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Effects of imidazolium-based ionic liquids with different anions on wheat seedlings

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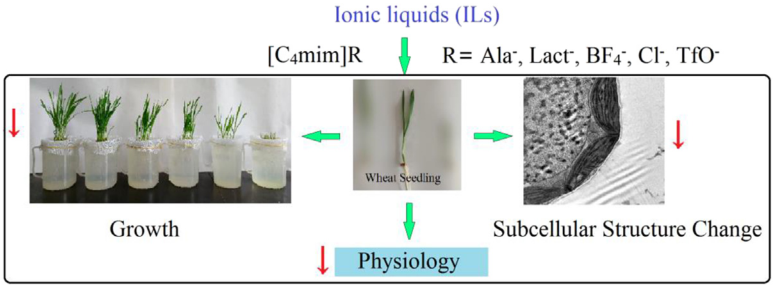
HIGHLIGHTS

- Effects of ionic liquids (ILs) with five different anions on wheat seedlings were studied for the first time.
- Effects of ILs on plant were studied from the subcellular level.
- Anion structure could affect the overall toxicity of ILs.
- ILs influenced the growth of plants may by limiting intracellular proteins transport.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of five imidazolium ionic liquids with different anions were studied in hydroponically grown wheat seedlings at concentrations of 50, 100, 150, 200, 250, and 300 mg L^{-1}. The results showed that shoots and roots grew shorter and dry weight decreased with increasing concentrations of ionic liquids. Moreover, the antioxidant enzyme activities decreased and malondialdehyde (MDA) content was greater in the leaves of wheat seedlings subjected to ionic liquid (IL) treatments. The order of influence of ionic liquids on these indexes was $[C_4mim][TfO] > [C_4mim][Cl] > [C_4mim][BF_4] > [C_4mim][Lact] > [C_4mim][Ala]$. A transmission electron microscope (TEM) was used to observe leaf and root cellular structures, such as chloroplast, nucleus, mitochondria, and rough endoplasmic reticulum, in wheat exposed to ionic liquids at a concentration of 150 mg L^{-1}. The results showed that the cellular structures of wheat were affected, and the degree of the effect of five ILs was consistent with the general trend of the measured indexes in this study. Ionic liquids influence the growth of plants by impeding growth, disrupting metabolic physiology and changing cellular structures. The degree of toxicity of imidazolium-based ionic liquids with different anions varies.

1. Introduction

Ionic liquids (ILs) are entirely composed of ions (generally the larger organic cations and inorganic or organic anions) and exist mostly in the liquid state at room temperature. They are also called low-melting-point salts (below 100 °C). Due to their unique physicochemical properties such as negligible vapor pressure, non-volatility, and non-flammability (Hallett and Welton, 2011), ILs are considered "green solvents" and are widely used in various applications such as synthesis or in electrochemical, biological and metal extractions (Yuan et al., 2010; Patel and Lee, 2012). However,

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it may be used as a solvent or catalyst, the ILs are eventually released into the environment. Some studies have demonstrated that ILs exhibit broad toxicity, which may be even greater than that of traditional organic solvents (Samortì et al., 2007; Ventura et al., 2013). Their high water solubility and resistance to biodegradability make it possible for ILs to become persistent organic pollutants in water, thereby causing contamination (Zhang et al., 2010). Consequently, it is of great practical importance to explore the ecological stability of ILs before they are applied on an industrial scale.

The toxicity of ILs depends on the type of cation and the length of the alkyl substituent, as well as on the type of anions (Matzke et al., 2008; Bałczewski et al., 2007). Matzke et al. (2008) studied the influence of various anion species ($(CF_3SO_2)_2N^-$, Cl^-, BF_4^-, $(CF_3)_2N_2^-$, octylsulfate and bis(1,2- benzenediolato) borate) on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids to batteries and found that the anion effects were not distinct. However, Ganske and Bornscheuer (2006) investigated the growth of three microorganisms in the presence of the ionic liquids [BMIM][BF_6] and [BMIM][PF_6], and found that anion structure affected the toxicity of ILs. Liu et al. (2015) assessed the biochemical toxicity of ionic liquids with different anions in soil on *Vicia faba* seedlings and defined anion toxicity as [BF_4]> [Cl]. Romeroa et al. (2008) reported toxicity of imidazolium ionic liquids to *P. phosphoreum* in the aqueous phase, and found that $[Cl]^-$ caused higher toxic effects than $[BF_4]^-$. Therefore, the contribution of anion structure to the overall toxicity of ILs is still controversial and needs to be further studied. The ILs are made with amino acid anions due to the resulting biodegradability and high biocompatibility; this has become the developmental direction of novel ionic liquids and been widely used in chemical transformations in recent years (Egorova et al., 2014; Zhu et al., 2012). However, the effect of amino acid-based ionic liquids on plants has not been reported so far. Moreover, lactic acid- and sulfonate-based ionic liquids have received considerable attention in extraction, catalysis and other fields on account of their relatively low price and solubility in water (Pavlovica et al., 2011; Montes et al., 2016; Requejo et al., 2015; Liang et al., 2013). However, related toxicity studies are less common. Imidazolium-based ILs are the most widely used, and inevitably enter the water environment due to their water solubility (Latała et al., 2005; Zhang et al., 2011a,b). Therefore, the imidazolium-based ILs 1-butyl-3-methyl-imidazolium alanine ([C$_4$mim][Ala]), 1-butyl-3-methyl-imidazolium lactate ([C$_4$mim][Lact]), 1-butyl-3-methyl-imidazolium tetrafluoroborate ([C$_4$mim][BF_4]), 1-butyl-3-methyl-imidazolium chloride ([C$_4$mim][Cl]), and 1-butyl-3-methyl-imidazolium trifluoromethansulfonate ([C$_4$mim][TfO]) were selected in this study. The growth (shoot length, root length, aboveground and underground dry weights) and physiological (the activities of superoxide dismutase, peroxidase, catalase and malondialdehyde content) indexes of wheat seedlings after exposure to the five ILs were measured to explore the phytotoxicity difference in imidazolium-based ionic liquids with different anions. Furthermore, the changes in leaf and root cell structures of wheat seedlings were observed by transmission electron microscopy to reveal the mechanism of action of ionic liquids on wheat seedlings at the subcellular level. This study provides a theoretical basis for estimating the environmental safety of ILs, which may be the basis for future strategies for the selection of non-toxic or low-toxicity ionic liquids.

2. Materials and methods

2.1. Ionic liquids

The ionic liquids 1-butyl-3-methyl-imidazolium alanine [C$_4$mim][Ala] (98% purity), 1-butyl-3-methyl-imidazolium lactate [C$_4$mim][Lact] (98% purity), 1-butyl-3-methyl-imidazolium tetrafluoroborate [C$_4$mim][BF_4] (99% purity), 1-butyl-3-methyl-imidazolium chloride [C$_4$mim][Cl] (99% purity), and 1-butyl-3-methyl-imidazolium trifluoromethansulfonate [C$_4$mim][TfO] (98% purity) were obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Science (Lanzhou, China). The structures of the tested ILs are shown in Fig. 1.

2.2. Wheat culture

Wheat (*Triticum aestivum* Liao Chun No. 18) seeds used in the experiments were provided by the Liaoning Academy of Agricultural Sciences. According to Chen et al. (2014), we screened out the full grain of wheat seeds. The wheat seeds were then soaked and disinfected with 0.1% $HgCl_2$ solution for 10 min, and washed thoroughly with distilled water. For each treatment, 30 seeds were placed in petri dishes ($d = 15$ cm) lined at the bottom with three layers of gauze. Eight mL of different concentrations of different ionic liquids were added to the dishes and then the seeds were placed in a constant temperature incubator for 24 h. When the seeds germinated uniformly, they were placed in plastic beakers (inner diameter: 20 cm; height: 15 cm) that had been prefilled to the top with Hoagland solution or Hoagland solution supplemented with different concentrations of ionic liquids and covered with nylon nets. The Hoagland solution is a hydroponic nutrient solution that contains essential elements for the growth of plants, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and trace elements (Fe, Cu, Zn, Mn, Mo, and B) (Hoagland and Arnon, 1937). Different concentrations of [C$_4$mim][Ala], [C$_4$mim][Lact], [C$_4$mim][BF_4], [C$_4$mim][Cl] and [C$_4$mim][TfO] (0, 50, 100, 150, 200, 250, 300 mg L^{-1}) were used in the culture preparation. The beakers were placed on the windowsill and exposed to natural light at approximately 22 °C for 10 h/d and 17 °C for 14 h/night at the Environmental Experimental Center, Liaoning University, China. For each treatment group, measurements were repeated three times.

2.3. Determination of growth responses

On the 8th d of cultivation, the lengths of the shoots and roots of the wheat seedlings were measured to assess the extent of growth under different IL conditions. Five wheat seedlings in each treatment were selected to measure shoot and root lengths according to Wang et al. (2009). After the shoot and root lengths were measured, the wheat seedlings were oven-dried at 100 °C for 30 min and then further dried at 80 °C until the weight remained constant to measure the dry weight of the aboveground and underground wheat seedlings (Lin et al., 2012).

2.4. Determination of physiological responses

On the 12th d of cultivation, 0.5 g fresh wheat leaves and 10 mL of protective enzyme extraction solution (ice-cold phosphate buffer solution, 0.2 M, pH 7.8) were homogenized by grinding and then poured into centrifuge tubes. The sample was centrifuged at 10,000 rpm for 15 min at 4 °C, and subsequently, the supernatant was used to assay antioxidant enzyme activities and malondialdehyde (MDA) content.

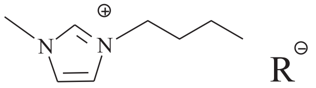

Fig. 1. The structure of the ILs.