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PROHEXADIONE-CA AND COPPER EFFECT ON GROWTH AND ACCUMULATION OF ENDOGENOUS POLYAMINES IN PEA PLANTS

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Abstract

Sergiev I., Todorova D., Brankova L., Alexieva V., 2016: Prohexadione-Ca and copper effect on growth and accumulation of endogenous polyamines in pea plants [Ca-proheksadiono ir vario poveikis žirnių augimui ir endogeninių poliaminų kaupimui]. – Bot. Lith., 22(1): 65–71.

The effect of retardant Prohexadione-Ca (BAS 125 10W, BASF Corp., Germany) and heavy metal copper on growth and endogenous concentrations of free and conjugated putrescine, spermidine and spermine in roots and leaves of pea plants were investigated. It was found that the polyamine concentrations increased seven days after the treatments. Most considerable increase of polyamines was detected in plants treated with copper and Prohexadione-Ca. The variations in polyamine concentrations in leaves and roots of pea plants suggest that Prohexadione-Ca had a protective effect against Cu stress of pea plants, which was expressed by additional accumulation of polyamines.

Keywords: heavy metal, pea, polyamines, Prohexadione-Ca, protection.

INTRODUCTION

Copper (Cu^{2+}) plays an important role in various plant physiological processes as photosynthesis, respiration, chlorophyll and cell wall synthesis, etc. (Hänsch & Mendel, 2009; Yadav, 2010). However, copper in excess causes phytotoxic and oxidative damages (Yadav, 2010). Polyamines (PAs) are nitrogen-containing organic molecules, widely distributed in higher and lower plants. The PAs common to all plant species are putrescine (Put), spermidine (Spd) and spermine (Spm). In plants, they occur as free molecules and can also be conjugated with small molecules (phenolic acids) or be bound to biomacromolecules such as proteins and nucleic acids (Groppa & Benavides, 2008). Conjugated PAs are implicated to play role in a variety of stress-induced plant responses and developmental processes, although the conjugation of polyamines is discussed also as a regulatory mechanism of free PAs pool (Bouchereau et al., 1999). PAs take place in plant responses under the variety of biotic and abiotic stresses such as temperature stress, UV stress, drought, salinity and heavy metal stress (Todorova et al., 2014).

Several studies have documented the changes in PA metabolism due to Cu excess in soil or nutrient solutions. Increased content of Cu in soil enhanced the accumulation of Put and Spm in barley and spinach (Bergmann et al., 2001). Accordingly, Choudhary et al. (2009) showed that Cu treatment led to substantial rise in PAs in *R. sativus* seedlings. Later, Szafranska et al. (2011) examined the effect of Cu on the regeneration of *Daucus carota* L. androgenic embryos of var. Feria and 1014 breeding line as well as on PAs and proline contents, lipid peroxidation and Cu accumulation after 16 and 24 weeks and found that higher tolerance of Feria to oxidative stress may result from the increased content of proline, Put and Spd. The authors concluded that variations in PA levels depend not only on the concentrations of heavy metal supplied, but also on plant species and cultivars, and PAs render better protection of cultivars, which possess higher constitutive PA levels. Groppa et al. (2007a, b, 2008) examined the PA metabolism in sunflower and wheat leaf discs treated with Cu. Put increased considerably after the treatment with Cu, Spd did not change noticeably, and Spm increased in sunflower, but it was reduced in wheat (Groppa et al., 2007a, b). So, the authors proposed that PAs are key biological compounds, which are probably involved in signalling pathways triggered under heavy metal stress conditions (Groppa et al., 2008).

Plant bioregulator Prohexadione-Ca (calcium 3-oxido-4-propionyl-5-oxo-cyclohexene carboxylate; Pro-Ca) is mainly used to inhibit disproportionate vegetative growth in fruit trees and other crop plants (Rademacher et al., 2006). Pro-Ca has a relatively short-life and possesses favourable toxicological and ecotoxicological properties such as bacterial, fungal and insect control since it acts as a structural mimic of 2-oxoglutaric acid. Prohexadione-Ca triggers pathogen resistance by inducing the formation of 3-deoxyflavonoids, in particular luteoforol, with phytoalexin-like properties (Spinelli et al., 2005). Additionally, treatments with Pro-Ca lead to decreased ethylene formation, which reduces fruit drop (Coatti et al., 2002) and controls the alternate fruit production of pear trees (Shehaj et al., 2015). To our knowledge, no data exist about protective effect of Pro-Ca against abiotic stress, particularly provoked by application of heavy metals. Here we examine the potential protective effect of this bioregulator against Cu stress of pea plants *via* alterations of endogenous polyamines and plant growth.

MATERIALS AND METHODS

Plant material and growth conditions

Young pea seedlings with fully developed 2nd leaf were treated with heavy metal Cu ($CuSO_4$ 2 µg/ml in the nutrient medium), Pro-Ca (0.01 mM, leaf applied by spraying) and combination between them. Plants were grown in a chamber at 12/12 h light/dark regime, 160 $\mu mol.m^{-2}.s^{-1}$ photon flux density, 26/22ºC day/night temperature, 60% air humidity. Samples were taken seven days after the treatments.

Biochemical analyses

To determine levels of free Put, Spd and Spm, the direct dansylation method was applied (Smith & Best, 1977). Conjugated PAs were measured in acid-hydrolysed supernatants as TCA-soluble bound PAs, and bound PAs in acid-hydrolysed pellets as TCA-insoluble bound polyamines, according to the method of Torrigiani et al. (1989). Three fractions of PAs were separated using TLC precoated plates of Silicagel G 60 (Merck) in a cyclohexane:ethylacetate (3:2, v/v) solvent system. Spots, visualised under UV light, were scraped off and eluted in 2 ml anhydrous acetone. Fluorescence was measured (excitation 360 nm, emission 505.5 nm) and the results were compared with dansylated PA standards on the same plate.

Fresh material was homogenized with 0.1% (w/v) trichloracetic acid for malondialdehyde (MDA) determinations. Malondialdehyde content was estimated as a parameter reflecting biomembrane integrity deterioration. It was determined as thiobarbituric acid-product according to Kramer et al. (1991) by using the molar extinction coefficient 155 mM^{-1} cm^{-1}.

For the assay of superoxide dismutase (SOD, EC 1.15.1.1), fresh plant material was homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (w/v). The homogenates were centrifuged at 12.000 × *g* for 15 min. The enzyme activity was determined according to Beauchamp & Fridovich (1971). Total SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue-tetrazolium (NBT). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

Statistical procedures

All experiments were repeated three times with three to five replications. The results reported in the figures are means of the values with standard error (SE). The significance of differences was statistically analyzed using Duncan's multiple range test at 0.05 significance level.

RESULTS

Copper decreased the fresh weight and length of roots and shoots of pea. Prohexadione-Ca decreased the length of shoots and did not influence the length of roots. It also slightly reduced the fresh weight of shoots and increased the fresh weight of roots. Applied in combination with Cu, Prohexadione-Ca entirely elimi-

Table 1. Length and fresh weight of shoots and roots of pea plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination (mean values ± SE). Different letters designate statistically significant difference at $p < 0.05$

Variant	FW roots		FW shoots		Length roots		Length shoots	
	[mg]	[%]	[mg]	[%]	[mm]	[%]	[mm]	[%]
Control	379.3 ± 31.4^{a}	100.0	552.3 ± 33.9^{a}	100.0	120.5 ± 8.2^{a}	100.0	105.5 ± 3.2^{a}	100.0
Cu	328.3 ± 25.6^{b}	86.5	468.0 ± 22.7^{b}	84.7	112.3 ± 3.6^{b}	93.2	81.5 ± 3.0^{c}	77.3
Pro-Ca	408.0 ± 28.2^{a}	107.6	519.0 ± 26.0^{a}	94.0	123.0 ± 6.6^{a}	102.1	91.5 ± 4.3^{b}	86.7
Cu + Pro-Ca	401.5 ± 18.3^{a}	105.8	526.0 ± 48.2^{a}	95.2	123.3 ± 2.7^{a}	102.3	89.8 ± 4.8^{b}	85.1

nated the negative effects of Cu on the fresh weight and length of roots and partly restored the decreased by Cu fresh weight and length of shoots (Table 1).

Copper significantly increased the content of malondialdehyde in the leaves (a marker for oxidative damages of the biomembranes), while the simultaneous application of Pro-Ca and Cu significantly diminished the level of this stress parameter. Similar trend was observed in relation to the activity of superoxide dismutase – Cu provoked 207% increase of SOD activity, and Cu + Pro-Ca treatment decreased it by 131% of the control level (Table 2).

The free forms of putrescine, spermidine and spermine were considerably increased by Cu and Prohexadione-Ca in leaves and in roots. The combined treatment of Cu + Pro-Ca led to an additional increase of free Put, Spd and Spm, but only in the

Table 2. Malondialdehyde content and superoxide dismutase activity in leaves of pea plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination (mean values ± SE). Different letters designate statistically significant difference at $p < 0.05$

Variant	MDA		SOD	
	[nmol/gFW]	[%]	[EU/gFW]	[%]
Control	89.6 ± 2.5^{a}	100.0	158.6 ± 14.2^{a}	100.0
Cu	118.8 ± 1.5^{d}	132.5	328.5 ± 23.4^{c}	207.1
Pro-Ca	69.8 ± 1.8^{c}	77.9	227.7 ± 18.7^{b}	143.5
Cu + Pro-Ca	101.3 ± 0.3^{b}	113.1	209.2 ± 16.2^{b}	131.9

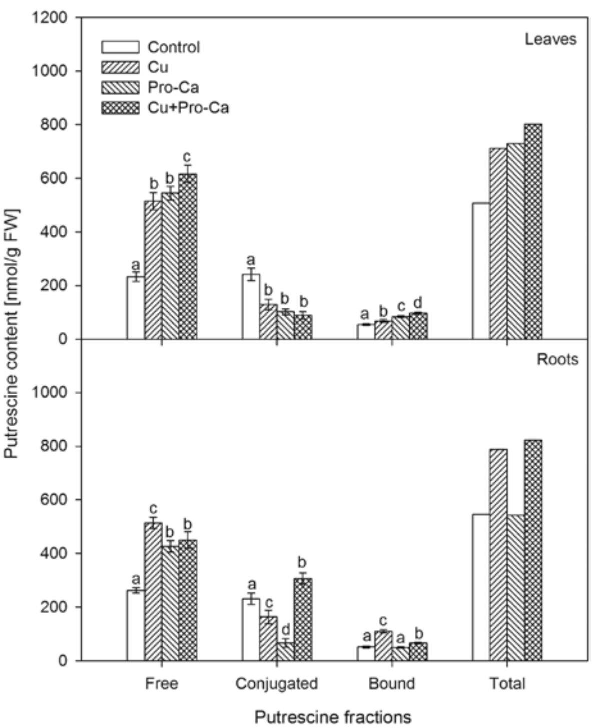

Fig. 1. Content of free, conjugated, bound and total putrescine in leaves (A) and roots (B) of pea plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination (mean values ± SE). Different letters designate statistically significant difference at $p < 0.05$

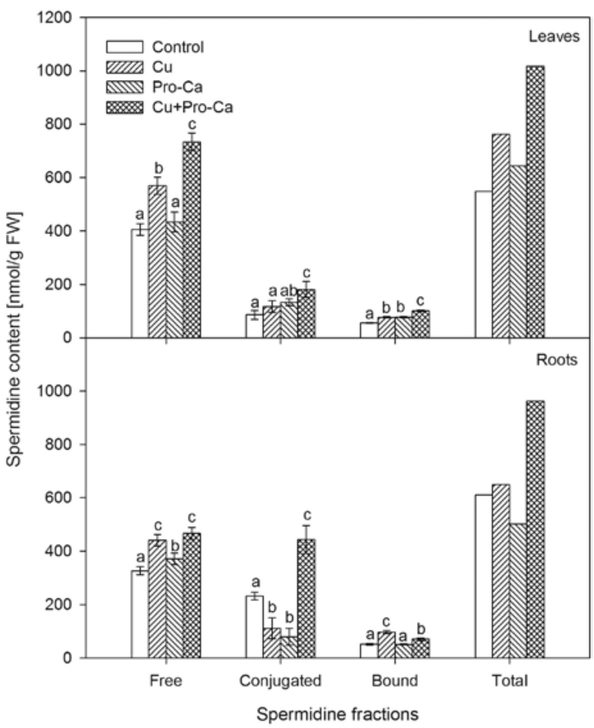

Fig. 2. Content of free, conjugated, bound and total spermidine in leaves (A) and roots (B) of pea plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination (mean values ± SE). Different letters designate statistically significant difference at $p < 0.05$

leaves (Figs 1–3).

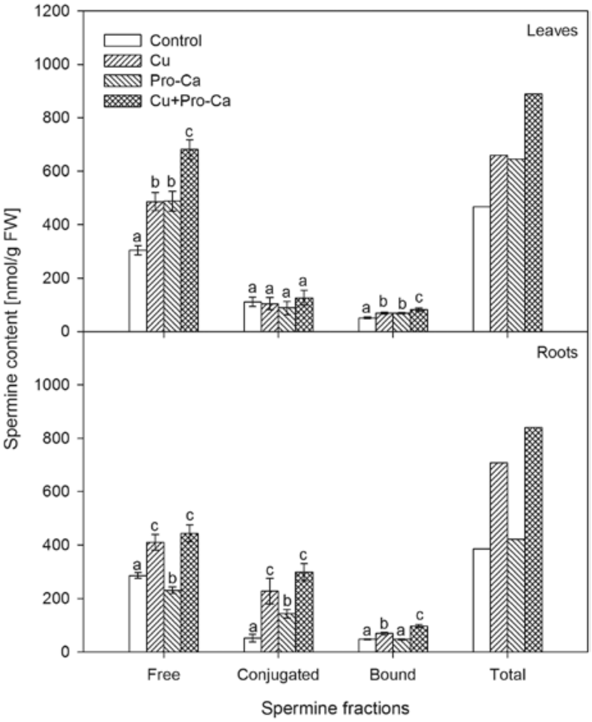

Fig. 3. Content of free, conjugated, bound and total spermine in leaves (A) and roots (B) of pea plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination (mean values ± SE). Different letters designate statistically significant difference at $p < 0.05$

There was a tendency of decrease of conjugated putrescine in leaves, while conjugated spermidine slightly increased and there was no change in conjugated spermine. In roots, the conjugated polyamines were most significantly increased by the simultaneous application of Cu + Pro-Ca, and Spm amount reached more than 5.5 times higher level than the control. Alone application of the chemicals caused the reduction of conjugated Put and Spd, especially after the treatment with Pro-Ca (by 71% and 65%, resp.) The bound forms of Put, Spd and Spm in leaves slightly increased in all treatments, while in roots the effect of copper either after alone or combined application was better expressed. By summarising the three polyamine fractions (free, conjugated and bound), it becomes obvious that the total polyamine content was significantly increased after the application of copper, but a synergistic increment was observed after the combined treatment with Cu and Prohexadione-Ca (Figs 1–3). The overall PAs content per whole plant also showed considerable augmentation after Cu application (by 39.7%) and Cu + Prohexadione-Ca (by 74%), while Prohexadione-Ca alone did not alter significantly PAs levels (Table 3).

Table 3. Total polyamine content per whole plants seven days after application of Cu^{2+}, Prohexadione-Ca and their combination. Values are calculated on the basis of the data presented in Figs 1–3

Variant	[nmol/g FW]	[%]
Control	3064.1	100.0
Cu	4280.7	139.7
Pro-Ca	3485.3	113.7
Cu + Pro-Ca	5333.1	174.1

DISCUSSION

It is well known that Cu in excess retards growth of various plant species (MARTINS & MOURATO, 2006; FARIDUDDIN et al., 2009; JANGID & SHRINGI, 2013). Our results also showed that Cu stress markedly decreased shoot and root fresh weight and length of pea plants (Table 1). In some other studies, the authors used exogenous compounds to improve growth of Cu-treated plants (FARIDUDDIN et al., 2009; CUI et al., 2010; SERGIEV et al., 2013). The results of our study also demonstrated that exogenous Prohexadione-Ca was able to improve shoot and root length and fresh weight of pea plants subjected to Cu stress (Table 1). Copper toxicity is mediated by the formation of free radicals (LUNA et al., 1994). The free radicals are highly reactive and can oxidize biological macromolecules such as lipids, and the lipid peroxidation disturbs membrane integrity and permeability (GILL & TUTEJA, 2010). SOD is a major enzyme that converts the highly toxic superoxide radical to hydrogen peroxide (GILL & TUTEJA, 2010). We found that Cu stress led to significant lipid peroxidation, and induced two-fold increase of SOD activity (Table 2). Similar tendency, but in different plant species, was reported by other authors (FARIDUDDIN et al., 2009; CUI et al., 2010; SINGH et al., 2010). After the simultaneous application of Pro-Ca and Cu, the activity of SOD increased less as compared to the Cu-treated variant. The decreased MDA amounts in the treated with Cu + Pro-Ca plants as compared to Cu-only treated plants might be an evidence that the oxidative stress was not largely spread out. The lower values of MDA as well as SOD activity in combine-treated plants indicate that Pro-Ca was capable to recover plant physiological status to regular level. It seems that Pro-Ca prevented the Cu-induced oxidative stress to be fully evolved and that Pro-Ca could protect pea plants against copper stress.

A number of environmental stresses, including heavy metals, cause significant accumulation of PAs in different tissues as a part of plant defence mechanisms (Todorova et al., 2014). In the present study, we found that an augmentation of polyamine pool occurred in plants treated with copper (Figs 1–3, Table 3). In hydroponically-grown *Erica andevalensis* with supplied increasing Cu concentration (1 to 500 μM) in the nutrient medium, the heavy metal led to a significant rise of PAs in shoots (Rossini Oliva et al., 2010). The increase in PAs found in shoots led the authors to suppose that PAs might play a protective function in plant cells at high metal concentration. Probably *de novo* synthesis of PAs took place in order to prevent the damages provoked by Cu, and the accumulation of PA could be involved in a protective mechanism against heavy metal stress by stabilizing the biomembranes as reported previously by other authors (Groppa et al., 2007a; Rossini Oliva et al., 2010). Since the total PAs levels in plants treated with Pro-Ca did not change significantly, the alterations in the concentrations of soluble polyamine fractions after the application of Prohexadione-Ca suggest that this could be a consequence of the interconversion of conjugated into free form and *vice versa*. However, when Pro-Ca was combined with Cu, it synergistically increased PAs levels (Figs 1–3, Table 3).

This gives a ground to suggest that Pro-Ca has a protective effect against Cu stress in pea plants, which is expressed by positive influence on the levels of endogenous polyamine fractions, and it could be proposed that leaf spraying with Pro-Ca could be useful approach to increase pea plant tolerance against Cu stress.

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CA-PROHEKSADIONO IR VARIO POVEIKIS ŽIRNIŲ AUGIMUI IR ENDOGENINIŲ POLIAMINŲ KAUPIMUI

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Santrauka

Buvo tiriamas retardanto kalcio proheksadiono (BAS 125 10W BASF, Vokietija) ir sunkiojo metalo vario poveikis žirnių šaknų ir lapų augimui bei endogeninėms jų laisvo ir konjuguoto putrescino, spermidino bei spermino koncentracijoms. Buvo nustatyta, kad praėjus 7 dienoms po apdorojimo kalcio proheksadionu poliaminų koncentracijos padidėjo. Ženkliausias poliaminų koncentracijos padidėjimas buvo aptiktas augalus paveikus vario jonais ir kalcio proheksadionu. Poliaminų koncentracijų svyravimai žirnių lapuose ir šaknyse parodė, kad Ca proheksadionas suteikė žirniams apsauginį poveikį nuo Cu streso, ir tas poveikis pasireiškė papildomu poliaminų kaupimu.