Chemosphere 191 (2018) 81–88

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Physiological and biochemical responses of wheat (*Triticum aestivum* L.) seedlings to three imidazolium-based ionic liquids in soil

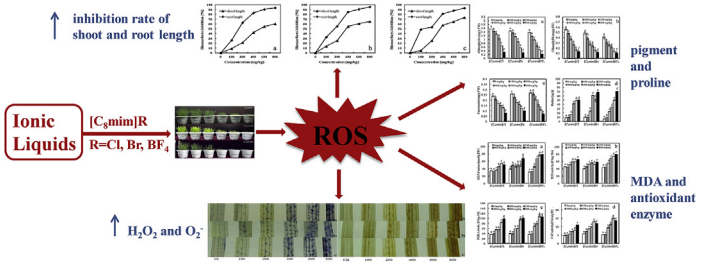
Yaqi Xu [1], Jun Wang [1], Lusheng Zhu*, Zhongkun Du, Jinhua Wang**, Kai Wei

College of Resources and Environment, Key Laboratory of Agricultural Environment in Universities of Shandong, Shandong Agricultural University, Taian, 271018, PR China

HIGHLIGHTS

- Toxic effects of [C_8mim]R (R = Cl, Br, BF_4) in soil on wheat seedlings were evaluated.
- [C_8mim]BF_4 most affected wheat growth, followed by [C_8mim]Br and [C_8mim]Cl.
- Oxidative damage is speculated to be the primary mechanism of IL toxicity.
- Toxic impact of different ILs anions on physiological indices appears to be marginal.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 July 2017
Received in revised form
17 September 2017
Accepted 23 September 2017
Available online 25 September 2017

Handling Editor: Frederic Leusch

Keywords:
Phytotoxicity
Reactive oxygen species
Oxidative stress
Lipid peroxidation
Antioxidant enzyme

ABSTRACT

Ionic liquids (ILs) are considered environmentally friendly solvents and are widely applied in various fields; however, some researchers have noted the toxicity of ILs to plants cultivated in nutrient solution. To evaluate the toxicities of ILs to wheat seedlings in soil, the natural growth environment of plants, a study was performed using three imidazolium-based ionic liquids with different anions: 1-octyl-3-methylimidazolium chloride ([C_8mim]Cl), 1-octyl-3-methylimidazolium bromide ([C_8mim]Br) and 1-octyl-3-methylimidazolium tetrafluoroborate ([C_8mim]BF_4). After 13 d of exposure to these three ILs at 0, 100, 200, 400, 600 and 800 mg kg^{-1} in brown soil, wheat seedlings were randomly sampled to evaluate growth (shoot length, root length, pigment content and proline content), lipid peroxidation, oxygen species (H_2O_2 and O_2^-) and activities of the detoxification enzyme glutathione-*s*-transferase and other antioxidant enzymes, including superoxide dismutase, catalase and peroxidase. The experimental results showed that all three ILs had inhibitory effects on the growth of wheat seedlings and induced the generation of reactive oxygen species, which indicated that the wheat seedlings suffered oxidative stress. Moreover, antioxidant enzyme activity was enhanced after exposure to [C_8mim]Cl, [C_8mim]Br and [C_8mim]BF_4, demonstrating that oxidative damage may be the primary underlying mechanism of IL toxicity in wheat.

1. Introduction

Ionic liquids (ILs), which are constituted entirely of ions, are deemed environmentally benign solvents due to their unique physicochemical properties, such as a low melting point, a low vapor pressure, high polarity, thermostabilization and recyclability.

* Corresponding author. College of Resources and Environment, Shandong Agricultural University, 61 Daizong Road, Taian, 271018, PR China.
** Corresponding author. College of Resources and Environment, Shandong Agricultural University, 61 Daizong Road, Taian, 271018, PR China.
E-mail addresses: xuyaqi612@163.com (Y. Xu), jwang@sdau.edu.cn (J. Wang), lushzhu@sdau.edu.cn (L. Zhu), dzk66@sdau.edu.cn (Z. Du), wjh@sdau.edu.cn (J. Wang), eti@sdau.edu.cn (K. Wei).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chemosphere.2017.09.108
0045-6535/

The configuration of ILs can be readily tailored by changing the ionic combinations or the length and branching of the alkyl chain (Matsumoto et al., 2004). As ILs can be synthesized as required to satisfy multiple demands, they have been widely applied in multiple fields, such as organic chemistry, catalysis and biocatalysis (Pham et al., 2010).

However, with large-scale use, ILs may be released into the environment, where they may remain in water and soil for a long time because of their resistance to photodegradation and their high solubility in water (Ventura et al., 2013). Additionally, ILs are not easily degraded by microorganisms (Coleman and Gathergood, 2013). Therefore, aquatic organisms and terrestrial plants may be impacted by these potential contaminants, and the toxicities of ILs have received increasing attention. For example, Du et al. (2014) studied the genotoxicity and cytotoxicity of 1-octyl-3-methylimidazolium bromide in zebrafish. Some researchers have also examined the toxic effects of ILs on plants (Biczak et al., 2016; Liu et al., 2016a, 2016b; Pawłowska and Biczak, 2016; Song et al., 2009, 2007). Liu et al. (2015) investigated toxic effects of [C_4min]Cl in soil on *Vicia faba* seedlings and found that ILs can inhibit growth and cause DNA damage. Toxic effects of ILs on bacteria and other microbes, earthworms, and other organisms have also been reported (Guo et al., 2015; Quraish et al.,2017; Shao et al., 2017; Sun et al., 2017). Nonetheless, there is a paucity of studies addressing the effects of IL toxicity on food crops, organisms that are relevant to human health.

Wheat (*Triticum aestivum* L.) is one of the world's three major food crops and has the second highest production value after corn. Some researchers have studied the toxicity of different ILs on wheat. Wang et al. (2009) reported that [C_8mim]Br can impede the generation of pigments as well as enzyme activity in wheat leaves, and Liu et al. (2014) studied the growth and physiological and biochemical responses of wheat seedlings affected by imidazolium-based ILs. However, the wheat seedlings in previous studies were mainly cultivated in nutrition solution; in contrast, the soil environment has more similarities to the natural environment, which is complex. Therefore, in the present study, we cultivated wheat in soil and determined the toxicities of three commonly used imidazolium-based ILs.

2. Materials and methods

2.1. Chemicals

Chengjie Chemical Company Limited (Shanghai, China) provided [C_8mim]Cl (1-octyl-3-methylimidazolium chloride, CAS No. 64697-40-1), [C_8mim]Br (1-octyl-3-methylimidazolium bromide, CAS No. 61545-99-1) and [C_8mim]BF_4 (1-octyl-3-methylimidazolium tetrafluoroborate, CAS No. 244193-52-0). All other chemicals were analytically pure and were purchased from either Sigma (St. Louis, Missouri, USA) or Solarbio Science & Technology Company (Beijing, China).

2.2. Wheat and soil

Wheat seeds (Jimai 22, *Triticum aestivum* L.) were provided by the College of Life Science, Shandong Agricultural University (Taian, China).

The soil used in the present study was obtained from the test field of Shandong Agricultural University (Taian, China). The physical and chemical properties of the soil are listed in Table 1.

2.3. Experimental design

Wheat seeds were disinfected with 10% sodium hypochlorite. After 10 min, the seeds were rinsed with distilled water and then immersed in distilled water at 25 °C for 24 h.

A preliminary experiment revealed feasible experimental concentrations of 0, 100, 200, 400, 600 and 800 mg kg^{-1}; these concentrations were prepared by dissolving the appropriate quantity (20 g of [C_8mim]Cl, 20 g of [C_8mim]Br and 20 g of [C_8mim]BF_4) in 1 L of deionized water. The recalculated volumes of the IL/deionized water solutions were then each mixed with 1 kg of air-dried soil.

One hundred wheat seeds were selected and sowed in pots containing the IL compound-exposed soil. Each concentration included three replicate pots. All pots were placed in a greenhouse at 25 °C with a 12:12-h light:dark cycle. After the 13-day cultivation period, 15 wheat seedlings in each experimental group were randomly sampled for determination of shoot and root lengths. Inhibition rates were then calculated.

2.4. Determination of shoot and root lengths

The shoot and root lengths were measured based on the method of Liu et al. (2016a). The shoot length was considered the length from the base of the stem to the tip of the longest leaf, and the root length was considered the length from the bottom of the stem to the tip of the longest root.

2.5. Determination of the pigment content

Fresh leaves (0.05 g) were immersed in 5 mL of 80% acetone and placed in the dark for 40 h. The absorbance of the supernatant was measured at 470 nm, 646 nm and 663 nm using a UV–visible spectrophotometer (UV-2600; Shimadzu), as based on the method of Song et al. (2007).

2.6. Determination of the proline content

Fresh leaves (0.45 g) were immersed in 4.5 mL of sulfosalicylic acid (3%, w/v), and the samples were heated for 10 min in a boiling water bath. After the mixture was cooled to room temperature, 1 mL of glacial acetic acid and 1 mL of 2.5% acidic-ninhydrin were added to the supernatant (1 mL); the samples were boiled in a 100 °C water bath for 30 min. The samples were placed in an ice bath to terminate the reaction, and proline was extract by the addition of 4 mL of toluene followed by vortex mixing for 30 s. After the two layers had separated completely, the absorbance of the upper layer was measured at 520 nm. The proline content was obtained by preparing a standard curve.

2.7. Determination of lipid peroxidation

To evaluate the level of lipid peroxidation, the malondialdehyde (MDA) content was determined based on the method of Song et al. (2009). Fresh leaves (0.2 g) were pulverized in a mortar with 6 mL of 0.1% trichloroacetic acid. The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C, and 1.5 mL of 0.6% thiobarbituric acid was added to 1.5 mL of the supernatant. The mixture was heated in a water bath at 95 °C for 30 min and then cooled rapidly on ice. The mixture was then centrifuged at 10,000 × g for 15 min, and the absorbance of the supernatant was measured at 532 nm and 600 nm.

2.8. Determination of enzyme activity

Fresh leaves (0.25 g) were homogenized in a mortar with 2.5 mL of prechilled phosphate buffer (pH 7.8) containing 1 mM ethylenediaminetetraacetic acid and polyvinylpyrrolidone (1%, w/v). The homogenate was centrifuged in a 2-mL centrifuge tube at