Microbe Interact. 23: 861-870.

Marrs, K. A. 1996. The functions and regulation of glutathione S-transferases in plants. Annu. Rev. Plant Biol. 47:127-158.

Martínez-Ferri, E., Moreno-Ortega, G., van den Berg, N., and Pliego, C. 2019. Mild water stress-induced priming enhance tolerance to *Rosellinia necatrix* in susceptible avocado rootstocks. BMC Plant Biol. 19:458.

Martínez-Ferri, E., Zumaquero, A., Ariza, M. T., Barceló, A., and Pliego, C. 2016. Nondestructive detection of white root rot disease in avocado rootstocks by leaf chlorophyll fluorescence. Plant Dis. 100:49-58.

Mendoza García, R. A., ten Hoopen, G. M., Kass, D. C. J., Sánchez Garita, V. A., and Krauss, U. 2003. Evaluation of mycoparasites as biocontrol agents of *Rosellinia* root rot in cocoa. Biol. Control 27:210-227.

Moreno-Pérez, A., Martínez-Ferri, E., Pliego-Alfaro, F., and Pliego, C. 2020. Elicitors and plant defence. JOJ Hortic. Arboric. 2:555600.

Moreno-Pérez, A., Zumaquero, A., Martínez -Ferri, E., López-Herrera, C., Pliego-Alfaro, F., Palomo-Ríos, E., and Pliego, C. 2023. A comparative transcriptome analysis of avocado embryogenic lines susceptible or resistant to *Rosellinia* necatrix exudate. Agronomy 13:1354.

Mori, I. C., Pinontoan, R., Kawano, T., and Muto, S. 2001. Involvement of superoxide generation in salicylic acid-induced stomatal closure in *Vicia faba*. Plant Cell Physiol. 42:1383-1388.

Moustaka, J., Tanou, G., Adamakis, I.-D., Eleftheriou, E. P., and Moustakas, M. 2015. Leaf age-dependent photoprotective and antioxidative response mechanisms to paraquat-induced oxidative stress in *Arabidopsis thaliana*. Int. J. Mol. Sci. 16:13989-14006.

Muir, C. D., Hangarter, R. P., Moyle, L. C., and Davis, P. A. 2014. Morphological and anatomical determinants of mesophyll conductance in wild relatives of tomato (*Solanum* sect. *Lycopersicon*, sect. *Lycopersicoides*; Solanaceae). Plant Cell Environ. 37:1415-1426.

Murchie, E. H., and Lawson, T. 2013. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. J. Exp. Bot. 64: 3983-3998.

Nazar, R., Umar, S., Khan, N. A., and Sareer, O. 2015. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. S. Afr. J. Bot. 98:84-94.

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N., and Ohashi, Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol. 39:500-507.

Oliveira, M. B., Junior, M. L., Grossi-de-Sá, M. F., and Petrofeza, S. 2015. Exogenous application of methyl jasmonate induces a defense response and resistance against *Sclerotinia sclerotiorum* in dry bean plants. J. Plant Physiol. 182:13-22.

Patakas, A., and Nortsakis, B. 1999. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. J. Plant Physiol. 154:767-774.

Peel, J. R., Mandujano Sánchez, M. C., López Portillo, J., and Golubov, J. 2017. Stomatal density, leaf area and plant size variation of *Rhizophora mangle* (Malpighiales: Rhizophoraceae) along a salinity gradient in the Mexican Caribbean. Rev. Biol. Trop. 65:701-712.

Pérez-Jiménez, R. M. 2006. A review of the biology and pathogenicity of *Rosellinia necatrix*-the cause of white root rot disease of fruit trees and other plants. J. Phytopathol. 154:257-266.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45.

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. M. 2012. Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28:489-521.

Pimentel, D., and Burgess, M. 2014. Environmental and economic costs of the application of pesticides primarily in the United States. Pages 47-71 in: Integrated Pest Management. D. Pimentel and R. Peshin, eds. Springer, Dordrecht, Germany.

Pliego, C., Kanematsu, S., Ruano-Rosa, D., de Vicente, A., López-Herrera, C., Cazorla, F. M., and Ramos, C. 2009. GFP sheds light on the infection process of avocado roots by *Rosellinia necatrix*. Fungal Genet. Biol. 46: 137-145.

Pliego, C., López-Herrera, C., Ramos, C., and Cazorla, F. M. 2012. Developing tools to unravel the biological secrets of *Rosellinia necatrix*, an emergent threat to woody crops. Mol. Plant Pathol. 13:226-239.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. 2012. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. New Phytol. 193:30-50.

Pospíšilová, J. 2003. Participation of phytohormones in the stomatal regulation of gas exchange during water stress. Biol. Plant. 46:491-506.

Qiu, X., Xu, Y., Xiong, B., Dai, L., Huang, S., Dong, T., Sun, G., Liao, L., Deng, Q., Wang, X., Zhu, J., and Wang, Z. 2020. Effects of exogenous methyl jasmonate on the synthesis of endogenous jasmonates and the regulation of photosynthesis in citrus. Physiol. Plantarum. 170:398-414.

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., Srivastav, A. L., and Kaushal, J. 2021. An extensive review on the consequences of chemical pesticides on human health and environment. J. Clean. Prod. 283: 124657.

Rohwer, C. L., and Erwin, J. E. 2008. Horticultural applications of jasmonates. J. Hortic. Sci. Biotech. 83:283-304.

Ruano-Rosa, D., Cazorla, F. M., Bonilla, N., Martín-Pérez, R., De Vicente, A., and López-Herrera, C. J. 2014. Biological control of avocado white root rot with combined applications of *Trichoderma* spp. and rhizobacteria. Eur. J. Plant Pathol. 138:751-762.

Sánchez-Chávez, E., Barrera-Tovar, R., Muñoz-Márquez, E., Ojeda-Barrios, D. L., and Anchondo-Nájera, ´A. 2011. Efecto del ácido salicílico sobre biomasa, actividad fotosintética, contenido nutricional y productividad del chile jalapeño. Rev. Chapingo Ser. Hortic. 17:63-68.

Sappl, P. G., Oñate-Sánchez, L., Singh, K. B., and Millar, A. H. 2004. Proteomic analysis of glutathione S-transferases of *Arabidopsis thaliana* reveals differential salicylic acid-induced expression of the plant-specific phi and tau classes. Plant Mol. Biol. 54:205-219.

Sappl, P. G., Carroll, A. J., Clifton, R., Lister, R., Whelan, J., Harvey Millar, A., and Singh, K. B. 2009. The *Arabidopsis glutathione* transferase gene family displays complex stress regulation and co-silencing multiple genes results in altered metabolic sensitivity to oxidative stress. Plant J. 58:53-68.

Schreiber, U., Bilger, W., and Neubauer, C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. Pages 49-70 in: Ecophysiology of Photosynthesis. vol. 100. E.-D. Schulze and M. M. Caldwell, eds. Springer Study Edition, Berlin.

Selvaraj, K., and Fofana, B. 2012. An overview of plant photosynthesis modulation by pathogen attacks. Pages 466-484 in: Advances in Photosynthesis - Fundamental Aspects. CBS Publishers, Daryaganj, New Delhi.

Sharma, N., Sharma, K. P., Gaur, R. K., and Gupta, V. K. 2011. Role of chitinase in plant defense. Asian J. Biochem. 6:29-37.

Sirhindi, G., Mushtaq, R., Gill, S. S., Sharma, P., Abd-Allah, E. F., and Ahmad, P. 2020. Jasmonic acid and methyl jasmonate modulate growth, photosynthetic activity and expression of photosystem II subunit genes in *Brassica oleracea* L. Sci. Rep. 10:9322.

Srinivasan, T., and Kirti, P. B. 2012. Protease inhibitors and stress tolerance. Funct. Plant Sci. Biotechnol. 6:59-66.

Srinivasan, T., Kumar, K. R. R., and Kirti, P. B. 2009. Constitutive expression of a trypsin protease inhibitor confers multiple stress tolerance in transgenic tobacco. Plant Cell Physiol. 50:541-553.

Szalai, G., Kellös, T., Galiba, G., and Kocsy, G. 2009. Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. J. Plant Growth Regul. 28:66-80.

Sztejnberg, A., and Madar, Z. 1980. Host range of *Dematophora necatrix*, the cause of white root rot disease in fruit trees. Plant Dis. 64:662-664.

Tanaka, Y., Sugano, S. S., Shimada, T., and Hara-Nishimura, I. 2013. Enhancement of leaf photosynthetic capacity through increased stomatal density in *Arabidopsis*. New Phytol. 198:757-764.

Teixeira de Sousa, A. J. 1985. Lutte contre *Rosellinia necatrix* (Hartig) Berlese, agent du «pourridié laineux»: Sensibilité de quelques espéces végétales et lutte chimique. Eur. J. For. Pathol. 15:323-332.

ten Hoopen, G. M., and Krauss, U. 2006. Biology and control of *Rosellinia bunodes*, *Rosellinia necatrix* and *Rosellinia pepo*: A review. Crop Prot. 25:89-107.

Thakur, M., and Sohal, B. S. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A review. ISRN Biochem. 2013:762412.

Thomma, B. P. H. J., Eggermont, K., Broekaert, W. F., and Cammue, B. P. A. 2000. Disease development of several fungi on *Arabidopsis* can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem. 38:421-427.

Tiwari, M., Kidwai, M., Dutta, P., Narayan, S., Gautam, N., Chawda, K., Shirke, P. A., Mishra, A. K., and Chakrabarty, D. 2022. A tau class glutathione-S-transferase (OsGSTU5) confers tolerance against arsenic toxicity in rice by accumulating more arsenic in root. J. Hazard. Mater. 426:128100.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., and Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91:37-46.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40:e115.

Vadez, V., Rao, S., Sharma, K. K., Bhatnagar-Mathur, P., and Devi, M. J. 2007. DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit. J. SAT Agric. Res. 5:1-5.

Vaghela, B., Vashi, R., Rajput, K., and Joshi, R. 2022. Plant chitinases and their role in plant defense: A comprehensive review. Enzyme Microb. Technol. 159:110055.

van den Berg, N., Mahomed, W., Olivier, N. A., Swart, V., and Crampton, B. G. 2018. Transcriptome analysis of an incompatible *Persea americana-Phytophthora cinnamomi* interaction reveals the involvement of SA- and JA-pathways in a successful defense response. PLoS One 13:e0205705.

van den Berg, N., Swart, V., Backer, R., Fick, A., Wienk, R., Engelbrecht, J., and Prabhu, S. A. 2021. Advances in understanding defense mechanisms in *Persea americana* against *Phytophthora cinnamomi*. Front. Plant Sci. 12:636339.

Verma, V., Ravindran, P., and Kumar, P. P. 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16:86.

Vlot, A. C., Dempsey, D. A., and Klessig, D. F. 2009. Salicylic acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 47:177-206.

von Caemmerer, S., and Farquhar, G. D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376-387.

Vos, I. A., Moritz, L., Pieterse, C. M. J., and Van Wees, S. C. M. 2015. Impact of hormonal crosstalk on plant resistance and fitness under multi-attacker conditions. Front. Plant Sci. 6:639.

Wang, C., He, J., Zhao, T.-H., Cao, Y., Wang, G., Sun, B., Yan, X., Guo, W., and Li, M.-H. 2019. The smaller the leaf is, the faster the leaf water loses in a temperate forest. Front. Plant Sci. 10:58.

Wang, Y., Mostafa, S., Zeng, W., and Jin, B. 2021. Function and mechanism of jasmonic acid in plant responses to abiotic and biotic stresses. Int. J. Mol. Sci. 22:8568.

Wong, H. L., Sakamoto, T., Kawasaki, T., Umemura, K., and Shimamoto, K. 2004. Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiol. 135:1447-1456.

Xu, Z., and Zhou, G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J. Exp. Bot. 59: 3317-3325.

Yoshida, C. H. P., Pacheco, A. C., Lapaz, A. d. M., Gorni, P. H., Vítolo, H. F., and Bertoli, S. C. 2020. Methyl jasmonate modulation reduces photosynthesis and induces synthesis of phenolic compounds in sweet potatoes subjected to drought. Bragantia 79:319-334.

Yu, X., Zhang, W., Zhang, Y., Zhang, X., Lang, D., and Zhang, X. 2018. The roles of methyl jasmonate to stress in plants. Funct. Plant Biol. 46:197-212.

Yuan, X., Wang, H., Cai, J., Li, D., and Song, F. 2019. NAC transcription factors in plant immunity. Phytopathol. Res. 1:3.

Zafar, Z., Rasheed, F., Mushtaq, N., Khan, M. U., Mohsin, M., Irshad, M. A., Summer, M., Raza, Z., and Gailing, O. 2023. Exogenous application of salicylic acid improves physiological and biochemical attributes of *Morus alba* saplings under soil water deficit. Forests 14:236.

Zhang, J., Wang, F., Liang, F., Zhang, Y., Ma, L., Wang, H., and Liu, D. 2018. Functional analysis of a pathogenesis-related thaumatin-like protein gene TaLr35PR5 from wheat induced by leaf rust fungus. BMC Plant Biol. 18:76.

Zhang, S., Li, Q., Ma, K., and Chen, L. 2001. Temperature-dependent gas exchange and stomatal/non-stomatal limitation to CO 2 assimilation of *Quercus liaotungensis* under midday high irradiance. Photosynthetica 39: 383-388.

Zheng, F., Chen, L., Zhang, P., Zhou, J., Lu, X., and Tian, W. 2020. Carbohydrate polymers exhibit great potential as effective elicitors in organic agriculture: A review. Carbohyd. Polym. 230:115637.

Zumaquero, A., Martínez-Ferri, E., Matas, A. J., Reeksting, B., Olivier, N. A., Pliego-Alfaro, F., Barceló, A., van den Berg, N., and Pliego, C. 2019. *Rosellinia necatrix* infection induces differential gene expression between tolerant and susceptible avocado rootstocks. PLoS One 14:e0212359.