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Research article

Changes in growth and physiological parameters of spring barley and common radish under the influence of 1-butyl-2,3-dimethylimidazolium tetrafluoroborate

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A R T I C L E I N F O

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A B S T R A C T

Ionic liquids (ILs) constitute a large group of chemical substances, which, thanks to their desirable properties, still attract attention of scientists and representatives of the industry. This may lead to a greater commercial use of these compounds, which will undoubtedly lead to the contamination of soils, constituting the basis of plant vegetation, with these substances. This paper presents effect of 1-butyl-2,3-dimethylimidazolium tetrafluoroborate [BMMIM][BF_4] on the growth and development of spring barley and common radish and on the physiological and biochemical changes in these plants. The used IL was characterized by relatively high toxicity for the monocotyledonous plant, which was exhibited by shortening of the plant length and their root length, decreasing the fresh weight yield. Moreover, [BMMIM][BF_4] led to the decrease in the content of all photosynthetic pigments in spring barley seedlings, reflecting the decrease in the fresh yield. Furthermore, the increase of malondialdehyde (MDA) level and changes in contents of hydrogen peroxide (H_2O_2) and the activity of antioxidant enzymes, that is, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) may suggest the occurrence of oxidative stress in spring barley. The decrease in the content of photosynthetic pigment and the increase of POD activity constitute the most reliable markers of oxidative stress and, at the same time, the signs of early aging of spring barley plants. Common radish was the plant with a very high tolerance for the used IL, which can be indicated by, that is, EC_{50} values, determined based on inhibition of root length, plant length, and fresh weight yield.

1. Introduction

Reactive oxygen species (ROS) are substances characteristic of normal plant metabolism, they are formed primarily as side products in the respiratory chain and of chemical reactions related to substrate oxidation. ROS, which are found in plant cells in low concentrations, play an important signaling role, used in regulating numerous physiological processes, such as plant immune reactions, gravitropism, phytohormone effect, leaf growth or development of flowers, seed germination, or cell death signaling. On the contrary, overproduction of ROS may lead to the occurrence of oxidative stress, which may result in lipid peroxidation, damage of proteins, nucleic acids or photosynthetic pigments; this inhibits stem and root growth, decreases yields, etc. Therefore, maintaining of ROS concentration at a balanced level constitutes one of the main problems encountered by plants during their entire vegetation period (Dragišic Maksimovic et al., 2013; Anjaneyulu et al., 2014; Rosalie et al., 2015).

Terrestrial plants are constantly vulnerable to a series of abiotic and biotic factors that cause oxidative stress in their organisms. Numerous scientific articles demonstrate, that the increase of ROS concentration occurs in plants subject to stress related to salinity, high and low temperatures, soil contamination with heavy metals and aluminum, effect of herbicides, ozone fumigation, or pathogen infestation (Sánchez-Rodríguez et al., 2010; Choi and Hwang, 2012; Anjaneyulu et al., 2014; Rachoski et al., 2015; Rosalie et al., 2015). Plants, being sedentary organisms, and thus unable to avoid stressors, have to develop defense system against oxygen radicals through evolution. The first line of defense against ROS includes non-enzymatic, low-molecule antioxidants as follows: reduced glutathione, tocopherol, carotenoids, flavonoids and ascorbic acid.

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The second line of defense against ROS comprise an enzymatic system, including enzymes such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) as well as glutathione peroxidase (GPX) and glutathione reductase (GR) (Sánchez-Rodríguez et al., 2010; Anjaneyulu et al., 2014; Gengmao et al., 2015; Rosalie et al., 2015).

It is increasingly common to include ionic liquids (ILs) in the abiotic factors causing oxidative stress in plants. ILs are one of the most promising and universal chemicals of the last few decades; they are characterized by ionic structure and the fact, that they can have both liquid and solid state in a wide temperature range. A great interest in ILs stems from their numerous desirable properties, such as low vapor pressure, polarity, high thermal and electrochemical stability, high ionic conductivity, non-flammability, and good catalytic properties. However, the most important feature of these chemical compounds consists in the ability to obtain theoretically assumed physical, chemical, and biological characteristics via selection of suitable cation, anion exchange or mixing 2 or 3 ionic liquids. Thus, in the literature, ILs are often called the "designer solvents." The above features of ILs enabled to consider them as the perfect substitutes for the volatile organic solvents, using them not only in the processes of biocatalysis, chemical analysis, separation and extraction, electrochemistry, but also in the pharmaceutical industry and biotechnology. What is more, ILs are successfully used in nanotechnology and as supercapacitors, as electrolytes in batteries, and as substances that increase corrosion resistance of metals (Messali et al., 2013; Cvjetko Bubalo et al., 2014a).

Ionic liquids, similar to all chemical compounds, may penetrate to soil environment, where they are retained by the soil colloids or occur in the soil solution. From there, they are absorbed by plants, influencing their growth and development, and also the size and quality of the yield. Thus, in the available literature there are increasingly common reports on the influence of ILs on terrestrial higher plants (Biczak et al., 2013, 2015; Cvjetko Bubalo et al., 2014b). In the mentioned works, the level of ILs effect on terrestrial higher plants was determined based on plant growth, fresh and dry weight level, and their appearance; however, Cvjetko Bubalo et al. (2014b) reports that the ILs' toxicity mechanism itself has not yet been entirely understood. Thus, in recent years, the scientific literature expresses a view that ILs cause oxidative stress in the studied organisms (Liu et al., 2014, 2015a, 2015b; Biczak, 2016; Biczak et al., 2016; Pawłowska and Biczak, 2016).

Considering the results of the presented study, the present experiment aimed at determining the growth inhibition and estimation of oxidative stress level in spring barley and common radish grown on soil with the addition of 1-butyl-2,3-dimethylimidazolium tetrafluoroborate. Due to low melting point (37 °C) and desired physical and chemical properties, [BMMIM][BF_4] has been successfully used as independent solvent or in mixtures with traditional solvents such as alcohols, acetonitrile, and DMSO. This has been widely used in chemical synthesis, electrochemistry, catalysis, and different biotechnological processes (Roy et al., 2014; Xu et al., 2015), which provides for a real possibility to use much post-production waste containing [BMMIM][BF_4], which will burden the natural environment. Currently, there are no reports describing phytotoxicity of ILs containing 3 alkyl substituents near the cation, and the existing studies of a similar nature concern only antimicrobial activity of compounds characterized by a similar chemical structure. The selection of IL containing tetrafluoroborate anion for the study was dictated by the information from the literature on the high toxicity of these anions because fluoride ion is created as a result of their hydrolysis, which is toxic and highly undesirable in the natural environment. Telesiński and Śnioszek (2009) and Telesiński et al. (2011) determined that the effect of fluorine on plants concerns, that is, the negative influence on the assimilation and photosynthesis processes that consequently leads to the inhibition of plant growth, etc. These phenomena stem from the destructive influence of fluorine on chloroplasts. The choice of spring barley for the study was dictated by the fact that it occupies fourth position among all the cereal species in terms of production and acreage, and radish is a popular vegetable, enriching the human diet in the number of micro- and macroelements and vitamins (Dragišic Maksimovic et al., 2013; Arias-Baldrich et al., 2015). In addition, the objective of the above study was to compare the effect of [BMMIM][BF_4] on mono- and dicotyledonous plants that may constitute the basis for the selection of such plant species, which in soils contaminated with ILs will cope with oxidative stress more efficiently, producing expected yields of required quality.

2. Materials and methods

2.1. Ionic liquid

The Ionic Liquid [BMMIM][BF_4] ($\geq$97% purity, CAS No 4028-0) used in the study was purchased from Sigma-Aldrich Chemical Co. The structure of the tested IL is illustrated in Fig. 1.

2.2. Evaluation of ILs phytotoxicity

Determination of phytotoxicity of IL, was performed according to the OECD/OCDE guidelines (2006). Following the recommendations of the guideline for phytotoxicity study, the following concentrations of IL were used: 0, 1, 10, 50, 100, 400, 700 and 1000 mg of compound per 1 kg of dry weight (DW) of the soil. When preparing the above-mentioned concentrations, appropriate amount of ILs was dissolved in acetone and subsequently mixed with quartz sand. After overhead evaporation of acetone, quartz sand containing IL was mixed with the soil. For each concentration of IL, 3 independent samples were prepared.

Control samples were prepared using the same procedure by adding acetone to the sand, however without IL. Granulometric analysis showed that in this study, loam was used as a soil. It contained about 9% of fraction with a diameter of <0.02 mm and organic carbon – 9.0 g kg^{-1}. The pH was 5.9. With the prepared medium, plastic pots were filled, and then 20 seeds of spring barley (*Hordeum vulgare*) and common radish (*Raphanus sativus* L. *radicula* Pers.) derived from the same source were seeded. Seed germination and seedlings growth (14 days) were carried out under strictly controlled conditions: soil moisture of 70% ppw, temperature of 20 ± 2 °C and constant illumination equal to 165 μmol m^{-2} s^{-1} for 16 h day/8 h night. Such stable growth and development conditions were ensured by vegetation hall, which belongs to the Department of Biochemistry and Ecotoxicology at Jan Długosz University in

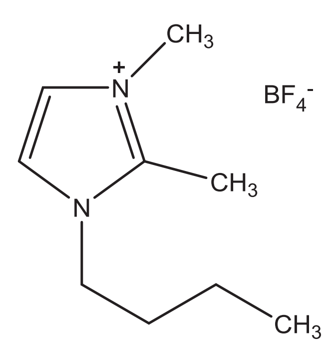

Fig. 1. Structure of the analyzed ionic liquid.