Journal of Plant Physiology 219 (2017) 37–44

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Research Paper

Mesophyll cell ultrastructure of wheat leaves etiolated by lead and selenium

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ARTICLE INFO

Keywords:
Etiolation
Lead
Selenium
Triticum aestivum

ABSTRACT

The ultrastructure of mesophyll cells was studied in leaves of the *Triticum aestivum* L. cv. "Trizo" seedlings after two weeks of growth on soil contaminated by Pb and/or Se. The soil treatments: control; (Pb1) 50 mg kg^{-1}; (Pb2) 100 mg kg^{-1}; (Se1) 0.4 mg kg^{-1}; (Se2) 0.8 mg kg^{-1}; (Pb1 + Se1); (Pb1 + Se2); (P2 + Se1); and (Pb2 + Se2) were used. Light and other conditions were optimal for plant growth. The (Se1)-plants showed enhanced growth and biomass production; (Pb1 + Se1)-plants did not lag behind the controls, though O_2 evolution decreased; chlorophyll content did not differ statistically in these treatments. Other treatments led to statistically significant growth suppression, chlorophyll content reduction, inhibition of photosynthesis, stress development tested by H_2O_2 and leaf etiolation at the end of 14-days experiment. The tops of etiolated leaves remained green, while the main leaf parts were visually white. Plastids in mesophyll cells of etiolated parts of leaves were mainly represented by etioplasts and an insignificant amount of degraded chloroplasts. Other cellular organelles remained intact in most mesophyll cells of the plants, except (Pb2 + Se2)-plants. Ruptured tonoplast and etioplast envelope, swelled cytoplasm and mitochondria, and electron transparent matrix of gialoplasm were observed in the mesophyll cells at (Pb2 + Se2)-treatment, that caused maximal inhibition of plant growth. The results indicate that Pb and Se effects on growth of wheat leaves are likely to target meristem in which the development of proplastids to chloroplasts under the light is determined by chlorophyll biosynthesis. Antagonistic effect of low concentration of Se and Pb in combination may retard etiolation process.

1. Introduction

Lead toxicity in plants is a well-known fact (Sharma and Dubey, 2005; Seregin and Kozhevnikova, 2008; Hadi and Aziz, 2015). Like some other heavy metals Pb induces oxidative stress in plants (Choudhury and Panda, 2005; Balakhnina et al., 2016), affects plant growth, leaf development and photosynthesis (Kosobryukhov et al., 2004; Islam et al., 2008), reduces stomatal aperture (Ghelich and Zarinkamar, 2013), increases stomatal resistance (Balakhnina et al., 2016) and induces chlorosis of leaves (Pandey et al., 2007). Chlorosis of leaves under heavy metal treatment is connected with inhibition of δ-aminolevulinic acid dehydratase (Prasad and Prasad, 1987). The effects of lead on the plant cell ultrastructure are mainly studied on the roots including root meristem (Wierzbicka, 1987; Tung and Temple, 1996; Jiang and Liu, 2010; Kaur et al., 2013) and rarely in leaves (Tung and Temple, 1996; Liu et al., 2008).

In excluder plants such as wheat, lead as well as other heavy metals is known to be accumulated mostly in the roots (Islam et al., 2008; Sharma and Dubey, 2005). Lead transport in roots has been described in detail (see Wierzbicka, 1987; Seregin and Kozhevnikova, 2008) while its transport to meristem at the base of leaf sheaths has not been sufficiently investigated.

Selenium is recognized as an essential micronutrient needed for humans and animals (Hartikainen, 2005). With regard to plants, microamounts of Se in the nutrient medium have also been shown useful (Feng et al., 2013; Haghighi et al., 2015). Extensive literature data shows that the application of Se increases plant resistance to chilling (Chu and Zhang, 2010), drought (Ibrahim, 2014; Nawaz et al., 2015), salinity (Hawrylak-Nowak, 2009) and heavy metals (Issa and Adam, 1999; Filek et al., 2009; Lin et al., 2012; Mroczek-Zdyrska and Wójcik, 2012; see also Hasanuzzaman et al., 2010; Sieprawska et al., 2015). However, high concentrations of Se may cause growth inhibition, chlorosis, withering and drying of leaves, suppressing of protein synthesis, and even death of plants (see Hartikainen et al., 2000; Terry

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http://dx.doi.org/10.1016/j.jplph.2017.09.008
Received 7 February 2017; Received in revised form 30 August 2017; Accepted 22 September 2017
Available online 28 September 2017

et al., 2000; Feng et al., 2013). Chlorosis of leaves under high concentrations of selenium is considered due to inactivation of porphobilinogen synthase activity (Padmaja et al., 1989). Few studies done on plant cell ultrastructure under the effect of high concentrations of selenium are inconsistent; Schiavona et al. (2012) showed the lack of morphological and ultrastructural alterations in *Ulva* sp. exposed to selenate, Reunova et al. (2007) demonstrated autolysis of the cells in green microalgae.

To find out the similarities and differences in the action of selenium and lead to higher plant chloroplast ultrastructure and functional activity of the leaf, and the possibility of diminution of negative effects of lead by low concentrations of selenium, the aim of the present work was to study ultrastructural organization of mesophyll cells, O_2 evolution and chlorophyll content in leaves, as well as H_2O_2 content as indicator of stress development in wheat plants treated by different concentrations of lead and selenium, both independently and in combination.

2. Materials and methods

2.1. Plant growth conditions

The seeds of *Triticum aestivum* L. cv. "Trizo" (species Lutescens) after sterilization by 3% H_2O_2 for 10 min were soaked in distilled water for 24 h and germinated on moist filter paper in the dark for 2 days. Equally well-germinated seeds were sown in plastic pots (18 seeds per pot). The pots were filled with slightly acidic soil with low available nitrogen; medium contents of phosphorus and selenium, and the higher content of potassium (see Balakhnina and Nadezhkina, 2017). The lead content in the soil did not exceed 6.27 mg kg^{-1}. Plants were grown under 300 μE m^{-2} s^{-1} illumination at 16/8 h day/night photoperiod and 22/20 °C day/night air temperature and investigated after 14-days growth.

2.2. Soil treatments

Nine variants of the soil treatments in 3 replicates were prepared for plant cultivation: (C) – the original soil; (Se1) – 0.4 mg kg^{-1}; (Se2) – 0.8 mg kg^{-1}; (Pb1) – 50 mg kg^{-1}; (Pb2) – 100 mg kg^{-1}; (Pb1 + Se1); (Pb1 + Se2); (Pb2 + Se1); (Pb2 + Se2). For this, 80 mg (Pb1) or 160 mg (Pb2) of $Pb(NO_3)_2$ were added per kg of dry soil as sours of lead. Selenium was applied in the form of Na_2SeO_4 at the concentration of 0.96 mg kg^{-1} (Se1) or 1.92 mg kg^{-1} (Se2), respectively. Weighted lead and selenium salts, calculated per 15 kg of air-dried soil, were dissolved in 1 L of distilled water. Soil (15 kg for each variant) was placed on the plastic film, aligned and sprayed by hand sprayer with a thin layer of salt solution or water (control). After thorough mixing, the soil was brought up to full field water capacity and used to fill in 1-L pots.

2.3. Electron microscopy analyses

Structural studies were carried out on the small leaf sections from the middle part of the second leaf, fixed with 2% glutaraldehyde in phosphate buffer with post fixation by 1% osmium tetroxide. After dehydration with the help of series of increasing alcohol and acetone concentrations the samples were embedded in Epon epoxide resin. Ultrathin sections for electron microscopy were cut using LKB ultratome (Sweden), contrasted with uranyl acetate and lead citrate, viewed and photographed with an electron microscope JEM 100B (Japan) (Semenova and Romanova, 2011). Quantitative measurements of organelles and inclusions were performed on ultrathin sections of mesophyll cells in the equatorial and sub-equatorial regions; the obtained data are estimates and not intended for statistical accuracy.

2.4. Chlorophyll measurement

Total chlorophyll (a + b) was measured spectrophotometrically (Lichtenthaler and Wellburn, 1985) in acetone extracts of leaf sections (0.1 g per 10 mL of 80% acetone cooled up to 2–4 °C) from the middle part of the second leaf.

2.5. Photosynthetic oxygen evolution measurement

Photosynthetic O_2 evolution was measured in the leaf slices (1–2 mm) suspended in a 50-mM K-phosphate buffer (pH 7.5) and placed in a water-jacketed cell of polarograph LP-7e (Prague) equipped with a standard Clark platinum electrode. The incident light intensity was 1200 μE m^{-2} s^{-1}, temperature was 25 °C. Before measurements 0.1 mL of 0.5 M $NaHCO_3$ sodium bicarbonate was added into the reaction medium as a source of carbon.

2.6. Hydrogen peroxide measurement

The determination of hydrogen peroxide was based on the peroxidative oxidation of luminol (Cormier and Prichard, 1968). Leaves (50–100 mg) were frozen in liquid nitrogen for 20 s. The frozen pieces were transferred to 0.4 mL of 2 M trichloracetic acid and homogenized. The extraction of H_2O_2 was carried out with 3 mL of 0.02 M K-phosphate buffer (pH 8.5). For the absorption of pheophytin and carotenoids, activated carbon (250 mg) was added to 3.4 mL of the homogenate, which was centrifuged for 20 min at 10,000g. The supernatant was decanted and titrated with 2 M KOH to pH 8.5. The H_2O_2 content was determined in 0.05–0.10 mL of the extract by adding 1 mL of a bioluminescent mixture containing peroxidase (1×10^{-6} M) and luminal (2.26×10^{-4} M) and calculated according to the standard curve (Lyubimov and Zastrizhnaya, 1992).

2.7. Statistical analyses

Biometric and chlorophyll analysis were conducted in 18 and 6–8 replications respectively. Statistical analyses were performed using the OriginPro 2015 software package program. All results were subjected to a one-way ANOVA with Bonferroni's test to confirm the variability of the data and validity of results. Values were expressed as means ± S.D., and differences between means were considered statistically significant at $P < 0.05$.

3. Results

3.1. Shoot growth

Two-week seedlings grown on (Se1)-soil showed an increase in length and biomass of shoots, compared to control plants, whereas (Se2)-treatment strongly suppressed the growth process (Table 1). (Pb1)-treatment resulted in a decrease in the length and biomass of the shoots by about 20%. Application of (Se1) showed a protective effect against (Pb1)-treatment, i.e. (Pb1 + Se1)-plants did not lag behind the control ones. (Se2)-treatment, on the contrary, increased the negative effects of (Pb1). Negative effects of (Pb2)-treatment were stronger than those of (Pb1), and application of (Se1) did not affect the action of lead. The joint action of (Pb2) and (Se2) led to the strongest inhibition of seedling growth, namely the length and biomass of (Pb2 + Se2)-plants did not exceed 44% and 38%, respectively, of the control values (Table 1).

3.2. Chlorophyll content, photosynthetic oxygen evolution and hydrogen peroxide concentration

The leaves of the plants were green under all soil treatments during the first week of growth. Two weeks later, the etiolation occurred in the