

Description for the general public

Plants interact with each other not only through competition for light, water and nutrients but also by the use of sophisticated chemical weapons. They are able to release different chemical compounds (allelochemicals) into the environment, causing a positive or negative impact on their neighbors. Due to the final consequence of application of these substances, such as growth restriction, or even death of neighboring plants, most observed and studied effects are negative. Compounds from different chemical groups could be allelochemicals. In the last 20 years it was demonstrated that among them are found non-protein amino acids. Generally, there are standard 20 amino acids, which are the building blocks of proteins for all plant and animal organisms. However, beyond the 'Great Twenty' there are over 200 other amino acids that are synthesized by various plants, and whose role is different than the formation of functional proteins. Some of them are toxic to insect herbivores or have an important role as intermediates in the biochemical reactions, or play a role of important signaling compounds. **A group of such non-protein amino acids include meta-tyrosine (*m-Tyr*)**, which is equivalent to the structural protein of the amino acid - phenylalanine. ***m-Tyr* is produced in the roots of plants of the fescues genus** - popular grasses used to set up pitches and golf courses. Previous studies conducted in the USA have shown that this compound, which is secreted into the soil by the roots of fescues, has a strong toxic effect towards many plants (including also *Arabidopsis thaliana* - the model plant used especially in genetic research). It is of great interest, that in animal's and human cells *m-Tyr* is considered as a marker of oxidative stress associated with increased production and accumulation of reactive oxygen species (ROS). ROS are "brothers" and "sisters" of oxygen molecules. They are produced in the cells of all organisms in many normal processes, e.g. breathing (respiration) and/or photosynthesis, in which the electrons are transferred to the oxygen molecule (when 4 electrons are transferred onto the oxygen molecule the water molecule is produced, but it not always happening, so if less electrons are transferred onto oxygen ROS are formed). High concentrations of *m-Tyr* is characteristic for people suffering from Alzheimer's disease, diabetes, or atherosclerosis, thus it is of great importance to know the mechanism of action of this compound. **The objective of the proposed research** is to link the negative impact of *m-Tyr* at root growth of young seedlings of tomato to changes in the metabolism of ROS. **We will be interested, particularly in the glutathione content.** It is, in addition to vitamin C (ascorbate) a compound responsible for removal of the ROS in cells. It occurs in tissues in the reduced and oxidized forms. Reduced form is required for maintenance of low amount of ROS. **We are also planning to examine how *m-Tyr* influences the activity of the three enzymes (superoxide dismutase, glutathione peroxidase and glutathione reductase)**, which are included in the so-called cellular antioxidant system (regulating concentration of ROS). Superoxide dismutase converts one of the ROS - superoxide anion to the less toxic hydrogen peroxide, which is then converted to water by glutathione peroxidase. Existing data suggest an increased level of superoxide anion in the roots of plants growing in the presence of *m-Tyr*. In contrast, glutathione reductase is responsible for maintaining a high concentration of the reduced form of glutathione in the cells and thus assure the high ability of the cells to scavenge ROS. Glutathione content depends also on the production of reactive nitrogen species, especially nitric oxide. The concentration of nitric oxide is regulated by formation of a nitrosogluthathione (GSNO). In turn, the amount of GSNO depends on activity of GSNO reductase, the enzyme responsible for converting this molecule to an oxidized glutathione and ammonia. As demonstrated in our last published studies, the activity and the amount of this enzyme increased in the plants treated with the *m-Tyr*. To complement the biochemical data measurement of transcription of genes encoding the aforementioned enzymes (superoxide dismutase and glutathione reductase) is also planned. **As research material we will use a roots of tomato seedlings (*Solanum lycopersicum* L., v. Malinowy Ożarowski)**, whose growth will be in 50 or 100% inhibited by supplementation with *m-Tyr* in a concentration of 50 or 250 μM for 24 or 72 hours. **Scientific hypothesis assumes that under the influence of *m-Tyr* the cellular system** which modulate the concentration of ROS **will be upregulated**. Thus, resulting in changes in the activity of enzymes and gene transcription of superoxide dismutase, glutathione peroxidase and glutathione reductase, as well as change the content of the oxidized and reduced forms of glutathione. We assume that changes in the enzyme would be upward. **Modification of the activity of glutathione reductase in turn will trigger modification in the ratio of oxidized to reduced form of glutathione.** The content of both forms of glutathione will be determined by use of high performance liquid chromatography - the method that allows a very thorough separation of the compounds present in the mixture. Transcription of the genes of the enzymes will be conducted by qRT-PCR (quantitative real-time polymerase chain reaction), using tomato genome, which is known since 2012. The enzymatic activity of superoxide dismutase will be determined after separating the proteins on the gel, it allow also to distinct isoforms of the enzyme, while the activity of glutathione reductase will be determined spectrophotometrically. **We hope that this research** will complemented the currently conducted analysis and **will allow us to propose a comprehensive model describing the mode of action of *m-Tyr* in plants**, taking into account the metabolism of reactive oxygen and nitrogen species **with a particular emphasis on the role of glutathione.**