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ORIGINAL PAPER

Regulation of the drought response of sweet pepper (*Capsicum annuum* L.) by foliar-applied hormones, in Mediterranean-climate greenhouse conditions

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Abstract Plant hormones play an important role in regulating stress responses and signaling in plants; many of them act to alleviate environmental stresses. However, the specific effects and physiological changes could be significantly altered according to the crop species, application concentration and frequency, and cultivation conditions. In this study, we investigated the effect of leaf-applied abscisic acid (ABA), gibberellic acid (GA_3), and indole-3-acetic acid (IAA) on plant growth before, during, and after water stress. The objective was to determine their effects on pepper plants (*Capsicum annuum* L.) in commercial greenhouse conditions, specifically their ability to mitigate water stress, through the study of different stress traits—such as plant growth, gas exchange parameters, chlorophyll content and fluorescence, ascorbate peroxidase activity, total phenolic compounds, and lipid peroxidation. While ABA and IAA heightened the water shortage in the leaves along the experiment, GA_3 diminished it. The effects of ABA involved short-term responses, such as stomatal closure and decreased transpiration, and long-term changes, affecting the ratios and concentrations of chlorophylls. Moreover, GA_3 complicated the crop management since the plants suffered high stress when treated with this hormone. The results obtained represent a first approach to studying the effect of foliar hormone application in sweet pepper and its ability to regulate (mitigate or amplify) the water stress suffered by the plant under greenhouse conditions.

Keywords Sweet pepper · ABA · GA_3 · IAA · Water stress

Introduction

Water stress is an important issue in crops cultivated under greenhouse conditions in Mediterranean countries (Alpi and Tognoni 1990). Its impact appears likely to assume greater significance as a result of the predicted shortage of water resources and global warming (Lobell et al. 2011). Thus, there is growing interest in understanding the underlying mechanisms of drought tolerance, and this includes responses that go beyond stomatal control. There are different mechanisms involved in the plant response to drought: those related to gas exchange (that prevent plant desiccation), those that are a result of stress (such as enzymatic oxidation activities, phenol content, or lipid oxidation), and those that can affect the production of photosynthates. Drought activates different responses that warn plants of the imminent stress (Ismail et al. 2002). Hence, plants rapidly activate their physiological and biochemical processes to promote the expression of different mechanisms which confer the drought tolerance. Traditionally, the mechanisms identified as responses to stress have been linked with the action of the phythormones since plant hormones levels are highly regulated and responsive to changes in the environment (Santner et al. 2009).

The plant hormones are a structurally-unrelated collection of small molecules derived from various essential metabolic pathways (Santner et al. 2009). Therefore, many of them are interconnected not only in their responses but

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also in their biosynthesis and degradation. However, the effects of plant hormones may vary depending on the developmental stage of the plant (San-Francisco et al. 2005), the species, or the culture conditions (del Amor and Cuadra-Crespo 2011). Indole acetic acid (IAA) is the major naturally-occurring auxin and polar auxin transport plays a major role in plant growth and development (Tantasawat et al. 2015). The roles of IAA range from virtually every aspect of plant growth and development to defense responses. Thus, exogenous application of IAA is potentially able to alleviate the adverse effects of water stress (Ashraf et al. 2006, Korovetska et al. 2016).

The hormones used most in commercial farming are the gibberellins, since they are involved in plant elongation and development and delay fruit maturation and abscission of leaves (Yilmaz and Ozguyen 2009). Gibberellic acid (GA_3) applied to tomato plants have been shown to induce marked stem elongation (Bukovac and Witter 1956) and to increase fresh weight (Bukovac and Witter 1956; Rappaport 1956). Also, GA_3 has the capability to regulate its own concentration, by repressing the expression of several genes whose products are involved in its biosynthesis and by promoting the expression of genes involved in GA_3 inactivation (Peng et al. 1999; Yamaguchi 2008). Thus, the versatility of this plant hormone and its capacity for interaction with other hormones indicate that gibberellins may be able to mitigate certain effects of water stress (Wang et al. 2008).

Nonetheless, the plant hormone that has been related most commonly to stress is abscisic acid (ABA). It has been associated with responses to stresses such as drought, extreme temperatures, and excess light (Hirayama and Shinozaki 2007; Thompson et al. 2000; Wang et al. 2015) and with other growth processes. In fact, it has proved to be an effective tool to modulate plant shoot growth and leaf abscission and to enhance the drought stress tolerance of several horticultural species, including pepper, tomato, melon, and artichoke (Leskovar et al. 2009). Notwithstanding, although ABA accumulation has been linked to a reduction in stomatal conductance (Fahad et al. 2014), many authors have reported negative exponential relationships between leaf conductance and ABA concentrations (Ismail et al. 2002); this contradictory information probably is due to the differences in sensitivity to hormones of the different plant genotypes (Pérez-Jiménez et al. 2013).

Since the variation in the previously-reported results concerning the effect of exogenous hormone applications in plants under drought conditions is large, this study is concerned with the analysis of the impact of plant hormone application, a common tool in agriculture, on sweet pepper (*Capsicum annuum* L.), grown in a commercial greenhouse, under water stress. Our objective was to analyze the effect of three different hormones on pepper plants and their ability to mitigate water stress, through the study of different stress indicators—such as plant growth, gas exchange parameters, chlorophyll content and fluorescence, ascorbate peroxidase activity, total phenolic compounds, and lipid peroxidation.

Materials and methods

Plant material and growth conditions

Sweet pepper plants, California type, were obtained from a commercial nursery. They were grown in 1.2-m-long bags filled with coconut fiber, in a greenhouse equipped with a computer-regulated drip-irrigation system, under controlled environmental conditions. Each bag had three plants and three $4\ l\ h^{-1}$ drippers. Irrigation management was according to local commercial soilless cultivation and the drainage percentage was maintained at 30 % (del Amor and Gomez-Lopez 2009). Sixty days after transplanting (DAT), the treatments started. Thirty-six plants were used for each treatment. Each treatment consisted of the application of a plant growth regulator (PGR) plus a surfactant (Tween 20) to the aerial part of the plants, every 2 weeks for 2 months: T0, distilled water +0.5 % (v/v) Tween 20; T1, 100 mg l^{-1} abcisic acid (ABA) +0.5 % (v/v) Tween 20; T2, 32.2 mg l^{-1} gibberellic acid (GA_3) +0.5 % (v/v) Tween 20; and T3, 32.2 mg l^{-1} indole-3-acetic acid (IAA) +0.5 % (v/v) Tween 20. The PGRs were sprayed early in the morning. Guard rows were placed at both ends to avoid contamination during the foliar application. The treated plants were sprayed completely and homogeneously with the PGR solutions (young and old leaves, and stems). Forty-two days after the start of the experiment (112 DAT), the irrigation was interrupted for 4 days.

Plant measurements and leaf samples were taken after the PGR treatment: before (112 DAT), during (116 DAT), and after water stress (120 DAT).

Growth parameters

In order to determine the effect of the water stress and foliar application of the PGRs on plant growth, 16 plants per treatment were harvested at the end of the experimental period. The dry (DW) and fresh weights (FW) of their leaves were measured. The effects of the PGRs on leaf water accumulation, calculated as the DW/FW ratio, and on the vegetative (leaf FW) and generative (stem + leaf FW) growth were studied.

Gas exchange

The net CO_2 assimilation (A_{CO_2}), internal [CO_2] (Ci), transpiration rate (E), and stomatal conductance (gs) were measured in the youngest fully-expanded leaf of each plant, using a CIRAS-2 (PP system, Amesbury, MA) with a PLC6 (U) Automatic Universal Leaf Cuvette, measuring both sides of the leaves. The cuvette provided light (LED) with a photon flux of 1300 m^{-2} s^{-1}, 360 or 800 µmol mol^{-1} CO_2, a leaf temperature of 25 °C, and 75 % relative humidity. The water use efficiency (WUE) of leaf gas exchange was calculated from the gas exchange data as A/E, where A is the carbon assimilated through photosynthesis and E is the amount of water lost via transpiration.

Chlorophyll content and fluorescence

Chlorophylls were extracted from samples of the youngest leaf with N,N-dimethylformamide, for 72 h, in darkness at 4 °C. Subsequently, the absorbance was measured in a spectrophotometer at 750, 664, and 647 nm, and the quantities of chlorophylls *a* (Chl *a*), *b* (Chl *b*), and $a + b$ (Chl $a + b$) were calculated according to the method of Porra et al. (1989). On the leaf used for gas exchange, the dark-adapted maximum fluorescence (Fm) and minimum fluorescence (Fo) and the light-adapted, steady-state chlorophyll fluorescence (F) and maximum fluorescence (Fm′) were measured with a portable modulated fluorometer, model OS-30P (Opti-Science, USA). The ratio between the variable fluorescence from a dark-adapted leaf (Fv) and the maximal fluorescence from a dark-adapted, youngest fully-expanded leaf (Fm)—called the maximum potential quantum efficiency of photosystem II (Fv/Fm)—was calculated. A special leaf clip holder was allocated to each leaf to maintain dark conditions for at least 30 min before reading.

Ascorbate peroxidase activity

Ascorbate peroxidase (APOX) was extracted from 0.9 g of fresh leaf material by homogenization in 9 ml of extraction buffer containing 50 mmol l^{-1} phosphate buffer (pH 7.4), 1.0 mmol l^{-1} EDTA, 1.0 g PVP, and 0.5 % (v/v) Triton X-100, at 4 °C. The homogenates were centrifuged at 10,000×*g* for 20 min, and the supernatant was used for the assays. The APOX activity was measured immediately in fresh extracts, as described by Nakano and Asada (1981), using a 1.0-ml reaction mixture containing 50 mmol l^{-1} potassium phosphate buffer (pH 7.0), 0.1 mmol l^{-1} H_2O_2, 0.5 mmol l^{-1} ascorbate, and 0.1 mmol l^{-1} EDTA. The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (e: 2.8 mmol l^{-1} cm^{-1}). One unit of APOX forms 1 µmol of oxidized ascorbate per minute under these assay conditions.

Total phenolic compounds

The total phenolic compounds were extracted from 0.4 g of frozen pepper fruits (−80 °C) with 4 mL of methanol and 0.1 mol l^{-1} HCl. The homogenate was centrifuged at 15,000×*g* for 20 min, at 4 °C. For the determination, Folin–Ciocalteu reagent was used, diluted with distilled water (1:10). The diluted reagent (2 ml) was mixed with 400 µl of supernatant; then, 1.6 ml of sodium carbonate (7.5 %) were added. The mixture was kept for 30 min in the dark and then centrifuged at 5000×*g* for 5 min. The supernatant was separated and its absorbance was measured at 765 nm, according to the methodology of Kähkönen et al. (1999). The total phenolic content was expressed as gallic acid equivalents, in mg ml^{-1} fresh material.

Lipid peroxidation

Lipid peroxidation was measured as the amount of thiobarbituric acid-reactive substances (TBARS), as determined by the thiobarbituric acid (TBA) reaction (Heath and Packer 1968). Lyophilized samples (0.1 g) were homogenized in 3 ml of 20 % (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3500×*g* for 20 min. To a 1.5-ml aliquot of the supernatant, 1.5 ml of 20 % (w/v) TCA containing 0.5 % (w/v) TBA and 0.15 ml of 4 % (w/v) BHT in ethanol were added. The mixture was heated at 95 °C for 30 min, then quickly cooled on ice, centrifuged at 10,000×*g* for 15 min, and the absorbance measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Balestrasse et al. 2006).

Statistical analysis

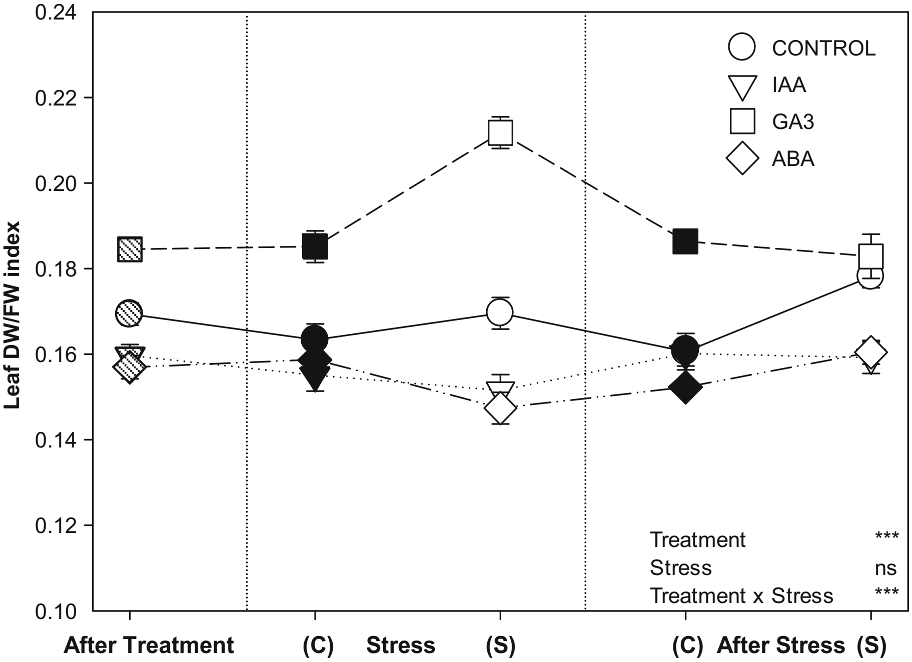
Twenty representative pepper plants were selected randomly for each treatment and distributed into four different samples. The data were tested first for homogeneity of variance and normality of distribution. Significance was determined by analysis of variance (ANOVA), and the significance ($P < 0.05$) of differences between mean values was tested by Duncan's New Multiple Range Test, using Statgraphics Centurion® XVI software (StatPoint Technologies, Inc.)

Results

Growth parameters

Control plants were affected by the water supply, as can be seen in the plant DW/FW ratios 4 days after application of the water stress (Fig. 1). The IAA treatment induced a decrease in the DW/FW ratio, indicating increased water accumulation in the leaf. Similarly, ABA also decreased

Fig. 1 Effects of the application of ABA, IAA, and GA_3 on the water accumulation index (dry weight/fresh weight; DW/FW) of sweet pepper leaves. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and *white symbols* mean stressed (S) plants



the DW/FW ratio, after the hormonal treatment and after the water stress; these differences were not shown during the stress period in non-stressed plants. However, the differences were more marked in stressed plants. In contrast, plants exposed to GA_3 exhibited an increase in their DW/FW ratios after the hormonal treatment and this was even more noticeable during the stress, before returning to pre-stress levels. In Fig. 2a, b, it is observable that GA_3 induced a growth response in the sweet pepper plants distinct from those of the other PGRs and the control (which did not differ). Plants treated with GA_3 elongated more than the control plants, while the IAA- and ABA-treated plants showed greater generative growth and shoot DW.

Gas exchange

The rate of photosynthesis in the control plants was not significantly affected by water stress (Fig. 3a). The hormonal treatments altered A_{CO_2} after their application and during and after the stress. Nevertheless, the values were equal 4 days after the hormonal treatment (C). The IAA treatment induced an increase in the A_{CO_2} after the treatment and a decrease during and after the water stress, when compared with the control plants. On the other hand, GA_3 decreased the rate of photosynthesis after its application and during and after the stress. However, ABA only decreased the rate of photosynthesis after the treatment; 4 days after the treatment and up to the end of the

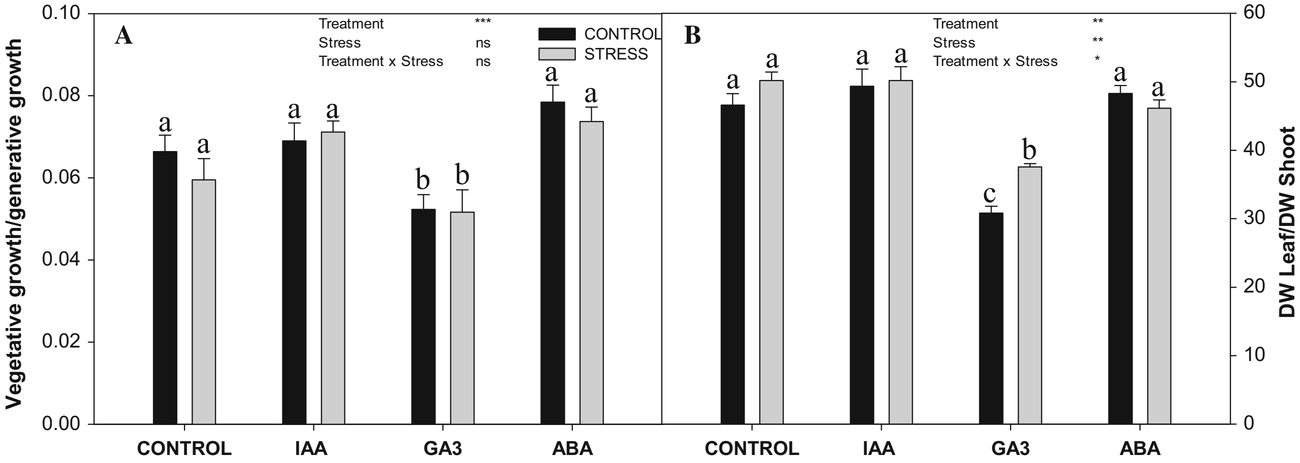


Fig. 2 Effects of the application of ABA, IAA, and GA_3 on **a** the vegetative/generative growth rate and **b** leaf dry weight (DW)/shoot DW, in sweet pepper plants. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. Control (C), stress (S)

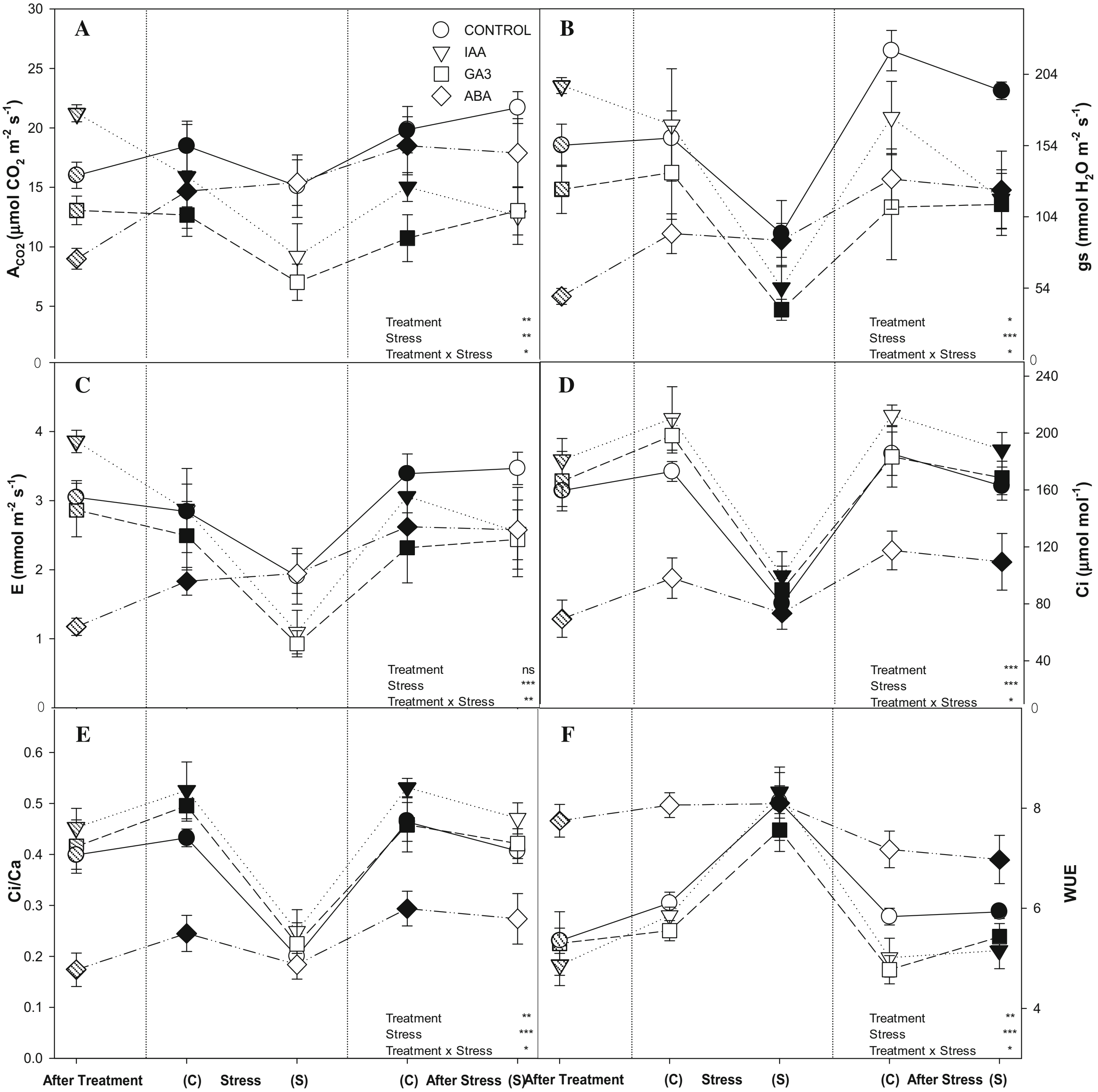


Fig. 3 Effects of the application of ABA, IAA, and GA_3 on **a** net CO_2 assimilation rate (A_{CO_2}), **b** stomatal conductance (gs), **c** transpiration rate (**e**), **d** internal CO_2 concentration, **e** water use efficiency (WUE) (A_{CO_2}/**e**), and **f** the internal CO_2 concentration/ambient CO_2 ratio (Ci/Ca), in sweet pepper. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and *white symbols* mean stressed (S) plants

experiment the rates shown by plants treated with ABA were the same as those of the control plants.

Control plants exhibited decreased gs during the water stress and an augmentation afterwards (Fig. 3b). The gs rose in IAA-treated plants after the hormonal treatment but decreased in ABA-treated plants. When treated plants are compared with control plants, the results show that the combined effect of the hormonal treatment and water stress did not change the gs during the stress for the treated plants; only plants treated with GA_3 exhibited more-intense stomatal closure during the stress. However, after the stress, the recovery of gs in plants treated with any of the hormones was slower when compared with control plants.

Concerning the values of E, the control plants showed a decrease during the water stress and an augmentation afterwards (Fig. 3c). Treatment with IAA or ABA changed the E values, similarly to the results obtained for gs after the hormonal treatment and after the stress. By contrast, differences were found between control and IAA- and GA_3-treated plants during the stress, these two hormones decreasing the transpiration rate.

The internal concentration of CO_2 (Ci) was lower during the stress in control plants and was markedly lower in plants treated with ABA during the whole experiment—except during the water stress, when it was equal to that of the control plants (Fig. 3d). On the other hand, IAA and GA_3 augmented the Ci measured 4 days after the hormonal treatment and IAA also produced an increase after the water stress, when compared with control plants. The same patterns were detected in terms of Ci/Ca (Fig. 3e).

The WUE was higher in control plants during the stress and clearly higher in the case of plants treated with ABA throughout the experiment, except during the water stress, compared with control plants (Fig. 3f). The WUE was reduced after the stress in plants treated with IAA or GA_3, relative to control plants.

Chlorophyll content and fluorescence

No changes were seen in control plants during the experiment (Fig. 4a). A rise in Fv/Fm was detected in plants treated with IAA after the hormonal treatment and after the water stress, while no differences were found in stress conditions, when compared with control plants. This is the opposite of the findings for GA_3—where an increase in Fv/Fm occurred during the water stress, a decrease after the stress, and no differences after the hormonal treatment. No differences were found in plants treated with ABA, compared with control plants, except after the water stress—when a decrease in the Fv/Fm value was detected.

Concerning the chlorophylls, the Chl *a* levels in control plants and plants treated with IAA did not change along the experiment while a decline was detected during the water stress in plants treated with ABA (Fig. 4b). Notwithstanding, the levels of Chl *b* and Chl *a* + *b* augmented in control plants during the water stress, while they decreased in plants treated with ABA (Fig. 4c, d). The Chl *b* and Chl

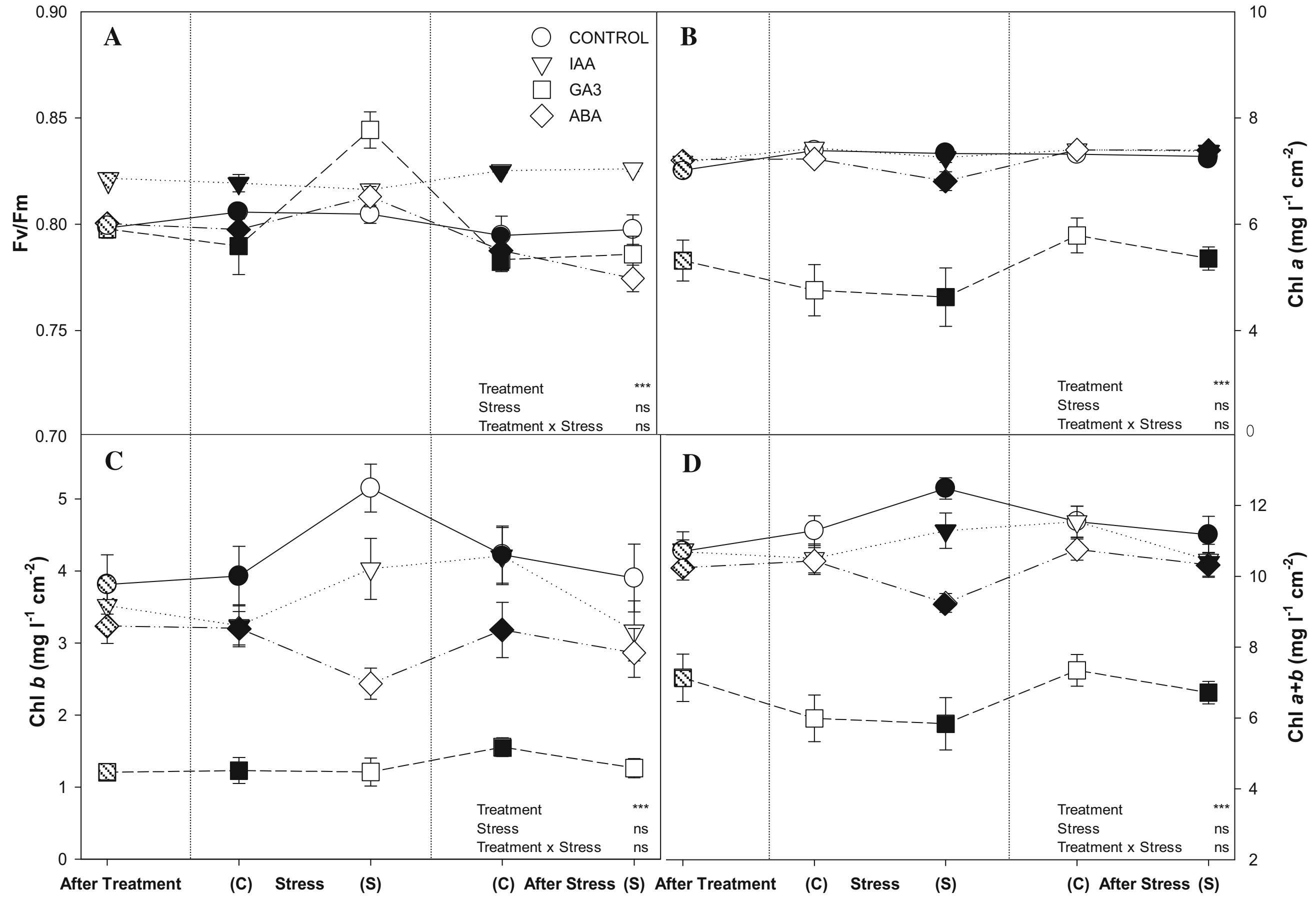


Fig. 4 Effects of the application of ABA, IAA, and GA_3 on **a** maximum potential quantum efficiency of photosystem II (Fv/Fm), **b** chlorophyll *a* content (Chl *a*), **c** chlorophyll *b* content (Chl *b*), and **d** chlorophyll *a* + *b* content (Chl *a* + *b*), in sweet pepper leaves. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and white symbols mean stressed (S) plants

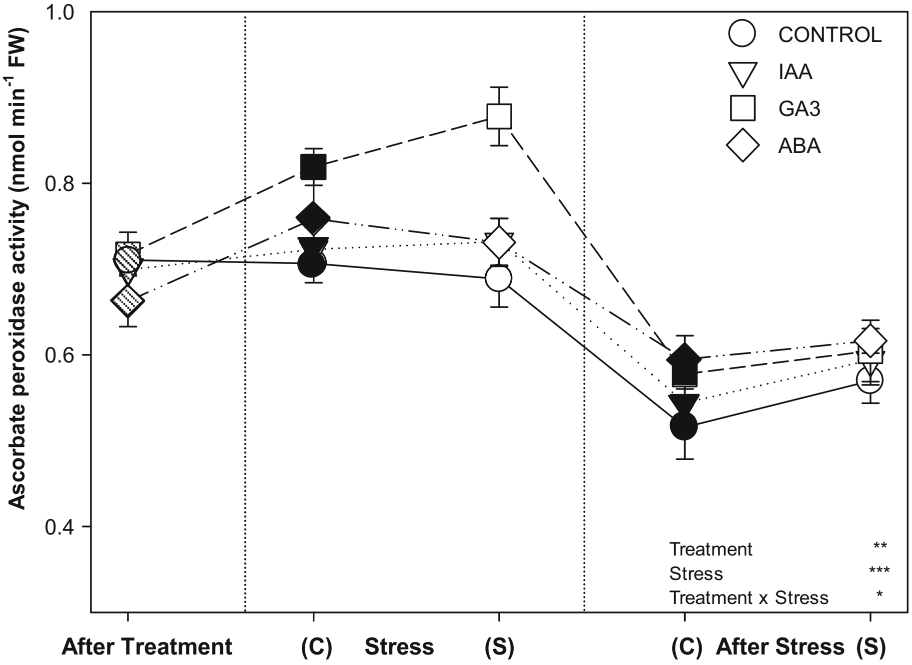


Fig. 5 Effects of the application of ABA, IAA, and GA_3 on ascorbate peroxidase activity in sweet pepper. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and white symbols mean stressed (S) plants

$a + b$ concentrations also rose in plants treated with IAA, but by a lower amount, and differed significantly from those of control plants. On the other hand, although plants treated with GA_3 did not show any variation in their Chl *a*, Chl *b*, and Chl $a + b$ levels during the whole experiment, these levels were markedly lower than those in control plants.

Ascorbate peroxidase activity

The APOX activity in control plants exhibited an increase after the water stress. No differences in activity were found among control plants and plants treated with IAA or ABA. However, the activity augmented under water stress. On the other hand, the APOX activity in all the treatments significantly decreased after the stress (Fig. 5).

Total phenolic compounds

The total phenolic content did not change in control plants or in plants treated with GA_3 along the experiment; however, the levels in plants treated with GA_3 were much higher than in control plants (Fig. 6). In contrast, plants treated with IAA augmented their gallic acid concentrations during the stress while plants treated with ABA augmented their levels after the stress.

Lipid peroxidation

No differences with time were found in control plants in terms of lipid peroxidation. Nevertheless, the treatment with GA_3 augmented the level of lipid peroxidation while ABA decreased it after the hormonal treatment (Fig. 7). In plants grown in control conditions, no differences were found 4 days after finishing the hormonal treatment, while an increase and a decrease, respectively, were detected under water stress in plants treated with GA_3 and ABA. After the stress, the levels of lipid peroxidation remained higher (GA_3) and lower (ABA) than in control plants, while no differences between control plants and plants treated with IAA were detected along the experiment.

Discussion

Untreated pepper plants subjected to soil drying do not show changes in their leaf turgor immediately after water stress, preserving their leaf water content during short periods of water deficiency (Ismail et al. 2002). This is probably due to the high sensitivity of the stomatal response to soil drying in *C. annuum* (Berkowitz and Rabin 1988, Aloni et al. 1991). However, when the drought is prolonged, shoot water deficit becomes unavoidable and some older leaves wilt, perhaps because of a weak hydraulic link with the main stem or weaker control of stomatal conductance (Zhang et al. 2006). Thus, in the present work, drought produced stomatal closure in control plants of sweet pepper, which reduced transpiration and carbon gain. Notwithstanding, the photosynthetic rate was not affected by the partial stomatal closure, provoking an increase in the WUE. Frequently, these mechanisms activated as responses to drought include responses that cause

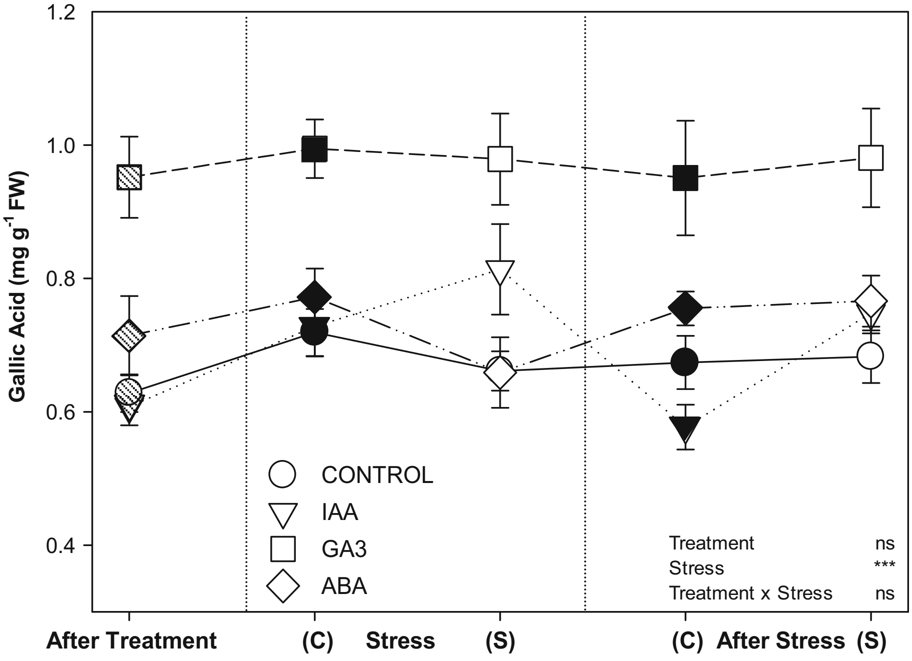


Fig. 6 Effects of the application of ABA, IAA, and GA_3 on gallic acid in sweet pepper. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and *white symbols* mean stressed (S) plants

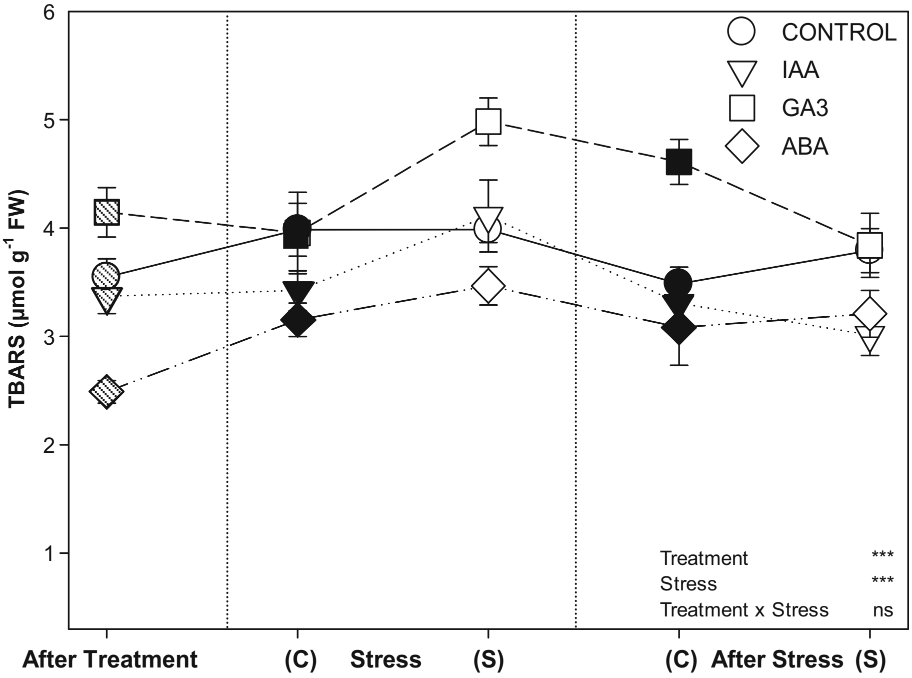


Fig. 7 Effects of the application of ABA, IAA, and GA_3 on TBARS in sweet pepper. *Vertical bars* indicate standard errors of means, only shown when larger than *symbols*. *White symbols* with *black stripes* mean plants before treatment, *black symbols* mean control (C) plants and *white symbols* mean stressed (S) plants

irreversible damage to physiological functions; this can be seen after the stress (Zhang et al. 2006). Nevertheless, all these effects were reversed after the stress in this study, implying that the short-term water shortage did not cause a non-reversible malfunctioning of the physiological mechanisms of the plant.

Among the three hormonal treatments, plants treated with ABA presented the most-similar responses to control plants under water stress. The levels of ABA are regulated by a variety of environmental conditions. Particular attention has been paid to changes in ABA levels in response to drought (Santner et al. 2009). When plants undergo water stress, endogenous ABA levels increase (Pustovoitova et al. 2004), playing a regulatory role in stress signaling (Rudús et al. 2009). Thus, this perturbation, together with the exogenous hormonal application in this experiment, altered the effect of the plant hormones, occasioning a combined effect (of the hormone and drought). In the case of plants treated with ABA, the effect would result from its increased accumulation. Nevertheless, in the case of plants treated with GA_3 or IAA, there would have been an interaction of the effects of each plant hormone. Especially relevant is the case of the IAA/ABA ratio—that is involved in important processes such as translocation of photosynthates (Kiselyova and Borzenkova 1998) and has been reported to decrease during water stress (Wang et al. 2008).

The ABA application produced two different kinds of responses: those noticeable after application and those noticeable in stress conditions, when the ABA concentration was probably increased. The first group is composed of response mechanisms that act rapidly against stress; these may be activated immediately to protect the plant from desiccation. This alludes to short-term responses provoked by an increase in the ABA concentration and the effects did not increase when the ABA concentration was modified by the drought. Gas exchange was affected by ABA through stomatal closure; thereby, photosynthesis and transpiration decreased, which had an effect on the WUE. However, ABA can also increase nutritional fluxes in the plant (Barickman et al. 2014), protecting it from the damage due to stomatal closure. Under water stress, ABA concentrations are expected to increase, regulating a second group of responses that prepare the plant for long-term adverse conditions—by modifying WUE and chlorophyll concentrations and alleviating lipid degradation. Also, these responses provoke changes in Ci and Ci/Ca.

IAA is a hormone involved in the uptake and further transport of nutrients within the plant, by regulating the sink action of developing tissues (Arteca 1996), and it has been implicated in every aspect of plant growth and development, as well as in defense responses (Santner et al. 2009). Thus, in this work, IAA increased the rate of photosynthesis after its application due to greater stomatal opening—which increased both CO_2 uptake into the leaf and loss of water vapor. During the stress, all these effects were reversed by the change in the endogenous ABA concentration, that modified the IAA/ABA ratio, and the total concentration of phenolics augmented as a response to stress.

Nevertheless, the IAA application increased the water accumulation in the leaf before, during, and after the water stress. This is contrary to the higher transpiration rate and gs values registered, when compared with control plants. Thus, it seems that IAA promoted water accumulation in the leaf tissues regardless of the gas exchange mechanisms. The results obtained in this experiment are in accordance with previous research (Ashraf et al. 2006) where IAA treatments were successful in alleviating the adverse effect of water stress, by increasing the relative water content, rate of photosynthesis, and gs. Notwithstanding, there are controversial results concerning the role played by IAA under water stress (Man et al. 2011), since both increases and decreases in its concentration during water stress have been reported.

Plants treated with GA_3 exhibited an increase in their elongation and also a reduction in their leaf water content—this was even more marked when the plants were subjected to water stress. The data do not show any evidence of higher transpiration rates or greater stomatal opening when leaves were sprayed with GA_3, contrary to previous reports (Aharoni et al. 1977, Santakumari and Fletcher 1987, Wachowicz et al. 2006). In fact, during water stress, stomatal closure was apparent in pepper plants treated with GA_3, accompanied by a reduction in the transpiration rate. GA_3 is able to reallocate plant resources from certain parts of the plant to others (Santner et al. 2009), in response to their needs. Hence, the lower amount of water in the sweet pepper leaves could have been due to reallocation of water from leaves to the rest of the plant, in an attempt to overcome the water imbalance produced by the stress.

In addition, GA_3 induced a reduction in the rate of photosynthesis, provoked partially by the stomatal closure and partially by other factors directly affected by the GA_3 treatment. After its application, GA_3 inhibited non-stomatal photosynthesis through drastic decreases in the amounts of Chls *a* and *b*. However, the chlorophyll fluorescence increased during the stress. This contrasts with results obtained in post-harvest experiments, where GA_3 applications delayed ripening and/or senescence; this was related to low chlorophyll degradation (Jordi et al. 1995; Wachowicz et al. 2006; Gambetta et al. 2014). These results—along with the data obtained regarding accumulation of phenolics, lipid degradation, and oxidative stress—indicate that sweet pepper plants suffered stress after being treated with GA_3, which was even more pronounced under water deprivation.

In this study, the influence of the exogenous application of three plant hormones on the response of sweet pepper plants to water stress has been evaluated. The results show that the effects went beyond stomatal control and the central role played by ABA. The effects of IAA are interesting in terms of increasing the shortage of water in the plants, although its effects in combination with those of ABA during water stress are still imprecise and deserve further studies. The effects of exogenous ABA were not additive concerning gas exchange—that just responded to the peak of ABA concentration, which regulated short- and long-term responses to drought. Finally, GA_3 application was counter-productive, since the plants suffered high stress when treated with this hormone. These three hormones have each been studied at a certain dose, but their effects can be positive or negative according to the dose. In any case, further studies will be necessary to figure out the exact role played by each hormone during drought.

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