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**ACTIVITY OF APOPLASTIC AND CYTOSOLIC PEROXIDASES UNDER THE AFTEREFFECT OF COPPER IONS IN *NICOTIANA TABACUM* PLANTS**

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The adaptation of plants to an excess of heavy metals in the environment and their recovery after elimination of the stressor is of interest in connection with the large-scale pollution of ecosystems and their remediation. The physiological and morphological reactions of plants under the action of stress factors have been well studied; however, there is much less information on plant recovery after the removal of the action of the stressor. It has been reported that copper ions are recycled from aging organs to young ones, which can contribute to the development of oxidative processes in various organs even after the stressor has been removed [1]. Antioxidant enzymes such as class III peroxidases (EC 1.11.1.7), which include guaiacol (GPO) and benzidine (BPO) peroxidases are involved in the hydrogen peroxide metabolism and their activity is specifically changes during plant development and depends on the type of plant tissue, the availability of substrates [2]. The study is aimed at the aftereffect of copper ions (100 and 300 µM) in plants of *Nicotiana tabacum* L.

Plants of *Nicotiana tabacum* L. were cultivated on a substrate – a mixture of perlite: vermiculite (1: 1) on Knop's medium with the addition of 0 (control), 100 and 300 μM/L CuSO4 during the first 20 days after germination, followed by cultivation on Knop's medium until reaching the age of 40 days. The activity of cytosolic and apoplastic guaiacol and benzidine peroxidase, the amount of Н2О2 was determined spectrophotometrically according to the standard method in the crude extract [3, 4]. Protein electrophoresis was carried out under non-denaturing conditions in a 10% polyacrylamide gel; peroxidase isoforms were detected according to Lee et al. [5]. Data analysis was performed in Excel and STATISTICA 10 for Windows 10 using the Mann-Whitney *U*-test.

During the recovery period the concentration of hydrogen peroxide in plant organs (root, stem, and leaves) was high compared to the control. The responses of the roots and shoots under the aftereffect of the stressor was different. The activity of cytosolic guaiacol peroxidase and apoplastic peroxidases in root tissues increased accordingly to the rise of Н2О2 amount. In plants pretreated with a lower copper concentration in leaves the activity of apoplastic peroxidases in the stem, cytosolic and apoplastic benzidine peroxidases increased. Pretreatment with a high copper concentration, on the contrary, led to a decrease in the activity of peroxidases during the period of plant recovery. Revealed universal and specific isoforms of peroxidases for root, stem, and leaf tissues. In the case of plant pretreatment with 300 µM Cu2+, a decrease in the enzymatic activity of individual isoforms was shown. We assumed that high concentrations of Н2О2 in root tissues stimulated the activity of cytosolic and apoplastic GPO, and apoplastic BPO.

The activity of peroxidases considered to be a marker of plant resistance to stress factors [1, 2]. The changes in enzyme activity indicate that the plants did not get rid of the symptoms of copper ion toxicity and still were stressed even after the treatment was remote for 20 days.

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Thus, plant organs differed in the content of H2O2 and the activity of class III peroxidases localized in different compartments (apoplast and cytosol) and in their ability to recover after the removal of the stressor. The aftereffect of excess copper ions led to an increase in the content of hydrogen peroxide in root and shoot tissues, which indicates the sensitivity of *N. tabacum* to this stressor and incomplete plant recovery in the post-stress period. The data obtained indicate the heterogeneity of tissues and organs in response to oxidative stress caused by the action of copper ions in the culture medium. The results show that copper had a long-lasting aftereffect. The accumulation of Н2О2 and the activity of peroxidases depend on the strength of stress and their tissue localization.

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