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Summary of the doctoral dissertation entitled „Phosphoromic study in evaluation of plant metabolics condition”

Phosphorus is a crucial element for the functioning of living organisms. The transformations of phosphorus compounds that occur as a result of vital processes determine the metabolic efficiency of each organism. Consequently, the efficiency of these processes determines the organism's readiness to carry out specific biochemical transformations that enable its development and maintain homeostasis. The research on these transformations is feasible by implementing a novel approach in metabolomics, namely phosphoromic research. This approach allows for the assessment of an organism's developmental potential, the extent of the influence of physiological stress on the organism's energy state, and the nature of the impact (positive, negative, or neutral) of selected physicochemical factors.

Among the methods used to determine the changes occurring in the metabolism of the plants tested, nuclear magnetic resonance spectroscopy ^{31}P NMR technique, played an important role, which allowed phosphorus profiles to be created. In turn, the content of phosphorylated adenine nucleotides (ATP, ADP and AMP) was determined using high-performance liquid chromatography (HPLC), which made it possible to establish the energy status and, in particular, the intensity of the catabolic or anabolic processes occurring in plants exposed to selected stressors. In addition, in order to trace the transformation of phosphorus compounds at the initial stage of plant development, changes in inorganic phosphorus and protein content were compared with the activity of acid phytase and phytic acid content – parameters thought to be useful so far mainly in cereal and legume species. For this purpose, a phytic acid extraction method was adapted and used in further studies to determine the effect of fungicide preparations on cucumber seed germination. A method for the extraction and determination of acid phytase activity in radish and cucumber sprouts was also developed, thus extending the application of this procedure. Determination of the aforementioned parameters provided new information on the nature of the quantitative transformations of phosphorus forms in plants at the initial growth stage, and their collation allowed a better understanding of the transformations of phosphorus compounds occurring in stressed plants.

The implementation of the present study in accordance with the research objectives has markedly enhanced the experimental procedures, thereby facilitating the determination of diverse forms of phosphorus in complex biological matrices. As a consequence, the presence of phosphonate compounds in plants has been irrefutably established, and it has been demonstrated that the phosphorus profiles (phosphoromes) associated with these substances are sufficiently distinctive to warrant consideration as genus or even species markers of interest. These results have

the potential to significantly expand the scope of plant chemotaxonomic research, influencing both the biological sciences discipline and the chemical sciences discipline. The findings of this research contribute to our understanding of the impact of diverse physiological stressors, including light colour, the presence of xenobiotics (in this case, fungicides) and transition metal ions, on the metabolism of germinating seeds. The effects of physical stressors were also analysed, and it was demonstrated that light colour affects not only standard biochemical markers (e.g. antioxidant compounds) but also the metabolism of phosphorus compounds. Light, causes changes in the activity of phytases - enzymes crucial in the germination process, thus influencing the free phosphorus (Pi) content. Depending on the wavelength, light leads to changes in the nature of the metabolic processes taking place and influences the energy status of plant cells. In plant cells growing under suboptimal light conditions, there was an increase in AMP content, due to the activation of biochemical pathways characterised by an increased demand for energy stored in ATP. With regard to the physiological stress of plants induced by the addition of copper(II), manganese(II) and zinc(II) ions, it was observed that the presence of these ions in the medium gave rise to disparate changes in plant metabolism. It was demonstrated that copper ions resulted in a reduction of the AEC parameter in comparison to the control conditions, and in the initial stages of plant development, contributed to an increase in the AMP content. The addition of zinc(II) ions resulted in an increase in adenine nucleotide content, whereas plants treated with manganese(II) ions exhibited a significant disruption of phosphorus metabolism. This was evidenced by a decrease in adenine nucleotide content and an accompanying decrease in free phosphate ion content. Furthermore, it was demonstrated that the application of fungicides, whether via seed treatment or foliar application, can exert a detrimental impact on the energy status of cucumber seedlings. With regard to cucumber seed treatment, the greatest differences in comparison to the control were observed in the case of Scorpion 325 SC (Agrecol). The active substances (azoxystrobin and difenoconazole) were observed to disrupt adenine nucleotide synthesis, with the greatest impact on ATP. However, when the preparations were applied as sprays, significant differences in the value of the AEC parameter between the control and the tested preparations were observed in the aboveground parts of the cucumber.

The complementary application of the ^{31}P NMR technique to the study of the phosphorus metabolome, complemented by the determination of the energy status of the organism based on adenylyl energy charge, enables the creation of dynamic phosphorus profiles reflecting the physiological state of plants and allows the set of methods used in monitoring metabolism to be extended, thus confirming the applicability of phosphoromic studies in the assessment of plant health.

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