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Carbon dioxide enrichment and brassinosteroid pretreatment alleviate chlorpyrifos phytotoxicity under suboptimal light and temperature conditions in tomato

Liwei Sun[a,b], Lin Ren[b], Tao Peng[a], Lihua Xin[a], Haifeng Qian[a,b], Zhengwei Fu[a,*]

[a] College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, PR China
[b] Department of Food Science and Technology, Ocean College, Zhejiang University of Technology, Hangzhou 310032, PR China

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ABSTRACT

Phytotoxicity is a daunting challenge for facility agriculture, and a crop is more susceptible to phytotoxicity under sub-optimum growth conditions. Although the practical values of brassinosteroid (BR) treatment or CO_2 enrichment in promoting crop yield have been confirmed, few studies have focused on the regulation of pesticide phytotoxicity, especially under sub-optimum growth conditions. In this study, the phytotoxicity of chlorpyrifos was evaluated mainly by monitoring the transcriptional responses of genes involved in various physiological processes in tomato leaves under chilling temperature and low light, which occur frequently during the cool season in China. Additionally, we investigated whether 24-epibrassinolide (EBR) pretreatment (0.1 μmol/L) or CO_2 enrichment (1000 μmol/mol) could mitigate this phytotoxicity. The treatment of chlorpyrifos decreased the transcriptions of photosynthetic and defense genes and increased those of antioxidant and detoxification genes. EBR pretreatment significantly increased the transcription of antioxidant, detoxification and defense genes, which could enhance the tolerance of the plants to pesticides and mitigate the phytotoxicity, as indicated by the increased mRNA level for photosynthetic genes. Similar observations were found with CO_2 enrichment, and the effects of CO_2 enrichment were even more pronounced than the effects of EBR pretreatment for the photosynthetic genes. Moreover, the changes of antioxidant enzymes activity and lipid peroxidation further confirmed that the chlorpyrifos exposure induced the oxidative stress, which could be ameliorated by either EBR pretreatment or CO_2 enrichment. These results strongly suggest a promising prospect for the practical application of BRs or CO_2 enrichment to alleviate the phytotoxicity of pesticides in facility agriculture.

1. Introduction

Since the first synthetic insecticide DDT became available during the 1940s, the world has witnessed a continuous growth in pesticide usage in agriculture in both the number of chemicals and the quantity (vander Werf, 1996). The use of pesticides, including insecticides, fungicides, herbicides and rodenticides, largely benefits agriculture by protecting crops from pests and enhancing crop yields (Carvalho, 2006). However, the debate on the risks and benefits of pesticides is ongoing, and a considerable amount of research has been devoted to understanding the adverse impacts of pesticides on non-target organisms. It is believed that many pesticides, which are deposited on plant surfaces, can exert toxicity or stress if the pesticides penetrate from the root or leaf surface into the living tissue (Verkleij et al., 2009), causing growth reduction, the perturbation of reproductive organ development, and the alteration of nitrogen and/or carbon metabolism (Wang et al., 2010; Xia et al., 2009c).

In addition to the phytotoxicity caused by pesticides, crops are constantly challenged with a variety of other biotic and abiotic stressors. In China, thermophilic crops, such as tomatoes and cucumbers, are mostly cultivated in unheated greenhouses during the cool season. Warm temperatures are maintained during sunny periods, but cloud cover can lead to chilling temperatures (Yu et al., 2002). Consequently, the sub-optimum growth conditions of chilling temperatures and low light can cause major losses in the productivity of facility agriculture. Additionally, the intensive farming in facility agriculture causes a high-occurrence of pests and diseases, and the resulting heavy use of pesticides, making the risk of phytotoxicity a more daunting challenge. Therefore, simple and

* Corresponding author at: 18, Chaowang Road, Hangzhou, Zhejiang 310032, PR China. Fax: +86 571 8832 0599.
E-mail address: azwfu@zjut.du.cn (Z. Fu).

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effective methods to reduce the pesticide residues or to enhance crop tolerance to pesticides are necessary to improve crop yields and control food safety.

Brassinosteroids (BRs) are a group of naturally occurring plant steroid hormones that play prominent roles in plant growth, reproduction and development. Additionally, treatment with BRs enhances the resistance to environmental stresses and the defense against bacterial, fungal and viral pathogens (Jiang et al., 2012; Xia et al., 2009c). Moreover, previous reports have shown that the exogenous application of BRs can alleviate the phytotoxic effects of pesticides and other persistent organic pollutants (Ahammed et al., 2012, 2013; Bajguz and Hayat, 2009; Sharma et al., 2012; Xia et al., 2006, 2009a). On the other hand, as the present atmosphere CO_2 level is a limiting factor for maximum photosynthesis, any increase in CO_2 above the ambient level could potentially benefit agriculture crops, especially C3 plants (Li et al., 2007; Vu et al., 2006). Accordingly, crop CO_2 enrichment has been a powerful practice in greenhouses for improving produce quality and increasing crop yield (Jiang et al., 2012; Li et al., 2007). The effects of elevated CO_2 level on important metabolic processes, such as photosynthesis and respiration, have been well documented (Sinha et al., 2011); however, few studies have been conducted to investigate the effects of CO_2 enrichment on the phytotoxicity in crop production.

Chlorpyrifos is an organophosphorus broad spectrum insecticide and is one of the most widely used active ingredients in agricultural insect control products. In China, the government has banned five highly toxic organophosphorus pesticides, and chlorpyrifos has become the dominant pesticide (Chen et al., 2012). Since gene transcriptional responses represent the primary interaction site and often ultimately relate with physiological processes, in this study we investigated the effects of chlorpyrifos on the transcription of genes involved in various physiological processes in tomatoes, which is a major crop in greenhouse cultivation, under chilling temperatures and low light. We also evaluated the effects of BR pretreatment and CO_2 enrichment on the phytotoxicity of pesticides. In addition to transcriptional responses, the antioxidant enzymes activity and lipid peroxidation were measured as well. The results from this study were expected to be helpful for understanding the underlying mechanisms of the regulation of BR pretreatment and CO_2 enrichment on phytotoxicity, which will potentially improve crop production in the future.

2. Materials and methods

2.1. Chemicals

Chlorpyrifos (48% active ingredient, commercial formulation) was purchased from Yinongnonghua Co., Ltd. (Jiangsu, China). A commercially produced BR, 24-epibrassinolide (EBR, CAS: 78821-43-9, purity > 90%), was purchased from Sigma, USA. The other chemicals were of either analytical or HPLC grade.

2.2. Plant materials and treatments

Tomato (*Solanum lycopersicum* cv. Zhongza No. 9) seeds were sown in a growth medium containing a mixture of vermiculite and perlite (3:1, v/v) with a photoperiod of 12 h:12 h (light:dark), a temperature of 25/17 °C, a light intensity of 200 μmol m^{-1} s^{-1} and a relative humidity of 75%. When the cotyledons fully expanded, the seedlings were transplanted into a container filled with Hoagland nutrient solution. The solutions were renewed every four days, and plants at the four-leaf stage were transferred to artificial climate incubators with chilling temperatures (15/10 °C) and low light (60 μmol m^{-1} s^{-1}) for the following experiments.

The plants were divided into three groups of equal size with different treatments: the EBR pretreatment group (in which the leaves of the plants were immersed in 0.1 μmol/L EBR solution for 10 s), the CO_2 enrichment group (in which the plants were cultivated with a 1000 μmol/mol CO_2 level during the whole experiment) and the control group. After 24 h, each group was subsequently divided into three sub-groups that were treated with chlorpyrifos at a concentration of 0.0, 0.3 or 3.0 mmol/L by immersing the leaves in a chlorpyrifos solution for 10 s. The actual concentrations of chlorpyrifos were verified analytically according to Standard Examination Methods for Drinking waters-Pesticides Parameters (Ministry of Health of the People's Republic of China, 2007). Briefly, the filtered water samples were extracted by dichloromethane, and the organic phases were dehydrated by anhydrous sodium sulfate. The solutions were then evaporated at 40 °C. The samples were analyzed by gas chromatograph (Agilent, 6890) using a flame photometric detector. The detection limit was 0.3 μg/L and the mean recovery was 94.8 ± 4.2%. It was found that mean measured concentrations were consistent and ranged from 91.7% to 103.6% of the nominal values. Since good agreement existed between the nominal and actual exposure concentrations, for simplicity, nominal concentrations were used when presenting in this paper. After 7 days of pesticide treatment, the plants were sampled. The leaves that were third from the bottom were harvested, frozen immediately for liquid nitrogen and stored at −80 °C until further analyses. There were at least four replicates for each treatment.

2.3. RNA isolation and quantitative real-time PCR

The leaves were ground in liquid nitrogen and total RNA was extracted with RNAiso Plus (Takara, Dalian, China). First-strand cDNA synthesis was performed using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). Real-time PCR with SYBR green detection was performed on the Mastercycler ep realplex (Eppendorf, Hamburg, Germany), according to the protocols established by the manufacturer (SYBR® Green Realtime PCR Master Mix, Toyobo). Oligonucleotide primers specific for the studied genes are summarized in Table 1, including those involved in photosynthesis (*psaB*, *psbA* and *rbcL*), protection against oxidative stress (*APX*, *MDAR*, *GR*, *GSH1, GSH2, CAT1* and *GPX*), detoxification (*GST1*, *GST2, GST3* and *ABC*) and defense (*PAL*, *HPL*). The quantification of target gene transcription was based on the comparative cycle threshold (Ct) method (Livak and Schmittgen, 2001), and *actin* was chosen as a reference gene for normalization.

2.4. Determination of antioxidant enzyme activity and lipid peroxidation

Catalase (CAT) activity in tomato leaves was measured as a decline in A240 according to the manufacturer's protocol (Beyotime Institute of Biotechnology, Haimen, China). Superoxide dismutase (SOD) activity was estimated with the colorimetric assay using a kit (S311) from Dojindo Molecular Technologies (Shanghai, China). Protein contents were determined following the method of Bradford (1976).

The level of lipid peroxidation was determined by quantifying the malondialdehyde (MDA) equivalents using 2-thiobarbituric acid (TBA) following the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Data analysis

Prior to conducting statistical comparisons, the data were assessed for normality and homogeneity of variances using the Kolmogorov–Smirnov one-sample test and Levene's test, respectively. Regarding the effects of chlorpyrifos, the statistical