Scientia Horticulturae 244 (2019) 322–329

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Heat shock, high CO_2 and nitrogen fertilization effects in pepper plants submitted to elevated temperatures

Margarita Pérez-Jiménez*, M. Carmen Piñero, Francisco M. del Amor

Departamento de Hortofruticultura, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), 30150, Murcia, Spain

ARTICLE INFO

Keywords:
Climate change
Heat shock
Nitrogen
Plant growth-promoting rhizobacteria
WUE
Capsicum annuum L.

ABSTRACT

The atmospheric CO_2 concentration and the frequency and severity of heat waves are increasing, and the nitrogen - key to plant development - supplied to crops leaches easily, polluting ground water. In this experiment, these three elements were combined, in order to unravel the physiological mechanisms involved in the plant response to this future scenario.

Sweet pepper seedlings were exposed to distinct nitrate inputs (nutrient solutions containing 12 mM, 5 mM and 0 mM of N) and plant growth-promoting rhizobacteria, a high CO_2 concentration (1000 μmol mol^{-1}), and a heat shock (43 °C), in a controlled environment. Physiological markers - such as photosynthesis rate, stomatal conductance (gs), water use efficiency (WUE), chlorophylls, chlorophyll fluorescence, lipid peroxidation, anions, and free amino acids - were measured.

Exposure to the high temperature did not lead to any measurable stress in the sweet pepper plants. In fact, it augmented photosynthesis and the nitrate concentration, particularly at the elevated CO_2 concentration. Heat shock did not suppose any detriment for the plants beyond the expected increases in gs and WUE. On the contrary, heat triggered many processes that ended up favoring photosynthesis; when combined with elevated CO_2, the result was even more beneficial for the plants.

On the other hand, nitrogen starvation produced serious damage - such as decreases in the photosynthesis rate and chlorophylls and increases in lipid peroxidation and the levels of anions, cysteine, leucine, and phenylalanine in the plant - which was ameliorated to a great extent by the action of high CO_2. Finally, the plant growth-promoting rhizobacteria hardly enhanced plant growth and development, giving results similar to those of the nitrogen withdrawal treatment.

1. Introduction

Climate change is a serious threat to crops since they need to be edaphoclimatologically adapted to the place in which they are grown. This scenario is predicted to worsen since the atmospheric CO_2 concentration ($[CO_2]$) is expected to reach nearly 1000 μmol mol^{-1} by the end of the century (IPCC, 2013). Thus, many extreme climatic events have arisen as a consequence of the rise in the concentration of CO_2 and other exhaust gases. These climate-change events - droughts, flooding, high radiation, and salinity – are seriously affecting crops (Röth et al., 2016). In general, changes in $[CO_2]$ affect various physiological processes in plants, and previous studies have highlighted that high $[CO_2]$ increases photosynthetic carbon fixation rates, which stimulates plant growth and development. In addition, this responsiveness of plants to elevated CO_2 enables them to mitigate the effect of some of the above mentioned abiotic stresses, such as salinity (Geissler et al., 2010 and 2010, Piñero et al., 2014) or drought (Wall et al., 2006; Vu and Allen, 2009). Nevertheless, our understanding of the interactions among these factors remains incomplete (Pérez-López et al., 2013).

The increase in the $[CO_2]$ along with other greenhouse gases is expected to cause an increase in mean global temperature of more than 2 °C (IPCC, 2013). In addition to rising mean annual temperatures, the frequency, duration, and severity of heat waves will increase and might cause physiological disorders in plants and compromise their survival (Jiang and Huang, 2001). Therefore, although the heat-threshold level varies considerably among developmental stages, this global warming is an emerging and major challenge to sweet pepper (*Capsicum annuum* L.) growth and development and it seriously affects pollination and yield (Hedhly et al., 2009; Mateos et al., 2013). Protein stability can decrease under heat stress, exposing hydrophobic patches that cause the aggregation of denatured proteins (Kim and Hwang, 2015). The processes of sensing and responding to heat are complex phenomena in

* Corresponding author.
E-mail address: margarita.perez3@carm.es (M. Pérez-Jiménez).

https://doi.org/10.1016/j.scienta.2018.09.072
Received 8 March 2018; Received in revised form 25 September 2018; Accepted 26 September 2018

plants and comprise the activation of numerous regulatory and signaling pathways that eventually lead to a fine metabolic adjustment to ensure cell survival (Röth et al., 2016). Although high CO_2 and heat shock have been extensively studied, little is known about how their combination affects plants, or how these stress effects are influenced by the supply of nitrogen (N), another major pollutant.

Since N is one of the most important mineral nutrients for plant growth and its availability is considered one of the main limiting factors in crop production (Glass, 2003), N fertilizer was often used in excess in the past; as a consequence, N has produced considerable impacts in the environment, such as water contamination or soil imbalance. This problem is now being addressed by reducing fertilizer applications, to limit pollution. However, N is involved in the biosynthesis of many important compounds and the lack of it could cause a serious imbalance, resulting in reduced growth and lower yield of plants (Rubio-Wilhelmi et al., 2011). Actually, N affects many aspects of plants - including growth, photosynthesis, stomatal conductance, maximum potential quantum efficiency of photosystem II, and chlorophyll content. Therefore, a balance between proper plant growth and respect for the environment could be difficult to achieve. In the last decade, efficient N use under low N supply has been considered as one way to resolve these problems (Zhao et al., 2014). Moreover, plant-growth promoting rhizobacteria (PGPR) have been shown to fix atmospheric N and enhance water and mineral uptake, thereby allowing reduction of the mineral N supply and also partly mitigating several abiotic stresses (del Amor et al., 2008; Bashan and de-Bashan, 2010; del Amor and Cuadra-Crespo, 2012). Thus, PGPR could suppose a good alternative to traditional mineral nutrition, contributing to plant growth and avoiding pollution.

This study is the first attempt at comprehending how heat, CO_2, and N (bio-fertilizer and mineral) interact in a climate change scenario. It addresses the problem of N leaching in the future context of high [CO_2] and heat waves. In order to stimulate the physiological mechanisms affected by these three factors $-CO_2$, heat shock, and N -seedlings of sweet pepper were exposed to differing N inputs, high [CO_2], and a heat shock in a controlled environment. The level of stress produced was assessed by measuring the degree of change in the photosynthesis rate, stomatal conductance, water use efficiency, chlorophyll content, chlorophyll fluorescence, lipid peroxidation, anion concentration, and free amino acid concentration.

2. Material and methods

2.1. Plant material and growth conditions

Sweet pepper plants (≈ 10 cm and 10 leaves), cv. Herminio (Syngenta), were obtained from a commercial nursery 21 d after germination. They were grown in 5-l black containers filled with coconut coir fiber (Cocopeat, Pelemix). Sixty-four plants were used for the experiment and were irrigated with different modified Hoagland solutions (Table 1). Sixteen plants were irrigated with solution 1 (0 mM; no N-supply) as a control, 16 plants were also irrigated with solution 1 but a commercial PGPR product (*Azospirillum brasilense* strain M3 and *Pantoea dispersa* strain C3, immobilized in a solid support; Biopron®, Probelte) was added (following the manufacturer's instructions) as a source of N, 16 plants received solution 2 (5 mM) containing an intermediate N concentration (5 mM; low N-supply), and 16 plants received solution 3 (12 mM) with a complete supply of N (12 mM; full N-supply). The plants were drip-irrigated four times per day for 15 d and then eight times per day over the next 3 d (heat stress) in order to maintain a minimum of 35% drainage, to avoid salt accumulation (Piñero et al., 2016). The pH of the nutrient solutions was adjusted to 5.7, using 85% phosphoric acid, every 2 d. The plants were grown in a climate chamber designed by our department specifically for plant research purposes (del Amor et al., 2010), with fully-controlled environmental conditions: 50/70% RH, 16/8 h day/night, 24/20/28 °C (20/0/8 h) for 15 d, and 38/30/43 °C (20/0/8 h) for 3 d (heat stress) (Fig. 1). Photosynthetically active radiation (PAR) of 250 μmol m^{-2} s^{-1} was provided by a combination of fluorescent lamps (TL-D Master reflex 830 and 840, Koninklijke Philips Electronics N.V., Eindhoven, the Netherlands) and high-pressure sodium lamps (Son-T Agro, Philips). The experiment was performed at two [CO_2]: elevated (e[CO_2]), 1000 μmol mol^{-1}, or ambient (a[CO_2]), 400 μmol mol^{-1}. The [CO_2] was regulated by injection of external compressed air or CO_2 (bottle [CO_2] ≥ 99.9%), controlled by a Dräger Polytron IR CO_2.

Table 1
Nutrient composition of the different solutions used in the experiment.

Nutrient (per liter)	Solution 1	Solution 2	Solution 3
$MgSO_4 \cdot 7H_2O$ (mg)	602.37	556.22	494.91
KH_2PO_4 (mg)	408.38	291.26	135.65
$Ca(NO_3)_2 \cdot 4H_2O$ (mg)		606.50	
$CaSO_4$ - 0.02 N (ml)	549.88	250.92	1066.88
KNO_3 (mg)			295.81
K_2SO_4 (mg)	446.16	474.85	258.05
KOH - 1 N (ml)	0.43	0.31	0.14

2.2. Gas exchange

The net CO_2 assimilation rate (A_{CO2}), internal [CO_2] (Ci), transpiration rate (E), and stomatal conductance (gs) were measured in the youngest fully open 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 density of 1300 m^{-2} s^{-1}, a [CO_2] of 1000 or 400 μmol mol^{-1}, a leaf temperature of 28 or 45 °C, and 70% 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.

2.3. Chlorophyll content and fluorescence

Chlorophylls *a* (Chl *a*), *b* (Chl *b*), and *a*+*b* (Chl *a* + *b*) were extracted from samples of the youngest fully open 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 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 open 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.

2.4. 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-cm^3 aliquot of the supernatant, 1.5 cm^3 of 20% (w/v) TCA containing 0.5% (w/v) TBA and 0.15 cm^3 of 4% (w/v) BHT in ethanol were added. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. It was centrifuged at 10,000 × *g* for 15 min, then the absorbance was measured at 532 nm. The value for non-specific