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Protective role of exogenous phytohormones on redox status in pea seedlings under copper stress

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ABSTRACT

The present work aims to provide insight on the role of phytohormone application in developing efficient practical defense strategies to improve plants tolerance under heavy metal contamination. For this purpose, pea (*Pisum sativum* L.) seeds were germinated in an aqueous solution of 200 μM $CuCl_2$ up to the 3rd day and then continued to germinate in the presence of distilled water (stress cessation) or were subjected to following combinations: Cu + 1 μM IAA and Cu + 1 μM GA_3 for 3 additional days. The results showed that copper excess induced oxidative stress in germinating seeds, which resulted in changes of the redox state of glutathione and cysteine, and proteomics revealed Cu-induced modifications of thiols (SH) and carbonyls (CO) (indicators of protein oxidation). However, application of IAA or GA_3 in the germination medium after 3 days of Cu exposure alleviated toxicity on seedlings, despite the persistence of Cu up to 6th day. This improving effect seems to be mediated by a cell Cu accumulation decrease and a protein reduced status recovery, since phytohormones modulate thioredoxin/ferredoxin systems in favor of protecting proteins against oxidation. In addition, an IAA and GA_3 protective effect was evidenced by a cellular homeostasis amelioration resulting from the balance conservation between the regeneration and consumption processes of glutathione and cysteine reduced forms. The exogenous effectors also induced modifications of profiles of SH and CO, suggesting changes in the regulation and expression of proteins that could be involved in defense mechanism against Cu stress.

1. Introduction

The functions of the components of redox status have been widely investigated in several plant systems, including seeds since they are considered as the sensor of the real physiological state in cells (Foyer and Noctor, 2005; Alkhalfioui et al., 2007). In addition, the regulation of the redox state of proteins, including the sulfhydryl groups of cysteine and methionine, is considered as a “switch” for the activity of several enzymes involved in specific signaling events(protein kinases, calcium signaling) and in cell cycle control (Alkhalfioui et al., 2007). However, under stress conditions, notably exposure to heavy metals, the cellular redox state can shift the redox balance toward an oxidizing state (Rouhier et al., 2010), which may result in altering many physiological processes associated with normal growth and development (Janas et al., 2010).

Copper is considered one of the toxic heavy metals that can affect one or more vital biochemical and physiological processes in plants and seedlings (Karmous et al., 2012; Chaoui et al., 2004; Chaoui and El Ferjani, 2014). A delay in embryo growth has been often recorded after Cu exposure, and has been associated with many disorders of germinative metabolism (Karmous et al., 2012), as well as cellular oxidative state generation, mainly described by reactive oxygen species (ROS) over-production (Ahsan et al., 2007; Chaoui and El Ferjani, 2014). Enzymatic and non-enzymatic defense mechanisms are activated to limit the induced oxidative stress damage (Wang et al., 2004). Indeed, several roles of glutathione have been reported, mainly in redox state

Abbreviations: CSH, reduced cysteine; CSS, oxidized cysteine; DTNB, 5,5′-dithio bis 2-nitrobenzoic acid; Fdx, ferredoxin; FNR, ferredoxin-NADP oxidoreductase; FTSC, fluorescein-5-thiosemicarbazide; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; IAF, 5′-iodoacetamide fluorescein; NTR, NADPH-dependent thioredoxin reductase; Trx, thioredoxin

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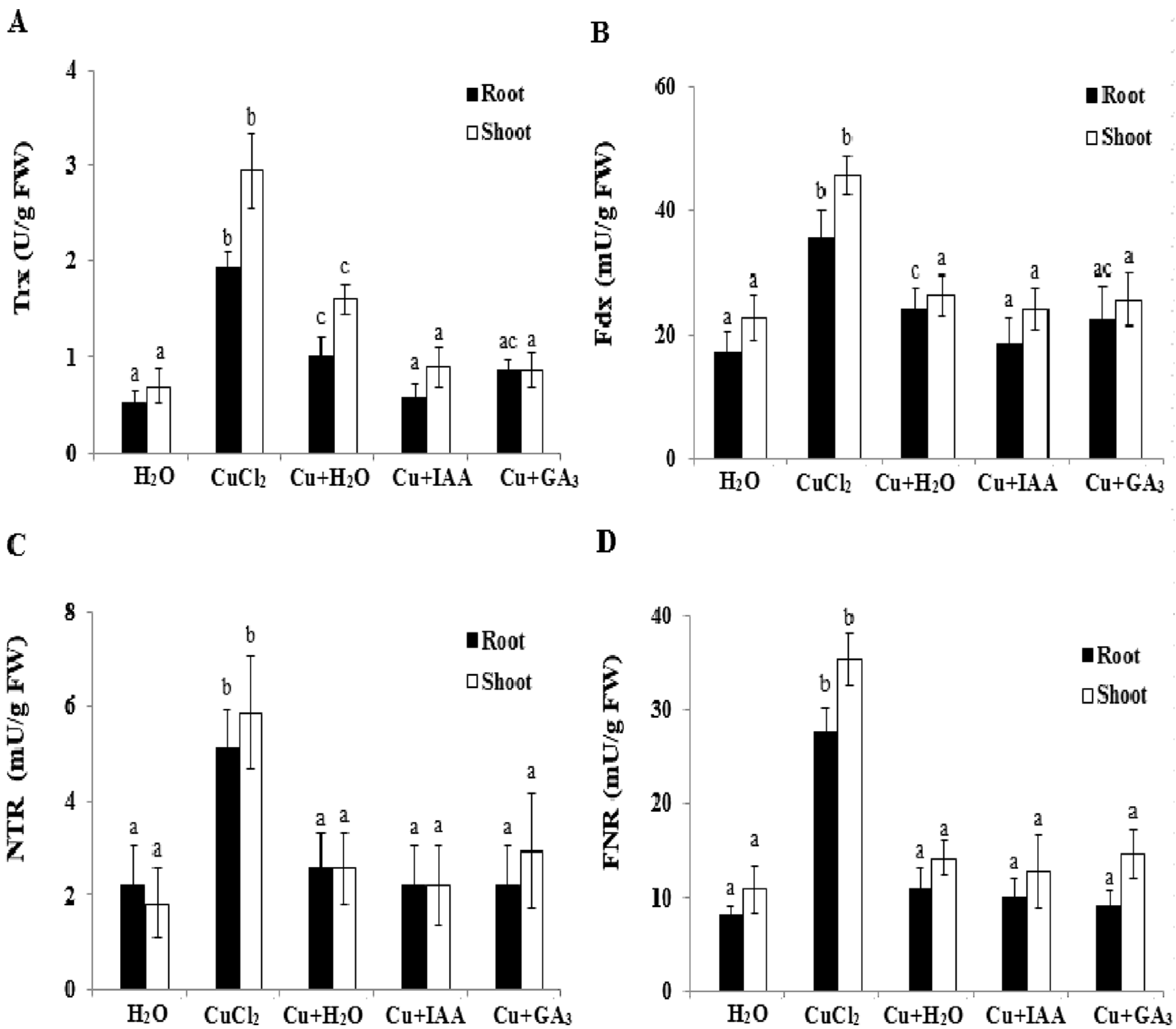

Fig. 1. Activities of Trx (**A**), Fdx (**B**), NTR (**C**) and FNR (**D**) in pea seedlings germinated for 3 days in the presence of distilled water (H_2O) or 200 μM $CuCl_2$ and then replaced up to 6 days either by H_2O or 200 μM $CuCl_2$ added combined with 1 μM IAA "Cu + IAA" or 1 μM GA_3 "Cu + GA_3". Values ± SE (n = 5) followed by a common letter for the same organ are not different at 0.05 level of significance, using ANOVA followed by Tukey's test.

homeostasis, regeneration of other antioxidants (Foyer and Noctor, 2005) and phytochelatins, as well as heavy metals sequestration in plants (Yadav, 2010). Likewise, GSH is implicated in the Asada-Halliwell cycle, exclusively existing in plants and essential for defense against oxidative stress (Foyer and Noctor, 2005). In this process, reduced glutathione (GSH) is converted to its oxidized form (GSSG; Foyer and Noctor, 2011), and in turn, the GSH is regenerated by glutathione reductase (GR) activity using NADPH as an electron donor, maintaining the cellular redox homeostasis by keeping optimum GSH/GSSG ratio (Gill et al., 2013). This parameter can also be changed by the activity of glutathione peroxidase (GPX). Plant glutathione peroxidases are functional peroxiredoxins distributed in several cell compartments and regulated during biotic and abiotic stresses (Navrot et al., 2006).

Scientists have been more interested in developing alternative ways and potential strategies to counteract the adverse effects of environmental stressors via the exogenous application of chemicals to improve plant tolerance, such as polyamines, brassinosteroids (Choudhary et al., 2012), nitric oxide (Hu et al., 2007), sulfur (Anjum et al., 2008), β-estradiol (Chaoui and El Ferjani, 2014), progesterone (Genisel et al., 2013), salicylic acid (Belkhadi et al., 2010), calcium (Sakouhi et al., 2016) and organic acids (malate, oxalate, citrate and benzoate) (Gao et al., 2012).

On these grounds, the aim of this investigation was to shed more light on the mechanism by which IAA and GA_3 interact with the redox components in germinating pea seeds subjected to 200 μM $CuCl_2$ treatment. The cysteine and glutathione contents were analyzed by HPLC, concomitant with the possible changes occurring within the accumulation profile of Cu in the two parts of the seedling and some enzyme activities involved in modulating the cellular redox homeostasis (Rouhier et al., 2008): thioredoxin (Trx), NADPH-dependent thioredoxin reductase (NTR), ferredoxin (Fdx) and ferredoxin-NADP oxidoreductase (FNR). The activities of GPX and GR were also measured to estimate the recycling of glutathione (Mittler, 2002). The oxidation state of the proteins was studied by quantifying their contents in thiol and carbonyl indicators. Proteins CO and SH were labeled with specific substrates, and analyzed by one- and two-dimensional electrophoresis.

2. Material and methods

2.1. Germination and treatment conditions

Pea (*Pisum sativum* L. var. douce province) seeds were disinfected with 2% sodium hypochlorite for 10 min, then rinsed three times with distilled water, and germinated at 25 °C in the presence of distilled water (control) or 200 μM $CuCl_2$. At day 3, Cu was replaced by the