Mild water stress-induced priming enhance tolerance to *Rosellinia necatrix* in susceptible avocado rootstocks

E. Martínez-Ferri¹, G. Moreno-Ortega¹, N. van den Berg²,³ and C. Pliego¹*

**Abstract**

**Background:** White root rot (WRR) disease caused by *Rosellinia necatrix* is one of the most important threats affecting avocado orchards in temperate regions. The eradication of WRR is a difficult task and environmentally friendly control methods are needed to lessen its impact. Priming plants with a stressor (biotic or abiotic) can be a strategy to enhance plant defense/tolerance against future stress episodes but, despite the known underlying common mechanisms, few studies use abiotic-priming for improving tolerance to forthcoming biotic-stress and vice versa (*cross-factor priming*). To assess whether *cross-factor priming* can be a potential method for enhancing avocado tolerance to WRR disease, ‘Dusa’ avocado rootstocks, susceptible to *R. necatrix*, were subjected to two levels of water stress (mild-WS and severe-WS) and, after drought-recovery, inoculated with *R. necatrix*. Physiological response and expression of plant defense related genes after drought-priming as well as the disease progression were evaluated.

**Results:** Water-stressed avocado plants showed lower water potential and stomatal limitations of photosynthesis compared to control plants. In addition, NPQ and qN values increased, indicating the activation of energy dissipating mechanisms closely related to the relief of oxidative stress. This response was proportional to the severity of the water stress and was accompanied by the deregulation of pathogen defense-related genes in the roots. After re-watering, leaf photosynthesis and plant water status recovered rapidly in both treatments, but roots of mild-WS primed plants showed a higher number of overexpressed genes related with plant defense than severe-WS primed plants. Disease progression after inoculating primed plants with *R. necatrix* was significantly delayed in mild-WS primed plants.

**Conclusions:** These findings demonstrate that mild-WS can induce a primed state in the WRR susceptible avocado rootstock ‘Dusa’ and reveal that *cross-factor priming* with water stress (abiotic stressor) is effective for increasing avocado tolerance against *R. necatrix* (biotic stressor), underpinning that plant responses against biotic and abiotic stress rely on common mechanisms. Potential applications of these results may involve an enhancement of WRR tolerance of current avocado groves and optimization of water use via low frequency deficit irrigation strategies.

**Keywords:** Abiotic and biotic stress, Drought recovery, Fungal pathogens, Gene expression, Priming, Physiological response, White root rot
Background

Avocado (Persea americana Mill.), a member of the Lauraceae family, is a very important fruit crop consumed worldwide in more than 50 countries. Avocado fruit is considered to be one of the top 15 healthiest foods according to surveys across the United States and Western Europe [1] and is becoming a key component of the consumer’s diet in many countries. Avocado health benefits have triggered its consumption in recent years (~4.6% increase of worldwide consumption every year; ~25% increase in Europe [2];) but production remains a step behind (~4.5% increase per year [3];), which raises concerns about the difficulties of satisfying this demand in the near future.

This gap between production and demand is aggravated by the incidence of avocado diseases, the soilborne pathogen Phytophthora cinnamomi Rands (Phytophthora root rot; PRR) being one of the major limiting factors of avocado production worldwide [4]. Given the importance of this pathogen, many studies have been focused on the control of PRR and positive results, derived from an integrated approach involving the use of phosphate, proper field management and commercially available rootstocks with partial tolerance to P. cinnamomi (‘Thomas’, ‘Duke 7’ and ‘Dusa’) [5, 6], have been achieved.

Another important soilborne disease affecting avocado groves in productive temperate regions such as South Africa, Israel, Italy and Spain (avocado exporters to the European market), is the white root rot (WRR) caused by Rosellinia necatrix Prill [7, 8]. In contrast to P. cinnamomi, control of this disease is a complex and difficult task, and, to date, no completely effective control methods have been developed [8, and references therein]. As for P. cinnamomi, breeding for R. necatrix tolerant rootstocks could represent an effective method for controlling the spread of this pathogen [9] but, although a breeding program is ongoing in Spain (Andalusian Institute of Agricultural Research and Training; IFAPA), no commercial rootstocks are currently available. Thus, alternative approaches, focused on achieving environmentally friendly strategies to decrease WRR incidence in avocado production areas, are necessary.

In this regard, many studies have shown that the pre-exposure of plants to a stress-inducing factor (priming concept) [10–12] allows them to become more tolerant to forthcoming biotic (i.e. pathogens [10, 13]) or abiotic (i.e. water stress, chemical compounds [14, 15]) stress episodes. This priming-induced tolerance seems to be associated with a more rapid and robust activation of cellular defense responses in primed plants compared to non-primed ones [11, 12, 16]. Although the mechanisms underlying the induction of the priming state are complex and diverse [17], it is well known that plant stress responses to biotic or abiotic factors share common pathways [18, 19] and even cross-tolerance can be achieved [20, 21]. For instance, levels of salicylic acid (SA), associated with reactive oxygen species (ROS) signalling and with the regulation of important plant physiological processes [22, 23], have been reported to increase under drought stress [18, 24–26] and pathogen attack [27–30]. More concretely, the accumulation of SA induces the transcription of non-expressor of pathogenesis related gene 1 (NPR1) that further activates genes encoding pathogenesis-related (PR) proteins [31, 32], shown to play an important role in either biotic [33–37] or abiotic stress [38–42] responses. Particularly, avocado tolerance to P. cinnamomi and R. necatrix has been linked to the induction of PR-genes and protease inhibitors, respectively [19, 43]; both are related with other abiotic stresses such as water stress [44, 45]. Thus, it is possible that exposure to one type of stress (i.e. abiotic stressor) could activate plant responses enabling tolerance to different types of forthcoming stresses (i.e. biotic stressor [46]); hereinafter referred as ‘cross-factor priming’. In fact, it has been reported that drought-primed Eucalyptus plants were more resistant to Neofusicoccum fungal infection compared to non-primed ones [16].

In this context, the present study aims to test whether drought-priming could be used in avocado to increase tolerance to WRR disease. For this purpose, the role of drought-priming in R. necatrix interaction with the susceptible avocado ‘Dusa’ rootstock was evaluated by assessing physiological status, stress-related gene expression, and disease progression response.

Results

Physiological response of avocado ‘Dusa’ rootstocks to mild and severe water stress levels and recovery after re-watering

To investigate the priming-induced response of ‘Dusa’ rootstock by mild and severe water stress (mild-WS and severe-WS), two sets of well irrigated plants (at field capacity, Fc ~ 0.4 v/v) were subjected to water deprivation until soil water content (SWC) reached 50 and 25% of Fc, respectively (Fig. 1). Throughout the experiment, a set of plants were irrigated daily to act as controls whereas, in the two sets of water-stressed plants, water lessening was done progressively to attain both water stress levels concurrently (after a 6 day lag; Fig. 2). Once these levels were reached, plants were re-watered and Fc values were achieved immediately. Daily irrigation was restored in all plants until inoculation with R. necatrix.

Physiological measurements were taken on the two water-stress levels and after re-watering. In consonance with the water stress severity, midday water potential decreased significantly compared to control plants (P < 0.05) reaching −1.01 ± 0.03 MPa in mild-WS and –
Fig. 1 Schematic illustration of the experimental design (a) and stages of aerial symptoms in ‘Dusa’ plants inoculated with R. necatrix (b). Control plants were watered to field capacity (Fc) throughout the experiment and water stressed plants were subjected to controlled substrate drying-up until they reached 50% of Fc (mild-WS) and 25% of Fc (severe-WS), respectively (t1). Afterwards, all plants were fully irrigated to assess drought recovery response (t2) and to carry out the pathogenicity test with R. necatrix.

Fig. 2 Time-course of mean values (±SE) of volumetric soil moisture of ‘Dusa’ non-stressed control plants (n = 36) and subjected to two water stress (WS) treatments: mild-WS and severe-WS (n = 38). The arrows indicate the time points where plants physiological status (t0), physiological measurements and root samplings (t1, t2) were done.
2.06 ± 0.09 MPa in severe-WS (Fig. 3a). Consistently, net CO₂ assimilation rates ($A_N$) and stomatal conductance ($g_s$) showed a marked and significant decrease in both stress levels ($P < 0.05$; Fig. 3b, c), $A_N$ being reduced in more than ~70% and ~90%, in mild-WS and severe-WS, respectively, while $g_s$ was almost completely suppressed in both treatments. Leaf relative water content (RWC) decreased only significantly ($P < 0.05$) in the severe-WS treatment showing values of ~87.5 ± 0.85% whereas in control and mild-WS, values were ~94%.

At the photochemical level, dark-adapted photochemical efficiency of photosystem II (PSII; $F_v/F_m$) was not significantly affected by water stress and mean values were close to 0.82 in all treatments (Table 1), indicating that water stress levels did not entail chronic photo-inhibition. The relative quantum yield of PSII photochemistry ($\Phi_{PSII}$) was not affected in the mild-WS treatment but was significantly reduced in the severe-WS (Table 1). Water stress treatments did not have an effect on the fraction of PSII centres in the open state.
water stressed plants (mild-WS and severe-WS) and drought-primed plants (recovery mild-WS and severe-WS)

Table 1: Maximal photochemical efficiency of PSII ($F_{v}/F_{m}$), relative quantum yield of PSII photochemistry (ϕPSII), maximum photochemical efficiency of the open reaction centres of PSII ($F_{o}/F_{m}^{*}$), fraction of PSII centres in open state ($q_{L}$), non-photochemical fluorescence quenching (NPQ) and coefficient of non-photochemical fluorescence quenching ($q_{N}$) in control non-stressed plants, water stressed plants (mild-WS and severe-WS) and drought-primed plants (recovery mild-WS and severe-WS)

| Treatment                | Control | Mild-WS | Severe-WS | Recovery Mild-WS | Recovery Severe-WS |
|--------------------------|---------|---------|-----------|------------------|--------------------|
| $F_{v}/F_{m}$            | 0.821 ± 0.00 | 0.820 ± 0.00 | 0.817 ± 0.00 | 0.825 ± 0.00 | 0.825 ± 0.00 |
| ϕPSII                    | 0.566 ± 0.01a | 0.556 ± 0.01a | 0.459 ± 0.02b | 0.562 ± 0.01a | 0.547 ± 0.01a |
| $F_{o}/F_{m}^{*}$        | 0.696 ± 0.01a | 0.661 ± 0.01b | 0.597 ± 0.01b | 0.703 ± 0.01b | 0.698 ± 0.01a |
| $q_{L}$                  | 0.587 ± 0.03ab | 0.649 ± 0.03a | 0.584 ± 0.03ab | 0.550 ± 0.02b | 0.533 ± 0.03b |
| NPQ                      | 0.510 ± 0.03c | 0.785 ± 0.05b | 1.224 ± 0.09a | 0.581 ± 0.05c | 0.599 ± 0.04c |
| $q_{N}$                  | 0.413 ± 0.02c | 0.534 ± 0.02b | 0.655 ± 0.02a | 0.439 ± 0.02c | 0.455 ± 0.02c |

Each value is the mean ± SE (controls n = 36, treatments n = 38). Different letters indicate significant differences among treatments within rows ($P < 0.05$)

(qL [47]); while the reverse was true for the maximum photochemical efficiency of the open reaction centres of PSII ($F_{o}/F_{m}^{*}$), which was significantly reduced as water stress became more severe (Table 1). These changes in $F_{o}/F_{m}^{*}$ were accompanied by a concomitant increase in other non-photochemical quenching related parameters (NPQ and $q_{N}$; Table 1).

Relative chlorophyll content (SPAD index) and leaf mass area (LMA) did not differ significantly between control and water stressed plants and no symptoms of leaf chlorosis were observed in any of the water stress treatments. Average SPAD values in all treatments were 59.4 ± 0.1 and LMA ranged from 76.8 g m$^{-2}$ to 83.4 g m$^{-2}$.

Within one week after re-watering and prior to inoculation with R. necatrix, all physiological parameters of stressed plants recovered similar values to those of control plants (Table 1 and Fig. 3). Hereinafter, these water-stressed-recovered plants will be referred as ‘primed plants’.

Molecular response of avocado ‘Dusa’ rootstocks to mild and severe water stress and recovery after re-watering

The expression of thirteen defense-related genes on roots of ‘Dusa’ avocado rootstock subjected to mild-WS and severe-WS and one week after re-watering, was analysed by performing a real time quantitative qPCR (qRT-PCRs). This selection included induced genes indicated in previous studies with ‘BG83’ (tolerant to R. necatrix) and ‘Dusa’ (tolerant to P. cinnamomi) avocado rootstocks after infection with the soilborne pathogens R. necatrix [43] and P. cinnamomi, respectively [19, 48–50]. In addition to their implication in pathogen defense, some of the selected genes are also involved in salt, oxidative, osmotic and water stress responses (Table 2).

Five primers were taken from literature and eight were developed in this research (Additional file 1: Table S1). 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. Water deprivation on avocado roots caused a significant repression of 6 and 3 genes in roots subjected to mild-WS and severe-WS, respectively (Table 2), with gene Contig00582, encoding the BTB/POZ and TAZ domain-containing protein 1-like, showing the highest repression in both treatments. In contrast, transcript levels of 6 genes (protease inhibitor-like, glutathione s-transferase, metallothionein like protein, NAC domain-containing protein 72, universal stress protein and miraculin) were significantly induced under both levels of water stress (Table 2 and Fig. 4).

Different gene expression patterns were detected in avocado roots of primed plants, in which the number of significantly repressed genes was reduced to two in both, mild-WS and severe-WS primed plants. A higher number of significantly overexpressed genes was observed in roots of mild-WS primed plants, being induced eight genes among which, four were repressed under water stress (NPR1, PR4, PR5, endochitinase). The highest induction level was found for the NAC domain containing protein 72, reaching fold change (FC) value of 177 in qRT-PCR experiments. Only three of the study genes (protease inhibitor-like, universal stress protein and miraculin) were significantly induced in roots of severe-WS primed plants.

Pathogenicity test on water stress primed ‘Dusa’ avocado rootstocks

In order to test whether priming with mild-WS and severe-WS could be used to induce tolerance to R. necatrix in avocado ‘Dusa’ rootstock, primed avocado plants were inoculated with wheat grains infected with R. necatrix. Disease progression was slightly faster in severe-WS primed plants than in non-primed control plants. Thus, visible above-ground WRR symptoms appeared 42 and 53 days post-inoculation, respectively. After 60 days post-inoculation, 50% of the non-primed control plants and severe-WS primed plants showed visible aerial symptoms (Fig. 5a).
Table 2 qRT-PCR expression data of selected contigs from non-inoculated ‘Dusa’ roots subjected to two different level of water stress (mild-WS and severe-SW) and after their recovery (primed plants)

| Citation of pathosystems | Contig/ GenBank ID | Annotation | Additional feature | Mild-WS Stress | Mild-WS Recovery | Severe-WS Stress | Severe-WS Recovery |
|--------------------------|--------------------|------------|-------------------|----------------|-----------------|-----------------|-------------------|
| BG83/R. necatrix          | Pa_Contig02817     | Basic 7s globulin-like | Salt and osmotic stress [53] | −1.80 ± 0.37 | 240 ± 060 | 7.11 ± 3.54 | 4.27 ± 2.63 |
|                          | Pa_Contig00582     | BTB/POZ and TAZ domain-containing prot. 1-like | Salt stress [54] | −10.16 ± 1.81 | −1.93 ± 0.32 | −12.76 ± 0.48 | −2.52 ± 0.65 |
| Dusa/P. cinnamomi        | Pa_Contig00535     | Endochitinase | Salt stress response [55] | 1.28 ± 0.10 | 2.35 ± 0.38 | 12.9 ± 0.16 | 3.29 ± 1.15 |
|                          | Pa_Contig00778     | Glutathione s-transferase | Salt, water and oxidative stress [56] | 2.17 ± 0.25 | −203 ± 083 | 2.75 ± 0.25 | 3.16 ± 0.50 |
|                          | Pa_Contig04910     | Metallothionein-like prot. | Oxidative and water stress [57, 58] | 2.79 ± 0.27 | 1.92 ± 0.17 | 1.48 ± 0.06 | 2.69 ± 0.70 |
| [19, 48, 49]             | Pa_Contig02540     | Miraculin | Water stress [59] | 2.38 ± 0.35 | 121 ± 010 | 3.25 ± 0.48 | 1.92 ± 0.00 |
| [43]                     | Pa_Contig00313     | NAC domain-containing prot. 72 | Salt and water stress [60–62] | 7.32 ± 1.38 | 177.00 ± 1.06 | 17.88 ± 1.43 | 3.27 ± 1.49 |
| [52]                     | KR056089           | NPR1 | Salt and osmotic stress [63] | −1.33 ± 0.19 | 1.21 ± 0.01 | −2.04 ± 0.15 | −2.71 ± 0.34 |
| [43]                     | Pa_Contig07140     | PR4 | Salt and water stress [64] | −1.91 ± 0.17 | 6.19 ± 0.44 | 1.31 ± 0.15 | 2.80 ± 0.86 |
| [48, 49, 51]             | Pa_Contig01450     | PRS | Salt and osmotic stress [55] | −1.51 ± 0.18 | 1.72 ± 0.12 | −1.76 ± 0.30 | 3.06 ± 1.51 |
|                          | Pa_Contig03407     | PR10 (Psemi) | Salt and water stress [66] | −2.22 ± 0.52 | −1.99 ± 0.15 | −1.78 ± 0.44 | 1.40 ± 0.16 |
| [43]                     | Pa_Contig05213     | Protease inhibitor-like | Oxidative stress [45] | 1.72 ± 0.15 | 2.84 ± 0.56 | 4.92 ± 0.61 | 4.28 ± 0.33 |
| [43]                     | Pa_Contig01245     | Universal stress prot. | Oxidative and water stress [67] | 1.23 ± 0.06 | 2.41 ± 0.14 | 1.63 ± 0.09 | 2.07 ± 0.19 |

The data are displayed as fold changes (FC) calculated by comparing treatments with non-stressed control plants. The expression data are the mean of three biological replicates with three technical replicates each. The numbers in bold indicate statistically significant results (t-test, P < 0.05).
Priming plants with mild-WS showed improved tolerance to WRR as indicated by a significant reduction in the area under disease progress curve (AUDPC) values \( (P < 0.05) \) (Fig. 5b). Although visible wilting symptoms in some leaves appeared 48 days post inoculation, 50% of the plants displayed aboveground WRR symptoms after 75 days post-inoculation (30 days after the first visible symptoms). After three and a half months post inoculation all non-primed control and severe-WS primed plants were at stage 5 (dead), while some mild-WS primed plants remained at stage 3.

**Discussion**

Plants have evolved diverse strategies to cope with different environmental stresses, but many studies have shown that most plant responses to biotic and abiotic stress rely on an assortment of common physiological and molecular mechanisms \[18, 19\]. Particularly, it has been reported that avocado ‘Dusa’ rootstock response to *R. necatrix* infection involves the impairment of water relations and photosynthesis \[68–70\] as well as the induction of genes related to water stress and pathogen defense responses \[43\]. These findings are in agreement with results of the present study on the response of ‘Dusa’ avocado to water stress. This response was dependent upon water stress intensity, since mild-WS and severe-WS treatments affected leaf water status differentially (i.e. decreased values of leaf water potential and RWC) as well as photosynthetic performance, shown by the enhancement of photoprotective mechanisms (i.e. NPQ and \( q_N \) values) and the decrease in gas exchange parameters (i.e. \( A_N \) and \( g_s \)). These physiological changes are consistent to those previously described in response to mild
and severe water stress in other woody plants [71, 72] and in avocado trees [73, 74]. ‘Dusa’ rootstock response to either *R. necatrix* infection or water stress treatments displayed water potential and *g* values that dropped below −1.0 MPa and 0.05 mol m⁻² s⁻¹, respectively, suggesting an oxidative burst in photosynthetic tissues [75, 76]. This agrees with the higher NPQ and qN values [77, 78] and with a potential vulnerability to cavitation that could limit water flow from roots towards the upperparts of the trees, especially in severe-WS [79, 80]. In the *R. necatrix*/avocado interaction, this limitation of water flow is consistent with the profuse invasion of root vascular system during pathogen root colonization [70, 81].

Molecular responses at the root level showed the up-regulation of six out of the thirteen tested genes under both water stress treatments (Table 2). These genes, besides being involved in the avocado response to soilborne pathogens (*P. cinnamomi* and *R. necatrix*), are also induced in the response of other horticultural and woody species (i.e. *Citrus* spp., *Malus domestica*, *Populus trichocarpa*) to water deficit [45, 82–86]. It is remarkable the increased overexpression of NAC transcription factor accordingly to the intensity of the water stress level, which could be supporting a major accumulation of ROS species under severe-WS since, among other functions, this gene has been associated with the up-regulation of ROS-scavenging genes under abiotic stresses [61]. On the other hand, mild-WS repressed seven out of the thirteen genes, three of which remained down-regulated in the severe-WS (Table 2). NPR1 and PR5 repression is in consonance with the ABA biosynthesis and signalling induced under water stress [87], known to exert an antagonistic effect on the salicylic acid (SA) pathway [88] in which NPR1 functions as a master regulator inducing the expression of pathogenesis related proteins (PR) such as PR5 [89, 90], which are potentially involved in the maintenance of osmotic adjustment in cells [65].

The results stated above indicate that pathways involved in the avocado response to gradually imposed water stress lead to the induction of genes expressed in incompatible interactions against fungal pathogens [43, 91].
[95]. In this regard, co-occurrence of water stress and soilborne pathogens could have a positive effect in achieving tolerance against the pathogen (i.e. cross-tolerance, [20, 21]) or a negative additive effect, making plants more susceptible [16, 91–94]. Additional studies on avocado are required to clarify this point.

Previous studies have suggested the use of ‘priming’ [10–12] with drought stress to achieve tolerance to forthcoming diseases [16]. This acquired tolerance is based on sustained changes on the basal levels of cellular and molecular defense in primed plants after cessation of stimuli compared to non-primed ones [11, 12, 16]. In the present study, water status and photosynthetic performance was completely restored in drought-primed plants one week after re-watering regardless of the pre-drought intensity. This fast recovery suggests that impairment of whole plant transpirational flow and photosynthesis did not lead to irreversable changes on avocado and can be indicative of some degree of drought adaption [78].

However, at the root level, re-watering induced the upregulation of defense related genes, suggesting a ‘primed state’ of the previously water stressed avocado plants. Gene overexpression, which could be associated with crosstalk between the different signaling pathways underlying plant tolerance/resistance to biotic and abiotic stress such as the abscisic (ABA), jasmonic (JA) and salicylic (SA) acids [95–98], was more remarkable in mild-WS compared to severe-WS. Particularly, the induction of NPR1 transcription factor in mild-WS primed plants suggests the activation of salicylic acid-mediated defense responses [52, 89, 90] and the deactivation of ABA-related responses after water stress [99]. In addition, this ‘primed state’ is accompanied by the significant accumulation of PR proteins (i.e. PR4 and PR5) which have been correlated with the development of systemic acquired resistance [48] and are considered the most promising candidates for developing multiple stress tolerance [89]. It is also remarkable that the expression of genes related with fungal cell wall degradation, such as endochitinase, was only up-regulated in plants recovered after mild-WS. Genes encoding metallothionein, universal stress protein, protease inhibitor and NAC domain containing protein 72 remained overexpressed in mild-WS primed plants. These genes are involved in the general plant response to stress [51, 58, 60, 62, 64, 67, 82, 100–103], playing the last two a fundamental role in avocado defense to R. necatrix [43]. It should be highlighted the marked overexpression of the gene encoding the NAC domain containing protein 72 (24 fold over mild-WS) in roots recovered from mild-WS compared to severe-WS, suggesting a higher promotion of root development [104, 105], although further studies are necessary to clarify its importance on the water stress recovery response.

The performed pathogenicity test shed light on whether this water-stress induced ‘primed state’ was effective for enhancing avocado tolerance to this necrotrophic pathogen. In this sense, the disease progression delay, observed in mild-WS primed plants in comparison with control and severe-WS primed plants, suggests an enhancement of plant ability to cope with R. necatrix infection after priming with mild water stress. This ability could be attributable to differential expression of key genes involved in the tolerance of avocado to soilborne pathogens such as NPR1 and NAC domain containing protein 72, as well as with a lower energy investment for overcoming a moderate water stress compared with severely stressed plants [72]. Moreover, although all of the overexpressed genes in mild-WS primed plants are involved in plant defense against fungi, not all have been described to be related with avocado tolerance to R. necatrix (i.e. NPR1, PR4, PR5 and endochitinase). However, their enhanced expression after drought-priming (i.e. abiotic factor) could also represent a benefit for avocado plants to overcome forthcoming fungal infection (i.e. biotic stressor).

Conclusions
In conclusion, this is the first study reporting the effectiveness of ‘cross-factor priming’ on the susceptible avocado rootstock ‘Dusa’ for increasing its tolerance to white root rot disease. Mild-WS induced a primed state in the WRR susceptible avocado rootstock ‘Dusa’ by overexpressing fungal defense related genes, revealing that plant responses against biotic and abiotic stress rely on common mechanisms. Although future experiments must be carried out on grafted plants, results presented here indicate the possibility of using moderate water stress as an approach to reduce R. necatrix impact on avocado orchards infected with the pathogen. These results reinforce the use of deficit irrigation strategies for disease management and water savings in cropping areas with limited water resources [74].

Methods
Plant material and experimental design
In order to test if water stress can be used as a priming factor for improving avocado tolerance to R. necatrix, a ‘cross-factor priming’ experiment was carried out in 2017 at the Institute of Agricultural Research and Training (IFAPA) (Málaga, south-eastern Spain, 36° 40′ 25″ N, 04° 30′ 11″ W, elevation of 32 m below sea level). One hundred and twelve 2-year old clonal ‘Dusa’ plants (Westfalia Estate, South Africa) propagated by Brokaw nursery (Brokaw España S.L.) using a modified Frohlich method [106], were grown in 16 L pots containing a
sterilised mixture of organic substrate and sand supplemented with a slow-release fertiliser (Basacote Plus 6 M, Compo Expert GmbH).

‘Dusa’ plants were kept in a greenhouse under day light illumination and semi-controlled conditions of air temperature (T) and relative humidity (RH). Photosynthetic photon flux density (PPFD), T and RH conditions inside the greenhouse were continuously registered by a quantum sensor (Apogee SQ-110, USA) and by a T/RH U23–001 HOBO® Pro v2 logger (Onset Computer Corporation, USA). Maximal midday values of PPFD varied between 440 and 1012 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 between 20 ± 10 °C by an automatic cooling system and heating when necessary. The RH values inside the greenhouse were always over 40%.

The experimental design is depicted in Fig. 1. At the beginning of the experiment (t₀), plant physiological status was tested non-destructively by measuring chlorophyll fluorescence at predawn. Plants were randomly distributed in rows into two sets of 56 plants to conduct two trials. For each trial, 18 plants were randomly assigned to a control group, in which soil moisture was maintained at field capacity (Fc) throughout the experimentation, and two sets of 19 plants were subjected to controlled substrate drying-up until they reached 50% of Fc (i.e. mild water stress, mild-WS) and 25% of Fc (i.e. severe water stress, severe-WS), respectively. Once these soil water content levels were attained (after ~16–17 days; t₁), full irrigation was restored in all plants and drought recovery response was assessed one week after re-watering (i.e. after ~23–24 days; t₂). Hereinafter, the term ‘primed plants’ refers to plants subjected to each of the water stress levels followed by a recovery period. The pathogenicity test with R. necatrix was performed at t₂ as described below.

Soil moisture was monitored in all plants with a wet sensor (HH2 Moisture meter, Delta-T Devices, Cambridge, England), previously calibrated for the substrate, which also allowed adjustment of volumetric soil moisture (v/v) for each water treatment (mild-WS and severe-WS) in relation to the soil water holding at field capacity (Fc~0.4 v/v). Once per week plants were fertilised with an NPK solution (Kristalon Blue 17–6–18, Yara, UK) supplemented with iron chelate (Sequestrene® Syngenta, Spain).

Throughout the experiment, physiological measurements and root samplings were carried out at t₁ and t₂. On each trial, 15 plants per treatment were measured at each sampling point. Roots were sampled from 9 plants per treatment not used for the pathogenicity test.

### Physiological measurements

Midday (12:00–14:00 am) leaf water potential was measured at t₁ (when mild-WS and severe-WS plants reached 50 and 25% of Fc) and at t₂ (one week after re-watering) using a Schölander pressure chamber (model 3005; Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). On each trial, 15 plants per treatment were measured at each sampling point. Measurements were done 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 [107].

Relative leaf water content (RWC), the specific leaf mass area (LMA) and relative chlorophyll content (SPAD index) were measured only at t₁ in the same plants as for leaf water potential determinations. For RWC determinations, leaf discs (2 cm²) 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). Afterwards, samples were oven dried at 80 °C for 48 h to get dry weight (D_W). RWC was calculated as follows:

\[
\text{RWC} = \left[ \frac{(F_W - D_W)}{(T_W - D_W)} \right] \times 100
\]

The specific leaf mass area (LMA) was calculated as the ratio between disc dry weight and disc area (g cm⁻²).

The SPAD index was non-destructively measured at midday on one leaf per plant using a hand-held SPAD 502 m (Minolta, Osaka, Japan). This index provides an estimation of leaf chlorophyll content consistent with leaf greenness [108]. For each plant, averaged SPAD values were calculated from three readings per leaf.

In vivo chlorophyll a fluorescence signals were measured with a portable fluorometer PAM-2100 (Heinz Walz, Effeltrich, Germany) at predawn (t₀) and midday (at t₁ and t₂) in one leaf per plant. The so-called saturation pulse method was used to determine all fluorescence parameters [109]. Dark-adapted parameters (i.e. minimal fluorescence (F₀)), maximal fluorescence (Fₘ) and maximal photochemical efficiency of PSII (Fᵥ/Fₘ = (Fₘ – F₀)/F₀) were determined at predawn (05:00–07:00 am). The steady-state fluorescence (Fᵦ), maximal fluorescence (Fₘ) and minimal fluorescence yield of a pre-illuminated sample (F₀) were assessed in light acclimated leaves (~450 μmol quanta m⁻² s⁻¹). The relative quantum yield of PSII photochemistry (ΦPSII = (Fₘ’’ – Fᵦ)/(Fₘ’’)) [110], the fraction of PSII centres in open state (gL) [47] and the extent of “Stern-Volmer” non-photo-chemical fluorescence quenching (NPQ = (Fₘ’ – Fₘ’’)/(Fₘ’’)) [111] were calculated.

Leaf gas exchange was measured at midday (11:00–14:00 am) at t₁ and t₂ in one mature exposed leaf. Measurements were performed with an open portable
photosynthesis system (model LI-6400, LI-COR, USA) equipped with a LED-light source (6400–02B), coupled to a sensor head/IRGA, and with a CO₂ mixer (6400–01) to modify the incoming air’s CO₂ concentrations. The operating flow rate was 500 mL min⁻¹ and CO₂ partial pressure was 400 ppm. Saturating photosynthetic photon flux density (1000 μmol m⁻² s⁻¹) was chosen as the default condition. Leaf temperature was kept at ~20 °C and relative humidity was adjusted to 50% (vapor pressure deficit ~1.4 kPa). Net CO₂ assimilation rates (A₅₀) and stomatal conductance (gₛ) were estimated with the equations of Von Caemmerer and Farquhar [112].

RNA extraction

Roots from 9 avocado plants from control, mild-WS and severe-WS were harvested at t₁ and t₂ in plants others than those used in the pathogenicity test. Three biological replicates were used for RNA extraction. Each replicate consisted in a bulk sample from three plants. RNA from ground root tissue was extracted using the CTAB extraction method [113], a simple and efficient method for isolating RNA from pine trees with slight modification. The chloroform:isoamyl alcohol step was repeated 3–5 times, depending on the stability of the interphase and colour of the sample. RNA quantity and quality were determined based on A₂₆₀/A₂₈₀ and A₂₆₀/A₁₈₅ ratio using a NanoDrop® ND-1000 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 manufacturer’s instructions. The cDNA as the template.

Quantitative real-time PCR

Single stranded cDNA was synthesized using iScript Reverse Transcription Supermix (Bio-Rad Laboratories Inc., California, USA) according to manufacturer’s instructions. The cDNA was analysed for genomic DNA contamination by PCR using gene specific primers F₃H-F (5’–TCTGATTTC GGGAGATGACTCGC–3’) and F₃H-R (5’–TGTTAG ACTTGGGCCACCTTTT–3’), which flank an intron of the eflavone 3-hydroxylase (F₃H) gene. PCR amplifications were carried out as previously described by Engelbrecht and van den Berg [48] using first-strand cDNA as the template.

The expression of thirteen avocado genes was investigated based on previous literature. The actin gene was used as endogenous control for normalization. Primer sequences for endogenous control gene and the thirteen avocado genes are presented in Additional file 1: Table S1. Primer pairs were chosen to generate fragments between 70 to 140 bp and were designed using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/). Primer specificity was tested by first performing a conventional PCR and confirmed by the presence of a single melting curve during qRT-PCR. Serial dilutions (1:10, 1:20, 1:50, 1:200) were made from a pool of cDNA from each treatment and time-points, and calibration curves were performed for each gene. For qRT-PCR, 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) in a total volume of 20 μl. The PCR conditions were as follows: 30 s at 95 °C, followed by 40 cycles of 1 s at 95 °C and 30 s at 60 °C, 3 min at 72 °C, 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 was analysed using the ΔΔCt method [116]. All reactions were done in triplicate.

Pathogenicity test in avocado plants

Inoculum was produced on wheat seeds according to Sztejnberg and Madar [117]. Briefly, seeds were soaked for 12 h in 250 ml Erlenmeyer flasks filled with distilled water. The flasks, each containing 100 g of seeds, were subsequently autoclaved after excess water drained off. After sterilisation, four 0.5 cm diameter fungal discs of a 2-week-old culture of R. necatrix grown on potato dextrose agar (PDA) were placed aseptically in each flask and incubated at 24 °C in the dark for three weeks until wheat grains were homogeneously covered by R. necatrix mycelium. Seven days after re-watering (t₂),  ‘Dusa’ rootstocks from each treatment (control n = 9, mild-WS n = 10, severe-WS n = 10) on each of the two trials, were inoculated with 3.75 g of colonized wheat seeds per litter of substrate. To ensure the spread of the inoculum, it was placed at eight points scattered around the stem (~3.5 cm apart) and introduced at two depths (~5 cm and ~15 cm, respectively). Disease progression was evaluated by measuring the aerial symptoms of WRR according to a scale: 1 = healthy plant; 2 = mild wilting; 3 = wilting; 4 = desiccated; and 5 = death. The disease index (DI) for each treatment and the area under the disease progress curve (AUDPC) was calculated as previously described by Teixeira de Sousa [118] and Campbell and Madden [119], respectively.
Statistical analysis
Data were analysed using the analytical software STATISTICA 7 (StatSoft, Inc., USA). Differences among treatments in physiological variables and AUDPC were evaluated by analysis of variance (ANOVA). On each sampling point, datasets obtained from the two trials were subjected to a two-way ANOVA, in which ‘trial’ and ‘treatment’ were the between-subjects factors. This analysis allowed to test whether the variability observed between the two trials was significantly different or not, and to what extent it was possible to merge datasets for performing a unique one-way ANOVA for each sampling point. Since no significant effect of ‘trial’ was observed in any of the variables analysed, data from the two trials were analysed jointly. Therefore, data depicted in the figures for each treatment are average values of the measurements taken in the two trials. 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, Fisher’s least significant difference (LSD) test was used to compare mean values. Statistical analysis of qRT-PCR data was carried out by Student’s t-test with Sigma Stat version 4.0 software (Systat Software GmbH).

Additional file

Additional file 1: Table S1. Primers used in the qRT-PCR experiments. (DOC 67 kb)

Abbreviations
ABA: Abscisic acid; AEC: net CO₂ assimilation rates; AUDPC: area under disease progress curve; Dlt: disease index; DW: dry weight; FC: Field capacity; Fc: Fold change; Fm/Fo, max: Maximum photochemical efficiency of PSII; Fv/Fm, max: maximum photochemical efficiency of the open reaction centres of PSII; Fv/Fo: Fresh weight g⁻¹; stomatal conductance; JA: Jasmonic acid; LMA: Leaf mass area; NPQ: Non-photochemical quenching of fluorescence; NPR: Non-expressor of pathogenesis related; PDA: Potato dextrose agar; PPFD: Photosynthetic photon flux density; PR: Pathogenesis-related; PRR: Phytophthora root rot; PSII: Photosystem II; qL: Fraction of PSII centres in open state; qN: Coefficient of non-photochemical quenching; qRT-PCR: real time quantitative PCR; RH: Relative humidity; ROS: Reactive oxygen species; RWC: Relative water content; SA: Salicylic acid; T: Temperature; Tm: Primer melting temperature; Tw; Turgid weight; WRR: White root rot; WS: Water stress; ψpsii: Relative quantum yield of PSII photochemistry

Acknowledgements
Authors would like to thank Mrs. J. Engelbrecht, Dr. A. Zumaquero and Dr. F. Pliego for their support at the laboratory and valuable comments to experimental design.

Authors’ contributions
CP and EMF planned and designed the experiment and obtained the fundings. CP, EMF, GMO and NB conducted the experiments, collected and analysed the data. CP, EMF and GMO prepared the draft. All authors wrote, reviewed and edited the manuscript.

Authors’ information
G. Moreno-Ortega is a graduate student at the Ph.D. Program of Advance Biotechnology, University of Málaga.

Funding
This research was supported by the RTA2017–00400–00–00 (INIA-AEI), AVA201601.14 and AVA2019:008 projects (20% Junta de Andalucía, 80% FEDER). The funding bodies had no role in the design of the study and no role in the collection, analysis, and interpretation of data or in writing the manuscript. C. Pliego is currently supported by an INIA-CCAA contract, co-financed by INIA (20%) and FEDER (80%).

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate
The acquisition, cultivation, and testing of plant materials, carried out in this study, followed national and local legislation.

Consent for publication
“Not Applicable”.

Competing interests
“Not Applicable”.

Author details
1IFAPA. Centro de Málaga. Cortijo de la Cruz s/n, 29140 Churriana, Málaga, Spain. 2Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa. 3Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Received: 5 July 2019 Accepted: 3 September 2019
Published online: 29 October 2019

References
1. Nordqvist C. What are the top healthful foods? Medical news today. MediLexicon, Intl. 20 Jun. 2017. Web. https://www.medicalnewstoday.com/articles/245259.php.
2. World Avocado Organization (WAO). 2019. https://avocadofruitoflife.com/ retail. Accessed 19 June 2019.
3. FAOSTAT. Fao.org. 2018. http://www.fao.org/faostat/en/. Accessed 18 mar 2019.
4. Pérez-Jiménez RM. Significant avocado diseases caused by fungi and cortenyes. Eur J Plant Biotechnol. 2008;21(1):1–24.
5. Coffey MD. Phytophthora root rot of avocado: an integrated approach to control in California. Plant Dis. 1987;71:1046–52.
6. Giblin F, Pegg K, Willingham S, Anderson J, Coates L, Cooke T, et al. Phytophthora revisited. Proc. of the New Zealand and Australia avocado Growers’ Conference, Tauranga, New Zealand. Avocado Growers Association: In; 2005. http://www.avocadosource.com/Journals/AUSNZ/ AUSNZ_2005/GiblinFiona2005.pdf.
7. Freeman S, Sztejnberg A, Rosellina. In: Singleton L, Mihail D, Rush M, editors. Methods for Research on Soilborne Phytopathogenic. St. Paul, Minnesota. USA. APS PRESS; 1992. p71–p73.
8. Pliego C, López-Herrera C, Ramos C, Cazorla FM. Developing tools to unravel the biological secrets of Rosellinia necatrix, an emergent threat to woody crops. Mol Plant Pathol. 2012;13(3):226–39.
9. Barceló-Murillo A, Zea-Bonilla T, Jurado-Valle I, Mimbida-Solano I, Vídy-Mercado I, Pliego-Aíllo F, et al. Programa de selección de portainjertos de aguacate tolerantes a la podredumbre blanca causada por Rosellinia necatrix en el Sur de España 2007. www.avocadosource.com/WACPS/Papers/WACPS_p637.pdf .
10. Conrath U, Beckers GJ, Flors V, García-Agustín P, Jakab G, Mauch F, et al. Priming: getting ready for battle. Mol Plant-Microbe Interact. 2006;19(10): 1062–71.
11. Bruce TJA, Mathews MC, Napior JA, Pickett JA. Stressful memories of plants: evidence and possible mechanisms. Plant Sci. 2007;173(6):603–8.
12. Pastor V, Balmer A, Gamir J, Flors V, Mauch-Mani B. Preparing to fight back: generation and storage of priming compounds. Front Plant Sci. 2014;5:1–12.
13. Conrath U. Molecular aspects of defence priming. Trends Plant Sci. 2011; 16(10):524–31.
14. Wang X, Vignjevic M, Juang D, Jacobsen S, Wollenweber B. Improved
tolerance to drought stress after anthesis due to priming before anthesis in
wheat (Triticum aestivum L.) var. Vinjett. J Exp Bot. 2014;65(22):6441–56.
15. Molassiotis A, Tanou G, Diamantidis G. No says more than ‘YES’ to salt
tolerance. Salt priming and systemic nitric oxide signaling in plants. Plant
Signal Behav. 2010;5(3):209–12.
16. Baradas C, Pinto G, Correia B, Castro B, Phillips AJ, Alves A. Drought x
disease interaction in Eucalyptus globulus under Neofusicoccum eucalyptorum
infection. Plant Pathol. 2018;67:87–96.
17. Pastor V, Luna E, Mauch-Mani B, Ton J, Flors V. Primed plants do not forget.
Environ Exp Bot. 2013;94:446–56.
18. Munné-Bosch S, Peruelo J. Photo- and antioxidative protection, and a role
for salicylic acid during drought and recovery in field grown Phylloxera
augustofolia plants. Planta. 2003;217(5):758–66.
19. van den Berg N, Mahomed W, Olivier NA, Swart V, Crampton BG.
Transcriptome analysis of an incompatible Persea americana-Phytophthora
cinamomi interaction reveals the involvement of SA- and JA-pathways in a
successful defense response. PLoS One. 2018;13(10):e0205705.
20. Ramegowda V, Senthil-Kumar M, Ishiga Y, Kaundal A, Udayakumar M,
Ceasar SA, Ignacimuthu S. Genetic engineering of crop plants for fungal
pathogenesis-related proteins. Plant Mol Biol. 2000;42(3):479–87.
21. Ceasar SA, Ignacimuthu S. Genetic engineering of crop plants for fungal
pathogenesis-related proteins. Plant Mol Biol. 2000;42(3):479–87.
22. Hayat S, Ahmad A. Salicylic acid: a plant hormone. Springer, Dordrecht:
Springer; 2007.
23. Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence: its role
in plant growth and development. J Exp Bot. 2011;62(10):3321–38.
24. Singh B, Usha K. Salicylic acid induced physiological and biochemical changes
in wheat seedlings under water stress. Plant Growth Regul. 2003;39(2):137–41.
25. Chini A, Grant JI, Seki M, Shinozaki K, Loake G. Drought tolerance
established by enhanced expression of the CCI-NBS-LRR gene, ADR1,
requires salicylic acid, EDS1 and ABI1. Plant J. 2014;38(3):10–22.
26. Miura K, Tada Y. Regulation of water, salinity, and cold stress responses by
salicylic acid during drought and recovery in field grown Phillyrea
latifolia plants. Planta. 2003;217(5):758–66.
27. Rejeib IB, Pastor V, Mauch-Mani B. Plant responses to simultaneous biotic
and abiotic stress: molecular mechanisms. Plants (Basel). 2014;3(4):458–75.
28. Hayat S, Ahmad A. Salicylic acid: a plant hormone. Springer, Dordrecht:
Springer; 2007.
29. Miura K, Tada Y. Regulation of water, salinity, and cold stress responses by
salicylic acid. Front Plant Sci. 2014;5:454.
30. Durrer J, Shah J, Klessig DF. Salicylic acid and disease resistance in plants.
Trends Plant Sci. 1997;2(7):266–74.
31. Dempsey DA, Shah J, Klessig DF. Salicylic acid and disease resistance in plants.
CRC Crit Rev Plant Sci. 1999;18(4):547–75.
32. Chaturvedi R, Shah J. Salicylic acid in plant disease resistance. In: Hayat S,
Ahmad A, editors. Salicylic acid: a plant hormone. Dordrecht: Springer; 2007.
p. 335–70.
33. Kumar D. Salicylic acid signaling in disease resistance. Plant Sci. 2014;228:
127–34.
34. Cao H, Bowling SA, Gordon AS, Dong X. Characterization of an Arabidopsis
mutant that is non-responsive to inducers of systemic acquired resistance.
Plant Cell. 1994;6(11):1583–92.
35. Delaney TP, Friedrich L, Rylas JA. Arabidopsis signal transduction mutant
defective in chemically and biologically induced disease resistance. Proc Natl
Acad Sci U S A. 1995;92(16):6602–6.
36. Okushima Y, Koizumi N, Kusano T, Sato H. Secreted proteins of tobacco
cultured BY2 cells: identification of a new member of pathogenesis-related
proteins. Plant Mol Biol. 2002;49(3):741–50.
37. Rodrigues FA, de Laia ML, Zingaretti SM. Analysis of gene expression
profiles of defence-related genes from Persea americana cv. ‘Fortune’ and
‘Murphy’ in response to infection with C. gloeosporioides f. sp. persea. Plant
Sci. 2010;177(5):532–40.
38. de las Mercedes Dana M, Pintor-Toro JA, Cubero B. Transgenic tobacco
plants expressing chitinases of fungal origin show enhanced resistance
to biotic and abiotic stress agents. Plant Physiol 2006;142(2):722–730.
39. Kumar S, Trivedi PK. Glutathione S-transferases: role in combating abiotic
stresses including arsenic detoxification in plants. Front Plant Sci. 2018;9:751.
40. Rodrigues FA, de Laia ML, Zingaretti SM. Analysis of gene expression
profiles under water stress in tolerant and sensitive sugarcanes. Plant Sci.
2008;176(2):286–302.
41. Cruz de Carvalho MH. Drought stress and reactive oxygen species:
production, scavenging and signaling. Plant Signal Behav. 2008;3(3):156–65.
42. Xiao JP, Zhang LL, Zhang HQ, Miao LX. Identification of genes involved in
the responses of potato (Solanum tuberosum L.) to salt stress. J Appl
Environ Sci. 2017;13:831–9.
43. Lv Z, Wang S, Zhang F, Chen L, Hao X, Pan Q, et al. Overexpression of a
novel NAC domain-containing transcription factor gene (NAC1) enhances
the content of artemisinin and increases tolerance to drought and Botrytis
cinerea in Artemisia annua. Plant Physiol Biochem. 2016;107:1961–71.
44. Tang Y, Liao K, Du H, Xu Y, Song H, Li X, et al. A stress-responsive NAC
transcription factor SNAAC1 confers heat and drought tolerance through
modulation of reactive oxygen species in rice. J Exp Bot. 2015;66(21):6803–17.
45. Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, et al. Overexpressing a NAM,
ATAF, and CUC (NAC) transcription factor enhances drought resistance
and salt tolerance in rice. Proc Natl Acad Sci U S A. 2006;103(35):12987–92.
63. Zhang JY, Qi SC, Qiao YS, Zhang Z, Guo ZR. Overexpression of the Malus × aphiflora MvNPR1 gene increased tolerance to salt and osmotic stress in transgenic tobacco. Mol Bi Rep. 2014;41(3):1553–61.

64. Wang N, Xiao B, Xiong L. Identification of a cluster of PR-like genes involved in stress responses in rice. J Plant Physiol. 2011;168(18):2212–24.

65. Mita RC, Sandeen, Kamnath M, Kumar S, Ghosh S. A thiamin-like protein of Cucumis biliscium confers tolerance to fungal pathogen and abiotic stress in transgenic Arabidopsis Sci Rep 2016;6(23540).

66. Wu J, Kim SG, Kang KY, Kim JG, Park SR, Gupta R. Overexpression of a pathogenesis-related protein 10 enhances biotic and abiotic stress tolerance in rice. Plant Pathol 2016;62(3):552–62.

67. Jung YJ, Melencion SM, Lee ES, Park JH, Alinapooy CV, Oh HT et al Universal stress protein exhibits a redox-dependent chaperone function in Arabidopsis and enhances plant tolerance to heat shock and oxidative stress Front Plant Sci 2015;6:1141.

68. Fleischmann F, Koehl J, Portz R, Beltraume AB, Ollswald W. Physiological changes of Fagus sylvatica seedlings infected with Phytophthora cinnola and the contribution of its elicitor 'cincolin' to pathogenesis. Plant Biol. 2005(7):650–8.

69. Clemenz C, Fleischmann F, Haberle KH, Matyssek R, Oßwald W. Photosynthetic and leaf water potential responses of Alnus glutinosa saplings to stem-base inoculation with Phytophthora alni subsp. alni. Tree Physiol. 2008;28(11):1703–11.

70. Martinez-Ferri E, Zumaquero A, Ariza MT, Barceló-Muñoz A, Pliego C. Nondestructive detection of white root rot disease in avocado rootstocks by leaf chlorophyll fluorescence. Plant Dis. 2016;100(1):49–58.

71. Galmés J, Abadía A, Cifre J, Medrano H, Flexas J. Photoprotection processes under water stress and recovery in Mediterranean plants with different growth forms and leaf habits. Physiol Plant. 2007;130:495–510.

72. Xu Z, Zhou G, Shimizu H. Plant responses to drought and rewatering. Plant Signal Behav. 2010;5(6):649–54.

73. Chávez-Zaleikis K, Patakas A, Kofidis G, Bosabalidis A, Nastou A. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Sci Hortic. 2002;95(1):23–30.

74. Moreno-Ortega G, Pliego C, Sarmiento D, Barceló A, Martinez-Ferri E. Yield and fruit quality of avocado trees under different regimes of water supply in the subtropical coast of Spain. Agric Water Manag. 2019;212:192–201.

75. Flexas J, Bota J, Galmés J, Medrano H, Riba-Carbo M. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol Plant. 2006;127(3):343–52.

76. Resco V, Ewers BE, Sun W, Huxman TE, Weltzin JF, Williams DG. Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C3 woody legume, Prosopis velutina New Phytol 2010;186(3):672–82.

77. Maxwell K, Zhang Y, Chalhoub BN. Chlorophyll fluorescence - a practical guide. J Exp Bot. 2000;51(345):659–68.

78. Chen D, Wang S, Cao B, Cao D, Leng G, Li H, et al. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. Front Plant Sci. 2016;7:241.

79. Nardini A, Salleo S. Limitation of stomatal conductance by hydraulic traits: a practical guide. J Exp Bot. 2011;62(1):168.

80. Long Y, Zhang T, Zhang J, Wang J, Li Y, Liu H, Zhang J, et al. Sp2ISP, an annexin-interacting universal stress protein, enhances drought tolerance in tomato. J Exp Bot. 2012;63(15):5593–606.

81. Bassett CL, Baldo AM, Moore JT, Jenkins RM, Soffer DS, Witsieniewski ME, et al. Genes responding to water deficit in apple (Malus x domestica Borkh.) roots. BMC Plant Biol. 2014;14:182.

86. González LP, Boscarillo Camargo RL, Takita MA, Machado MA, Dos Soares Filho WS, Costa MGC. Rootstock-induced molecular responses associated with drought tolerance in sweet orange as revealed by RNA-Seq. BMC Genomics. 2019;20(1):110.

87. Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C. How tree roots respond to drought. Front Plant Sci. 2015;6:547.

88. Jiang CJ, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, et al. Absciscic acid interacts antagonistically with salicylic acid signaling pathway in rice-Magnaporthe grisea. Mol Plant-Microbe Interact. 2010;23(6):791–8.

89. Ali S, Gani BA, Kamili AN, Bhat AA, Mir ZA, Bhat JA, et al. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiol Res. 2018;212–213:29–37.

90. Barker R, Naidoo S, van den Berg N. The nonexpression of pathogenesis-related genes 1 (NPR1) and related family: mechanistic insights in plant disease resistance. Front Plant Sci. 2019;10:102.

91. Suleman P, Al-Musallam A, Menezes CA. The effect of solute potential and water stress on black scorch caused by Chalara paradoxa and Chalara radicillo on date palms. Plant Dis. 2011;85(1):263–2.
110. Genty B, Briantais JM, Baker NR. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA). 1989;990(1):87–92.

111. Bilger W, Björkman O. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynth Res. 1990;25(3):173–95.

112. von Caemmerer S, Farquhar GD. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 1981;153(4):376–87.

113. Chang S, Puryear J, Cairney J. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep. 1993;11(2):113–6.

114. Unterberger A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3—new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.

115. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics. 2007;23(10):1289–91.

116. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.

117. Steenjberg A, Madar Z. Host range of Dematophora necatrix, the cause of white root rot disease in fruit trees. Plant Dis. 1980;64(9):45.

118. Teixeira de Sousa AJ. Lutte contre Rosellinia necatrix (Hartig) Berlese, agent du "pourrié laineux": Sensibilité de quelques espèces végétales et lutte chimique. Eur J For Pathol. 1985;15:323–32.

119. Campbell CL, Madden LV. Temporal analysis of epidemics. I: Descriptions and comparisons of disease progress curve. In: Campbell CL, Madden LV, editors. NY: Wiley; 1990. p 161–162.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.