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Original Article

Hydrogen peroxide is involved in drought stress long-distance signaling controlling early stomatal closure in tomato plants

O peróxido de hidrogênio está envolvido na sinalização de longa distância do déficit hídrico controlando o fechamento estomático precoce em plantas de tomate

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Abstract

It has long been hypothesized that hydrogen peroxide (H_2O_2) may play an essential role in root-to-shoot long-distance signaling during drought conditions. Thus, to better understand the involvement of H_2O_2 in drought signaling, two experiments were carried out using tomato plants. In the first experiment, a split-root scheme was used, while in the second experiment, the tomato plants were grown in a single pot and subjected to drought stress. In both experiments, H_2O_2 and catalase were applied together with irrigation. Control plants continued to be irrigated according to the water loss. In the split-root experiment, it was verified that the application of H_2O_2 to roots induced a clear reduction in plant transpiration compared to untreated or catalase-treated plants. In the second experiment, we observed that H_2O_2-treated plants exhibited similar transpiration when compared to untreated and catalase-treated plants under drought stress. Similarly, no difference in water use efficiency was observed. Thus, we conclude that the increase in H_2O_2 in the root system can act as a long-distance signal leading to reduced transpiration even when there is no water limitation in the shoot. But it has little effect when there is a reduction in the shoot water potential.

Keywords: reactive oxygen species, root-to-shoot communication, split-root, water loss, water relations.

Resumo

Tem sido hipotetizado que o peróxido de hidrogênio (H_2O_2) pode desempenhar um papel essencial na sinalização de longa distância entre a raiz e a parte aérea sob condições de seca. Assim, para melhor entender o envolvimento do H_2O_2 na sinalização da seca, dois experimentos foram realizados com plantas de tomate. No primeiro, foi utilizado o esquema de raízes divididas, enquanto no segundo, os tomateiros foram cultivados em um único vaso e submetidos ao déficit hídrico. Em ambos os experimentos, o H_2O_2 e a catalase foram aplicados juntamente com a irrigação. As plantas do grupo controle continuaram a ser irrigadas de acordo com a perda de água. No experimento de raiz dividida, verificou-se que a aplicação de H_2O_2 nas raízes induziu uma clara redução na transpiração da planta em comparação com plantas não tratadas ou tratadas com catalase. No segundo experimento, observamos que plantas tratadas com H_2O_2 apresentaram transpiração semelhante quando comparadas com plantas não tratadas e tratadas com catalase sob seca. Da mesma forma, não foi observada diferença na eficiência do uso da água. Assim, concluímos que o aumento do H_2O_2 no sistema radicular pode atuar como um sinal de longa distância levando à redução da transpiração mesmo quando não há limitação hídrica na parte aérea. Mas tem pouco efeito quando há redução do potencial hídrico da parte aérea.

Palavras-chave: espécies reativas de oxigênio, comunicação raiz-parte aérea, raízes divididas, perda de água, relações hídricas.

1. Introduction

Drought stress is the main threat for agricultural crops production worldwide, and forecast models suggest that drought occurrences will become even more often (Lesk et al., 2016; Kim and Jehanzaib, 2020). Therefore, plants will be more vulnerable to frequent and severe water scarcity. On the other hands, plants have evolved a complex and intricate set of physiological, biochemical and molecular pathways for drought stress adjustment (Osakabe et al., 2014; Villate et al., 2021; Warsi et al., 2021). For example, from the perception of drought stress, a series of physical and chemical signals are triggered leading to the reprogramming of plant development through mechanisms such as gene expression regulation, modification of hormonal homeostasis, cell wall readjustment and

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stomatal closure (Augé and Moore, 2002; Yang et al., 2021). Interestingly, responses to drought in the shoot, especially stomatal closure, can begin even before leaf water status is reduced (Augé and Moore, 2002; Holbrook et al., 2002). In other words, the reduction in soil water potential is sufficient to trigger adaptive responses in the shoot. Such effects indicate the existence of an efficient drought stress signaling between the root and the shoot.

Thus, some molecules are postulated to act as a drought signal between the root and the shoot. These molecules must be able to modulate ABA biosynthesis in the shoot, since ABA is intricately related to stomatal closure under drought conditions (Hsu et al., 2021). Indeed, it has recently been shown that H_2O_2 biosynthesis is an essential part of the ABA signaling pathway capable of regulating stomatal closure mechanisms under water deficit conditions (An et al., 2016; Niu and Liao, 2016; Skowron and Trojak, 2021).

H_2O_2 is a reactive oxygen species produced under adverse environmental conditions throughout plants life cycle. However, under adverse conditions, H_2O_2 biosynthesis is intensified and, thus, at high levels, it can cause damage to proteins, membrane lipids and nucleic acids (Singh et al., 2017; Xie et al., 2019; Javed and Athar, 2021). Therefore, H_2O_2 concentrations are commonly used as an indicator of the occurrence of oxidative stress in plants subjected to stressful conditions.

On the other hand, in recent decades, H_2O_2 has been widely investigated as a secondary messenger in signaling processes and in response to biotic and abiotic stresses (An et al., 2016; Choi et al., 2017; Nazir et al., 2019; Xie et al., 2019; Silva et al., 2022). For example, as mentioned earlier, H_2O_2 is an essential component of the ABA signaling pathway that induces stomatal closure in drought stress conditions (Niu and Liao, 2016; Singh et al., 2017; Hsu et al., 2021). Therefore, during exposure to drought, there is an accumulation of foliar ABA that induces the activation of anion efflux channels and the inhibition of H^+-ATPase (Mak et al., 2014). At the same time, ABA stimulates the production of H_2O_2 through NADPH oxidase (Rboh, "Respiratory burst oxidase homolog"). H_2O_2 is associated with an increase in cytosolic Ca^{2+} which, together with ABA, induces membrane depolarization, culminating in K^+ efflux (Daszkowska-Golec and Szarejko, 2013). Together, these events result in the loss of guard cell turgor and, consequently, in stomatal closure (Mak et al., 2014).

In addition to its local action on guard cells, it has recently been attributed an important role to H_2O_2 as a signaling molecule capable of mediating systemic responses to different environmental conditions (Mittler et al., 2011; Gilroy et al., 2014; Choi et al., 2017). In fact, recent work has demonstrated the existence of a propagating wave of H_2O_2 in response to environmental stimuli (Choi et al., 2017). Thus, from the point of perception of a particular stressor agent, for example a wound, the induction of H_2O_2 biosynthesis occurs, which propagates through the activation of the NADPH oxidase of neighboring cells (Gilroy et al., 2016; Fraudentali et al., 2020). It is not a direct transport of the H_2O_2 produced at the site of stress, but a cell-to-cell communication that acts as a long-distance signal to activate adaptive mechanisms in organs distant from the stress occurrence region (Gilroy et al., 2014, 2016). Furthermore, the H_2O_2 wave propagation speed can reach an incredible 8.4 cm min^{-1}, which would allow a fast and efficient activation of response mechanisms even in organs distant from the stress occurrence site (Mittler et al., 2011; Gilroy et al., 2014).

It is important to emphasize that the H_2O_2 signaling pathway does not act in isolation, but it is intrinsically associated with other signaling pathways, especially hormonal (Mittler and Blumwald, 2015; van Breusegem and Mann, 2018). Gaion et al. (2018) found that tomato plants grafted on rootstocks with a constitutive response to gibberellins are more tolerant to drought stress. These authors hypothesized that greater tolerance to drought stress was mediated by higher concentrations of H_2O_2 present in tomato mutant. However, it is not clear whether the action of H_2O_2 during drought stress is local, mediating stomatal closure through ABA, or acted as a messenger molecule between the root and the shoot. Therefore, the objective of the present work was to evaluate the involvement of H_2O_2 in drought stress root-to-shoot communication regulating plant water loss.

2. Material and Methods

2.1. Plant and growth conditions

Tomato seeds (cv. BS II0020) were germinated in trays containing a commercial substrate mixture (Carolina Soil® - composed by peat, expanded vermiculite, organic residue, class A agro-industrial organic residue) combined with coconut fiber with a ratio of 1/1, supplemented with 1 $g.L^{-1}$ of NPK (10/10/10) and 4 $g.L^{-1}$ of limestone. Two experiments were carried out to better understand the role of H_2O_2 in the signaling between the root and shoot during drought stress. The plants were grown under natural light with a photoperiod of 12/12 h (light/dark), mean temperature of 25.6/18.6 °C (day/night), and relative humidity of 61.2 ± 19.

2.2. Split-root experiment

Twenty-eight days after sowing (DAS), plants were transplanted with the root system divided and then placed in two 500 mL pots filled with the same substrate mentioned above. Initially, both pots were watered daily to maintain the water level within the field capacity. Water withdrawn was performed 50 DAS by total suspension of irrigation in one of the pots that contained part of the plants root system. Thus, the drought was applied to only part of the root system, while the other part of the root system was watered regularly without compromising the water status on plants shoot (Holbrook et al., 2002). Control plants were watered regularly. Biochemical treatments were applied to one pot only. One day before water suspension, the same pots that were no longer irrigated and one pot from control plants received the following treatments: a) only water; b) water + 1 mM H_2O_2; c) water + 100 µM catalase (CAT, EC 1.11.1.6). The content of H_2O_2 and CAT applied was determined to be capable of inducing stomatal closure and neutralizing the effect of H_2O_2 on stomatal closure, respectively, both based on articles and on our previous dose-response trials (Shin and Schachtman, 2004; An et al., 2016; Li et al., 2016; Sun et al., 2016).

After the onset of stress condition, the water availability in both watered and non-watered pots were monitored for 7 days using tensiometers (SoilControl model M-WaterMeter) (Supplementary Figure S1).

Pots were weighed daily to calculate water loss and the amount of water to be replaced to maintain water availability (watered pot). Growth analyzes, electrolyte extravasation and water relations were performed 7 days after the onset of drought stress.

2.3. Growth analysis

Plants height was determined using a graduated ruler. Leaf area was obtained by scanning the leaves and subsequent image analysis using the Quant software (Vale et al., 2003). The fresh mass of both roots and shoots was determined using an analytical balance and then the material was stored in paper bags and dried at 60 °C for 72 hrs. The dry mass of the roots and shoots was determined using an analytical balance.

2.4. Electrolyte extravasation

Electrolyte extravasation was verified by obtaining five leaf discs from each plant, following the recommendations by Dionisio-Sese and Tobita (1992).

2.5. Leaf temperature and relative water content

Leaf temperature readings were taken every day at 1 pm during the seven days of imposed drought stress using an infrared thermometer (Fluke 59 Max) placed on the terminal leaflet of the third leaf. Relative water content (RWC) was obtained using four leaf discs, as recommended by Turner (1981).

2.6. Water relations

To analysis the plant transpiration at the first day of induction of drought stress, the pots were sealed with plastic film to prevent water loss through drainage (bottom of the pots) and by evaporation (upper part of the pots). Thus, the pots were weighed daily, which allowed us to determine the water daily loss from the plant-pot system exclusively via plant transpiration (total transpiration) as well as transpiration per leaf area (total transpiration/leaf area). In addition, the water use efficiency was calculated based on the dry mass gain from the beginning to the end of the evaluation period, and the total plant transpiration in that period calculated as well (Medeiros et al., 2012; Oliveira et al., 2012). The percentage of open stomata was obtained from the third leaf of each plant using a "super glue" (Super Bonder®) to obtain impressions of the abaxial surface of the epidermis on glass microscope slides and counting using an optical microscope (Martin and Stimart, 2005).

2.7. Water loss of detached leaves

Water loss from detached leaves was determined using the third leaf of the plants at 68 DAS. For this, the petiole of each leaf was inserted into a 2 mL tube (Eppendorf) containing a solution of artificial xylem sap (Carvalho et al., 2011). Thus, after the first evaluation at 9 am, the leaves were submitted to the following treatments: a) only H_2O; b) water + 1 mM H_2O_2; c) water + 100µM of CAT, following the weighing at 2-hour intervals until 21:00 hrs. The water loss values were obtained from the sum of the mass of water lost at each interval.

2.8. Drought stress experiment

To evaluate the role of H_2O_2 in long-distance signaling that regulates plant water loss under drought conditions, a second experiment was carried out. In this experiment, the plants were grown in a condition like that described in the first experiment, but without having their roots divided. Thus, the plants were subjected to a drought condition until the first signs of leaf wilting were observed. Therefore, at 28 DAS, the tomato plants were transplanted into 500 mL pots filled with the same substrate mentioned above. At 50 DAS, drought stress was applied through complete watering suspension, while control plants were maintained at field capacity by replacing transpired water daily. One day before the plants drought stress induction, the following treatments were applied: a) only water; b) water + 1 mM H_2O_2; c) water + 100 µM catalase; water + 1 mM H_2O_2 + 100 µM catalase (applied separately), whose concentrations were based on articles and our previous dose-response trials (Shin and Schachtman, 2004; An et al., 2016; Li et al., 2016; Sun et al., 2016).

Daily after the application of drought stress, to monitor the availability of water, the water potential of irrigated and non-irrigated pots was measured at 13 pm for six consecutive days (period of induction of drought stress and appearance of the first signs of wilting of the plants) (Supplementary Figure S2). After 6 days, at the end of the experiment, samples were collected for growth and water relations analysis as described in items growth analysis, leaf temperature and relative water content and water relations.

2.9. Experimental design and statistical analysis

The split-root experiment was conducted on a completely randomized design, 2 x 3 factorial scheme [two irrigation regime (full irrigation and partial irrigation) and three biochemical treatments (control, H_2O_2 and catalase)], with four replications for each treatment, from which the mean and standard error of each treatment were obtained. The drought stress experiment was conducted on a completely randomized design, 2 x 4 factorial scheme [two irrigation regimes (well-irrigated and drought stress) and four biochemical treatments (control, H_2O_2, CAT and H_2O_2+CAT)], with four replications for each treatment, from which the mean and standard error of each treatment were obtained. All data were subjected to analysis of variance (ANOVA), considering mean comparisons according to the Scott-Knott test at $p<0.05$. The analysis was conducted using RStudio (R. RStudio, Inc., Boston, MA, USA).

3. Results

3.1. Split-root experiment

In this study, tomato plants, cultivar BS II0020, were grown with their roots divided between two pots. Throughout the experiment, one of the pots was irrigated regularly maintaining the water level within the field capacity. Thus, it was possible to preserve the turgidity in plants shoot, regardless the treatment applied to the other pot (irrigated or non-irrigated). In fact, an intelligent experimental design added to the application of exogenous molecules allowed us to evaluate the involvement of hydrogen peroxide (H_2O_2) in the long-distance signaling that regulates water loss by plants.

When comparing the fresh mass of the shoot under full irrigation (both pots irrigated), it was found that plants treated with catalase grew more than plants treated with H_2O_2 or control plants (Figure 1a). In addition, we can observe that the suspension of irrigation in one of the pots reduced the shoot fresh mass only for the plants treated with catalase when compared to full irrigation, although this was not different from the other exogenous treatments under partial irrigation (Figure 1a). Regarding the roots fresh mass, the application of catalase stimulated root growth under full irrigation (Figure 1a). On the other hand, under partial irrigation, we observed a reduction in root fresh mass of untreated plants that showed lower root growth, while plants that received H_2O_2 and catalase showed intermediate and higher values, respectively, for root fresh mass (Figure 1a).

In relation to shoot dry mass, there was no difference between treatments, regardless of condition applied (Figure 1b). Regarding the root dry mass, the exogenous treatments did not affect root growth (Figure 1b). On the other hand, the suspension of irrigation reduced the dry mass of the roots only in plants without exogenous treatment, which exhibited lower root dry mass in comparison to other treatments (Figure 1b). There was no effect of exogenous treatments on the plants leaf area. Furthermore, we noticed a reduction in leaf area on untreated plants when exposed to partial irrigation, but it did not differ from the other treatments (Figure 1c). In relation to height, there was no difference between treatments, regardless of condition applied (Figure 1d).

There was no effect of exogenous treatments on the plants electrolyte extravasation and foliar temperature, independent of the water regime applied (Figure 2a and 2b).

Further, the relative water content found in the plants did not differ between water regimes applied (Figure 3a). Difference was found only in plants under full irrigation, whereas untreated plants had higher relative water content compared to plants treated with H_2O_2 or catalase (Figure 3a). In turn, the application of H_2O_2 was able to induce a reduction in both total transpiration (total plant water loss) and transpiration per cm^2 of leaf area when compared to control plants, regardless the cultivation condition used (Figure 3b and 3c). It is noteworthy that the peroxide-induced reduction in transpiration was more

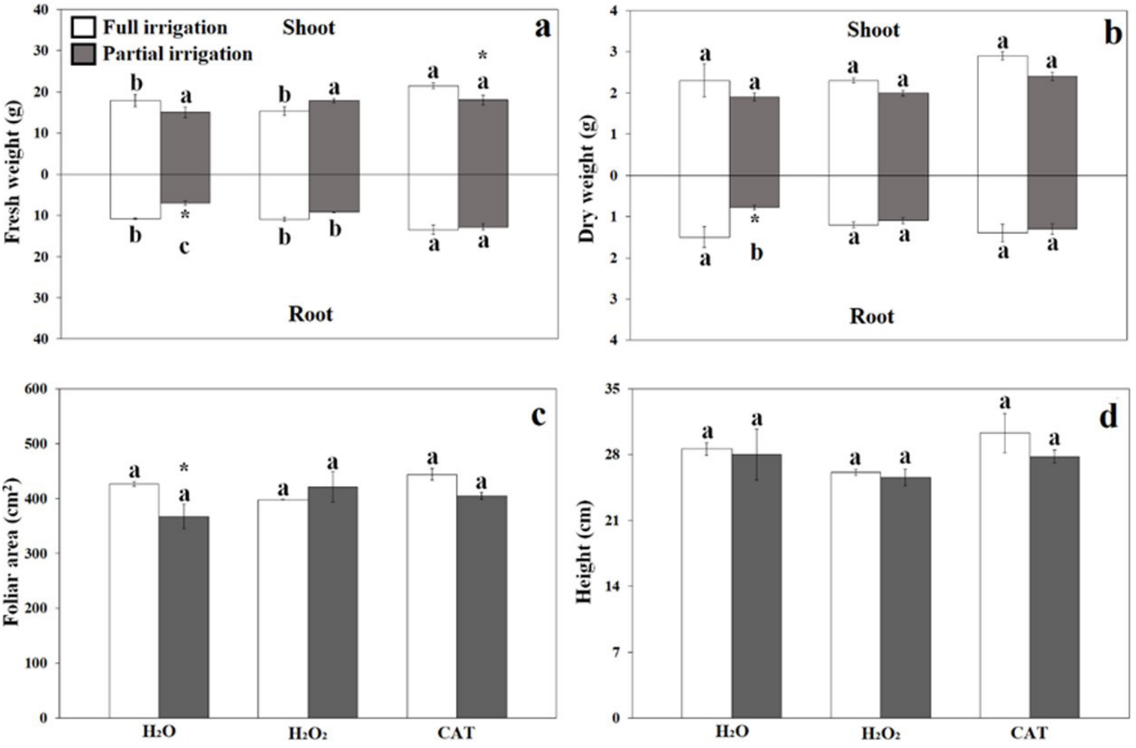

Figure 1. Growth analysis of tomato BS II0020 grown in split-root scheme under full or partial irrigation. (a) fresh weight; (b) dry weight; (c) foliar area; (d) height. Control plants received full irrigation throughout the experiment. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means between biochemical treatments within each condition, and the asterisks the differences of the same biochemical treatment between the conditions, calculated by Scott-knott test at 5% probability.

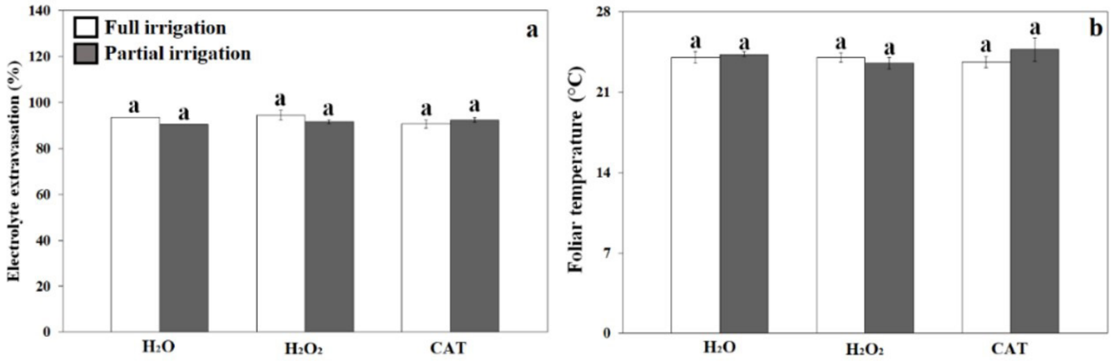

Figure 2. Electrolyte extravasation (a) and foliar temperature (b) analysis of tomato BS II0020 grown in split-root scheme under full or partial irrigation. Control plants received full irrigation throughout the experiment. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means between biochemical treatments within each condition, calculated by Scott-knott test at 5% probability.

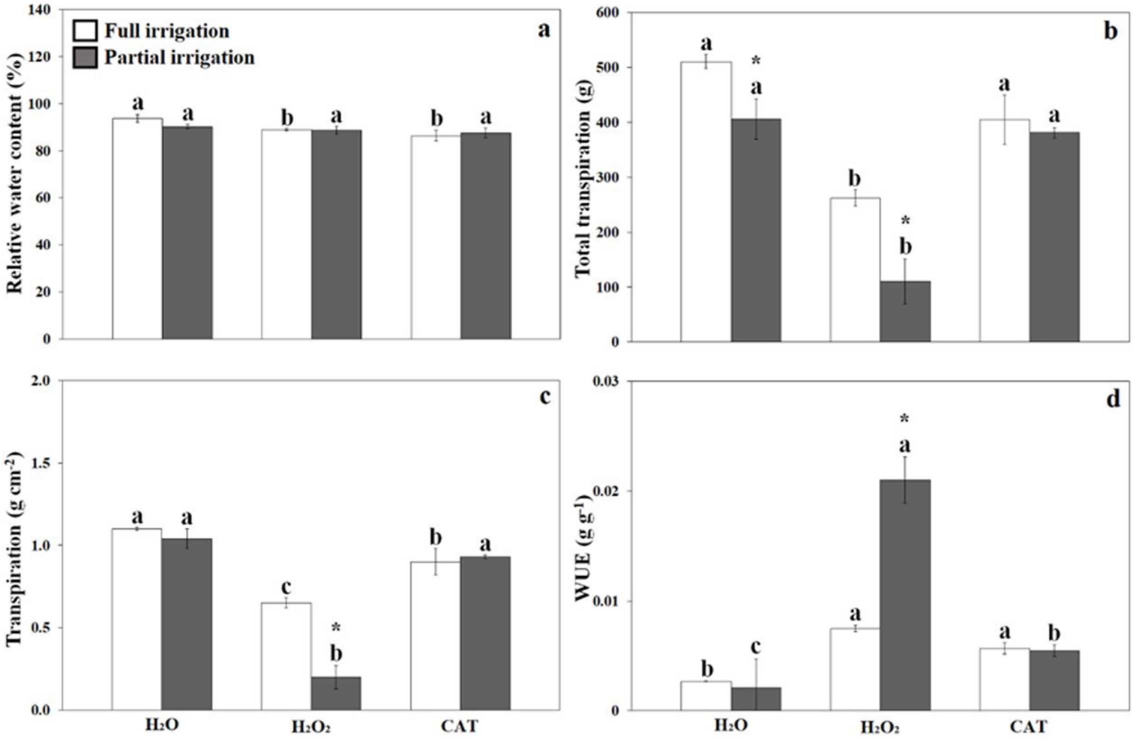

Figure 3. Water relations of tomato BS II0020 grown in split-root scheme under full or partial irrigation. (a) relative water content; (b) total transpiration of plants throughout the evaluation period; (c) transpiration per cm^2 of leaf area; (d) water use efficiency. Control plants received full irrigation throughout the experiment. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means among biochemical treatments within each condition, and the asterisks the differences of the same biochemical treatment between the conditions, calculated by Scott-knott test at 5% probability.

pronounced in plants under partial irrigation. On the other hand, although partial irrigation reduced total transpiration in untreated plants, the same finding was not observed in plants that received catalase (Figure 3b). This finding demonstrates that the application of catalase prevented the reduction of transpiration under partial irrigation conditions, at least at the plant level. Regarding the transpiration by foliar area, it was found that the partial irrigation inhibited transpiration only in H_2O_2-treated plants, while untreated and catalase-treated plants were not affected by partial irrigation (Figure 3c). In relation to water use efficiency, plants treated with H_2O_2 showed an increase in water use efficiency at full and partial irrigation conditions when compared to untreated plants. Moreover, catalase-treated plants exhibited intermediated values, independent of the water condition (Figure 3d).

H_2O_2-treated plants exhibited the lowest percentage of open stomata regardless of the irrigation condition adopted (Figure 4a). On the other hand, untreated and catalase-treated plants showed a higher percentage of open stomata in both conditions, not differing from each other (Figure 4a). To evaluate the effects of the *in loco* treatments, without the need for long-distance transport, a test was carried out to evaluate the water loss of detached tomato leaves. Here, it was also observed that the treatment with H_2O_2 reduced drastically the loss of water in the leaves (Figure 4b). While leaves treated with catalase showed similar water loss in comparison to the control treatment (Figure 4b).

3.2. Drought stress experiment

In this experiment, tomato plants were grown in two different conditions, irrigated and drought. Thus, it was possible to evaluate the effects of treatments with H_2O_2, catalase and the combination of both on growth and water relations, especially transpiration of tomato plants when there was a reduction in the shoot water level.

Regarding to shoot fresh mass under irrigated conditions, no difference was observed among exogenous treatments (Figure 5a). On the other hand, when exposed to the drought, all plants showed a similar reduction in shoot fresh weight in comparison to the irrigated condition (Figure 5a). The root fresh weight was positively affected by treatment with H_2O_2, exhibiting greater root growth when compared to the other treatments, under irrigated condition (Figure 5a). In turn, drought stress inhibited root fresh weight only in plants treated with H_2O_2. In this condition, treatments did not differ from each other (Figure 5a).

The accumulation of shoot dry mass was influenced by exogenous treatments (Figure 5b). Plants that received catalase or catalase + H_2O_2 exhibited greater accumulation of shoot dry mass when compared to untreated plants (without exogenous treatment) and plants that received only H_2O_2 (Figure 5b). In addition, all plants grown under drought condition showed a reduction in shoot dry mass, without differing from each other (Figure 5b). In relation to the root dry mass, no difference was observed between the plants regardless of the water condition or exogenous treatment (Figure 5b).

Regarding leaf area, it appears that the treatment with catalase + H_2O_2 provided greater development of leaf area in tomato plants under irrigated conditions, when compared to the other treatments (Figure 5c). The restriction of water availability under drought condition led to an inhibition of leaf area expansion only in plants that received catalase or catalase + H_2O_2 (Figure 5c). Furthermore, under drought stress, we found that plants treated with catalase had the lowest values of leaf area when compared to the other treatments (Figure 5b). Plant height was not influenced by treatments with H_2O_2 and/or catalase (Figure 5d). When plants were grown under drought conditions, we observed a lower plant height, regardless the exogenous treatment used (Figure 5d).

In terms of relative water content, irrigated plants treated with H_2O_2 showed an increase in leaf water content compared to untreated plants (Figure 6a). On the other hand, when plants were submitted to drought stress, there was a reduction in the relative water content in all plants, except in plants treated with catalase + H_2O_2 (Figure 6a). Leaf temperature did not differ in relation to exogenous treatment (Figure 6b). However, under drought stress, all plants showed an increase in leaf temperature, not differing from each other in this condition (Figure 6b).

Total transpiration and average daily transpiration of tomato plants showed similar pattern, both were reduced as a function of H_2O_2 application, when compared to other treatments under irrigated conditions (Figure 6c and 6d). However, when exposed to drought stress, all plants showed a similar reduction in total transpiration and average daily transpiration (Figure 6c and 6d). Moreover, in the drought stress condition, there was no difference between treatments for both parameters (Figure 6c and 6d).

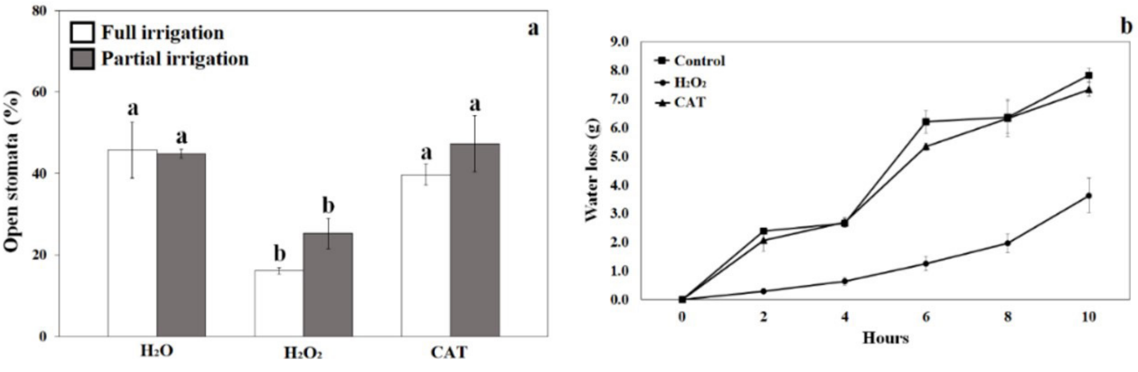

Figure 4. Open stomata (a) and water loss by detached leaves (b) of tomato BS II0020. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means among biochemical treatments within each condition, and the asterisks the differences of the same biochemical treatment between the conditions, calculated by Scott-knott test at 5% probability.

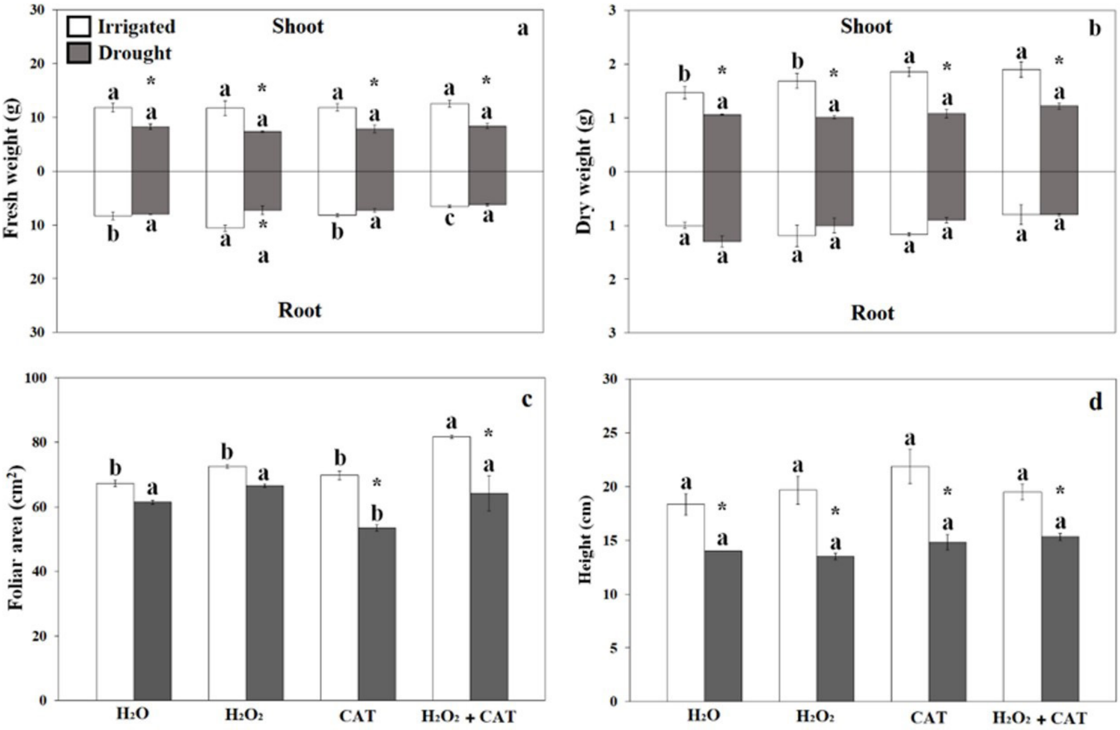

Figure 5. Growth analysis of shoots and roots of tomato BS II0020 grown under irrigated or drought conditions. (A) fresh weight; (b) dry weight; (c) foliar area; (d) height. Control plants received full irrigation throughout the experiment. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means between biochemical treatments within each condition, and the asterisks the differences of the same biochemical treatment between the conditions, calculated by Scott-knott test at 5% probability.

Under irrigated conditions, it is noted that the application of H_2O_2 reduced the transpiration by foliar area, when compared to the control plants (Figure 6e). In turn, the drought stress led to a reduction in transpiration as a function of leaf area in all treatments, with no difference among them (Figure 6e).

Under irrigated condition, all plants subject to some exogenous treatment, catalase and/or H_2O_2, showed higher water use efficiency in comparison to control plants (Figure 6f). On the other hand, the drought stress induced an increase in water use efficiency only in untreated plants, which showed higher water use efficiency in relation to the other treatments (Figure 6f).

4. Discussion

4.1. Partial root-zone drying

The growth analysis data show that our strategy using a split-root experiment was successful, as the untreated plants showed no restriction for shoot growth (shoot fresh, dry weight and height) when under partial irrigation (Figure 1). It was possible to apply the drought stress to part of the root system without affecting the shoot growth. On the other hand, root growth of untreated plants was negatively affected by partial irrigation (Figure 1a and 1b). Actually, it has been shown that plants grown in pots and subjected to severe water deficit can exhibit a lower root system growth (Morales et al., 2015; Xu et al. 2016). However, treatments with H_2O_2 and catalase favored the maintenance of root growth under partial irrigation (Figures 1a and 1b). Indeed, these compounds can modulate root development in different ways, although both modulates auxin hormone signaling (Su et al., 2018; Zhou et al., 2018; Kora and Bhattacharjee, 2020). For example, exogenous H_2O_2 treatment causes a significant growth of the adventitious roots in bean (*Vigna radiata*) plants (Kora and Bhattacharjee, 2020). Whereas mutant *Arabidopsis thaliana* plants unable to synthesize catalase become insensitive to the application of indolebutyric acid, which reduces the emission of lateral roots (Su et al., 2018). Thus, the H_2O_2 and catalase application can stimulate root growth by modulation of auxin signaling.

On the other hand, more recently, it has been shown that some plants have the ability to redistribute water between well-watered roots and those exposed to drought conditions (Miranda et al., 2021). For example, Rangpur lime (*Citrus limonia* Osbeck) plants, a citrus rootstock, showed a capacity for root hydraulic redistribution that was associated with its ability for osmotic regulation, aperture stomatal and transpiration adjustment (Miranda et al., 2021). This

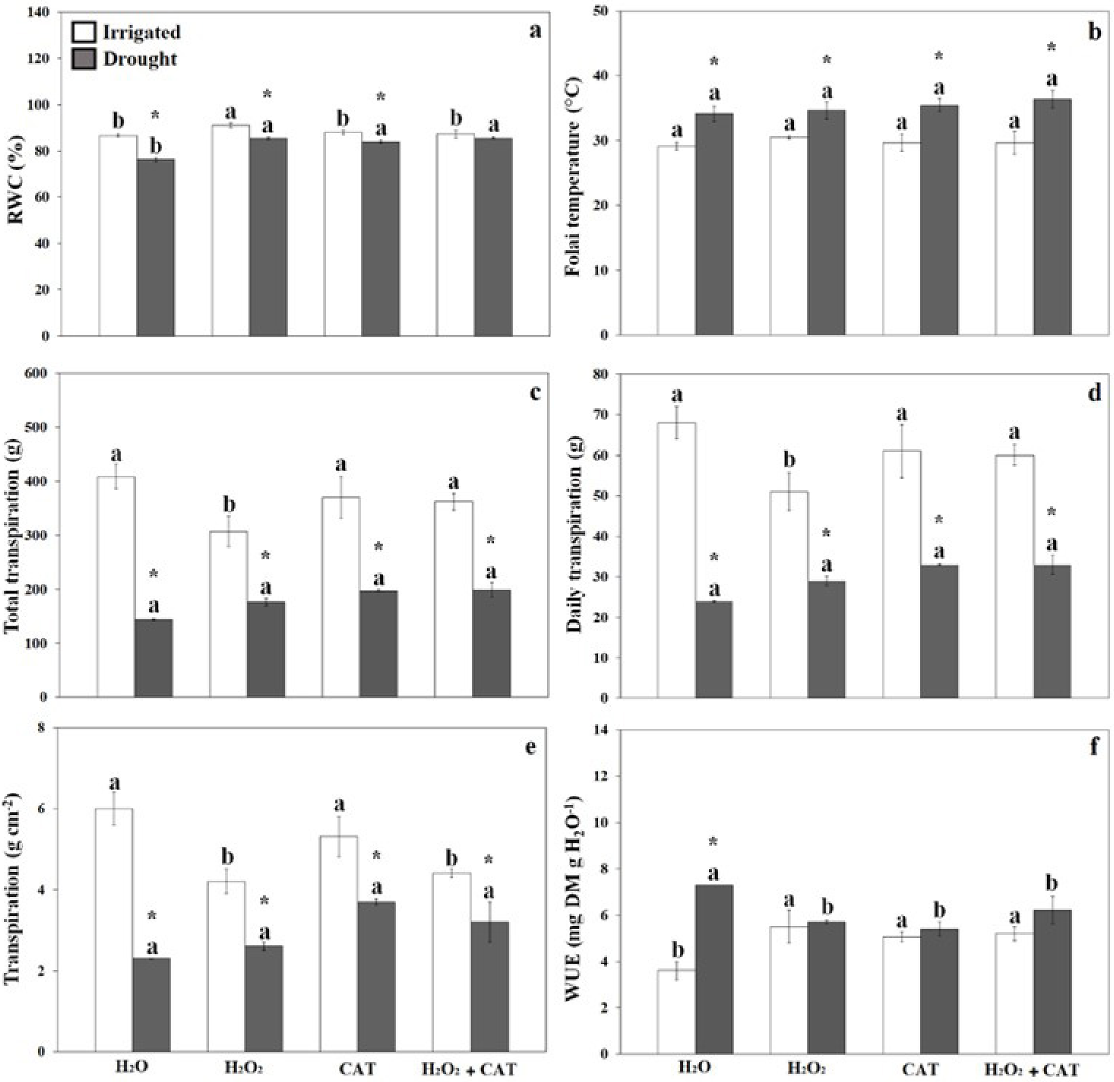

Figure 6. Water relations analysis of tomato BS II0020 cultivated under irrigated or drought conditions. (a) relative water content; (b) leaf temperature; (c) total transpiration of plants throughout the evaluation period; (d) average daily transpiration; (e) transpiration per cm^2 of leaf area; (f) water use efficiency (shoot dry mass/total transpiration). Control plants received full irrigation throughout the experiment. The values are the means of each treatment (n= 4), followed by the standard error. The letters over the bars represent the differences in the means between biochemical treatments within each condition, and the asterisks the differences of the same biochemical treatment between the conditions, calculated by Scott-knott test at 5% probability.

hydraulic redistribution could even be an important part of the communication of the drought stress between the different plant organs (Christmann et al., 2013).

In addition, partial irrigation or H_2O_2 application did not increased electrolyte extravasation, there was no enhance in oxidative stress (Figure 2a). We can confirm that H_2O_2 levels did not reach harmful levels to the plant, but it could act as a long-distance signaling molecule during partial root-zone drying (Gilroy et al., 2016; Verma et al., 2019). In addition, the maintenance of leaf temperatures and relative water content (Figure 2b and 3a) indicates that there was no change in the shoot water status (Rojo et al., 2016; García-Tejero et al., 2017; Zhang et al., 2019). The maintenance of shoot water status, allow us to isolate the effect of H_2O_2 and catalase application on the long-distance signaling that control plant transpiration in response to soil moisture reduction.

Our results demonstrated that the irrigation suspension applied to part of the root system alone did not generate long-distance signaling between roots and shoot capable of inducing stomatal closure (Figure 4a). Thus, although partial root-zone drying induced lower total transpiration, there was not a reduction in transpiration per cm^2 of foliar surface or improvement in water use efficiency in plants control or plants treated with catalase (Figure 3). In turn, plants treated with catalase showed no reduction in transpiration or increase of water use efficiency in comparison to full irrigation condition. This demonstrated that the catalase, an H_2O_2 scavenger, application impaired the plant ability to respond to the decline in soil water potential. On the other hand, the application of H_2O_2 induced a reduction in transpiration even when under full irrigation (Figure 3b and 3c). This transpiration reduction was accompanied by a strong stomata closure (Figure 4). Therefore, these data show that the long-distance root-to-shoot signaling that induces the reduction of transpiration, when plants are exposed to reduced soil water availability, may be dependent on the accumulation of H_2O_2 in the roots. Furthermore, the reduction in the transpiration rate for peroxide-treated plants was accompanied by an increase in the efficiency of water use and lower water loss of detached leaves, when compared to the control plants (Figure 3d and 4b).

In fact, H_2O_2 has been shown to act at the cellular level together with the ABA inducing stomatal closure (Jiang and Zhang, 2002; Verma et al., 2019; Yoshida et al., 2021). In addition, when plants are treated with H_2O_2 inhibitors or scavengers, a reduction in the ability of plants to close stomata in response to drought stress or even the application of ABA is observed (An et al., 2016; Niu and Liao, 2016). Thus, making it more difficult for plants to minimize transpiration under extreme conditions to increase water use efficiency.

4.2. Drought experiment

The plant growth results showed us a similar pattern among exogenous treatments within each growth condition, irrigated or drought conditions (Figure 5). We observed that the shoot dry mass was higher in plants treated with catalase or catalase + H_2O_2 under irrigated conditions (Figure 5b). In fact, catalase is an important enzyme of the plant antioxidant system, being a key factor in protecting the structure and functioning of the photosynthetic apparatus, which may explain the improvement in dry mass accumulation in plants that received catalase exogenously (Brisson et al., 1998; Arias-Moreno et al., 2017; Palma et al., 2020). When exposed to drought stress, plants exhibited reduction of shoot growth and similar values among exogenous treatments, except for leaf area, where only the plants treated with catalase isolate or with H_2O_2 suffered reduction on leaf area expansion (Figure 5c). This is probably associated with the ability of plants to adjust water loss under drought conditions, since leaf expansion is one of the most sensitive processes to reduce water availability (Liu et al., 2003).

In the irrigated condition, we can observe a clear effect of H_2O_2 application on tomato plants water use efficiency (Figure 6). Indeed, all transpiration parameters evaluated (*i.e.*, total transpiration, daily and per leaf area transpiration) were reduced by the application of H_2O_2 in tomato plants (Figure 6). Thus, the reduction in transpiration provided an increase in the relative water content in plants treated with H_2O_2 (Figure 6a). Furthermore, following the application of H_2O_2 that led to reduced transpiration of irrigated plants, we observed an increased water use efficiency in plants treated with H_2O_2, although it did not differ from the plants treated with catalase (Figure 6f). When grown under drought stress, only untreated plants exhibited increased water use efficiency that was greater than plants treated with H_2O_2 and catalase (Figure 6f).

In fact, H_2O_2 has been shown to be an important long-distance signaling molecule considering different environmental stresses, including being able to regulate stomatal conductance in sugarcane plants (Mittler et al., 2011; Silva et al., 2015; Gilroy et al., 2016; Khedia et al., 2019). Thus, from the region where the stress occurs, a wave of H_2O_2 would be generated which would travel rapidly from cell to cell; reaching 8.4 cm min^{-1}; inducing a systemic response throughout the plant (Gilroy et al., 2016; Verma et al., 2019). Similar to a plant subjected to drought stress, in which the root system is the first organ to experience reduced water availability, it becomes essential that a rapid signaling should be generated from the roots and induces the reduction of transpiration in the shoot (Jia and Zhang, 2008; Christmann et al., 2013; Lu et al., 2020). This should occur before the shoot water status is compromised (Holbrook et al., 2002; Devireddy et al., 2018; Gupta et al., 2020). We demonstrated that catalase application cancels out the effect of H_2O_2 application, since plants treated with catalase and H_2O_2 exhibited values like transpiration in control plants, except for leaf area transpiration (Figure 6e). However, the lower transpiration per leaf area of plants treated with catalase and H_2O_2 seems to be more associated with greater leaf area than the reduction in transpiration *per se* (Figure 6e and 5e).

When exposed to drought stress, we observed similar responses for growth and water relations between exogenous treatments. These results indicated that, under a more severe water deficit condition, which impairs the shoot water status, the signaling with H_2O_2 is possibly not essential for communication between the root and shoot, as well as to trigger the long-term drought acclimatization responses. Nevertheless, the importance of H_2O_2 in the signaling cascade that leads to stomatal closure due to the accumulation of abscisic acid in guard cells has been widely evidenced (An et al., 2016; Niu and Liao, 2016; Takahashi et al., 2020).

In this work, we provide evidence that H_2O_2 can acts as part of a long-distance signaling involved with stomatal closure and, consequently, reduction of water loss, especially in the early stages of drought stress, when there is still no reduction in the shoot water potential. Therefore, H_2O_2 could behave as a long-distance messenger, regulating stomatal opening according to fluctuations, even daily, in water availability. When the drought stress becomes more severe and negatively affects the shoot, other signals of a different nature (*e.g.*, hormones, chemical elements, reactive nitrogen species, electrical currents, hydraulic signals and pH changes) could act, alone or together, as long-distance messengers in an intricate signaling network that ensures plant adjustment to the water deprivation (Christmann et al., 2013; Karuppanapandian et al., 2017; Eisenach and De Angeli, 2017; Li et al., 2017; Sarwat and Tuteja, 2017; Fichman and Mittler, 2020; Mahmood et al., 2020). Finally, our results led us to develop a schematic representation where the accumulation of H_2O_2 in the root system would act as a long-distance signaling and so, trigger

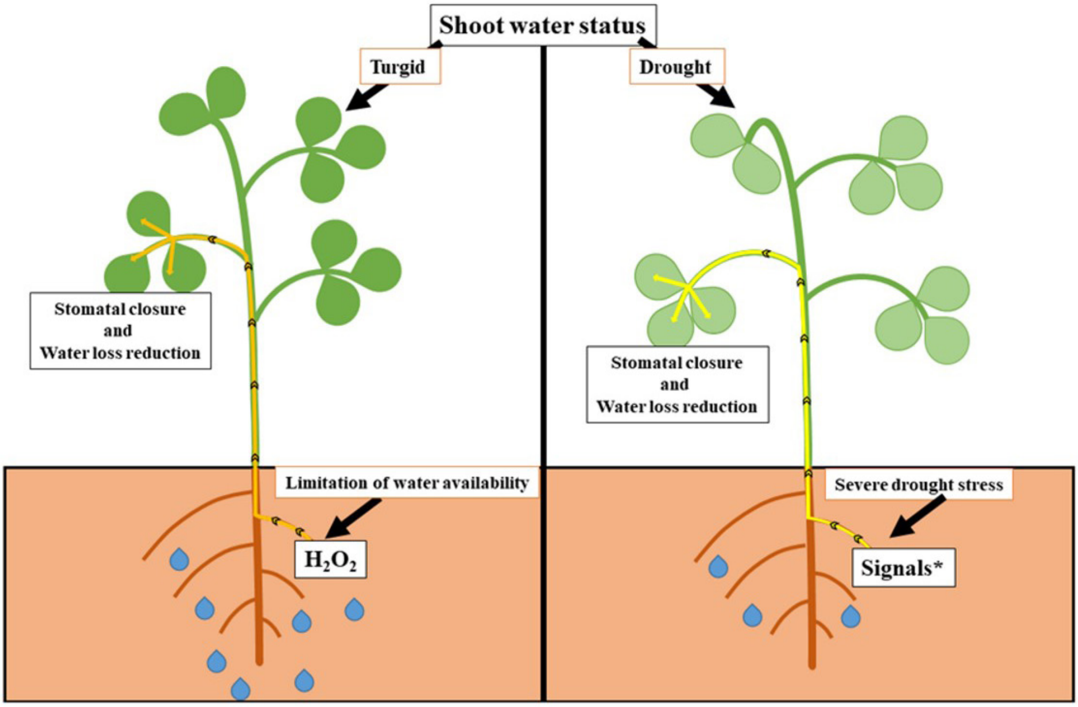

Figure 7. Schematic representation of the proposed model for the role of H_2O_2 in drought stress responses in tomato plants before and after the decline in shoot turgor. Thus, when there is a mild drought stress, the H_2O_2 produced by the roots can travel to the shoot where it will induce stomatal closure and thus reduce water loss, even before there is a reduction in the leaves water status. On the other hand, when drought stress becomes severe, other signals become part of the drought response complex, such as hormones, pH changes and electrical current, among others. Currently, H_2O_2 appears to exert a lesser effect on drought signaling. *Several signals, such as hormones, chemical elements, reactive nitrogen species, electrical currents, hydraulic signals and pH changes (Christmann et al., 2013; Silva et al., 2015; Karuppanapandian et al., 2017; Huber et al., 2019; Fichman and Mittler, 2020; Mahmood et al., 2020).

the stomatal closure and the reduction of transpiration, during small fluctuations in soil water availability. When drought stress is severe and negatively affects the shoot, other signals could act independently of H_2O_2 and modulate stomatal closure (Figure 7).

5. Conclusion

We concluded that in addition to the well-documented *in loco* effect of H_2O_2 inducing stomatal closure, the increase in H_2O_2 in the root system can act as a long-distance signal leading to reduced transpiration in tomato plants (Figure 7). However, H_2O_2 seems to act mainly in conditions where the shoot water status has not yet been affected. In this condition, the exogenous H_2O_2 application to the roots induced reduction of total transpiration and transpiration by leaf area, as well as increased water use efficiency, in both conditions, full and partial irrigation. When drought stress becomes more severe, affecting of the shoot water content negatively, root-H_2O_2 does not seem to be essential for reducing plant transpiration, possibly by the involvement of signals of other natures. Finally, further studies can help us to better understand the mechanisms involved in the action of H_2O_2 as a long-distance signal, thus allowing its use as an important tool to improve the efficiency of water use in agricultural crops.

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Supplementary Material

Supplementary material accompanies this paper.
Supplementary informations

Supplementary figure S1. Water potential of full-irrigated (control) and non-irrigated (drought stress) pots during the experiment. Tensiometer readings were taken at 8:00 h and 13:00 h.
Supplementary figure S2. Water potential of full-irrigated (control) and non-irrigated (drought stress) pots during the experiment. Tensiometer readings were taken at 13:00 h.

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