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# Effects of 1-butyl-3-methylimidazolium chloride on the photosynthetic system and metabolism of maize (*Zea mays* L.) seedlings

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ABSTRACT

Ionic liquids (ILs) are widely used in various chemical processes. However, a growing number of studies have found that ILs are potentially toxic to different types of living organisms, including crops. The present study analysed the effects of 1-butyl-3-methylimidazolium chloride ([$C_4$mim]Cl) on the photosynthetic system and metabolism of maize seedlings. Results showed that [$C_4$mim]Cl could significantly reduce maize leaf chlorophyll level and cause extensive leaf bleaching. The activity of photosystem II (PSII) was significantly inhibited when seedlings exposed to higher concentration of [$C_4$mim]Cl. The maximum quantum yield of PSII and the potential efficiency of PSII were reduced by 63% and 88% under 800 mg/L [$C_4$mim]Cl treatment in comparison with the control treatment. The RNA sequencing analysis performed to examine gene expression profiles of maize leaves under [$C_4$mim]Cl treatment revealed 639 differentially expressed genes (DEGs), 115 of which were categorized into different metabolic pathways. Among these DEGs, the seven genes involved in the photosynthetic Calvin cycle were down-regulated by [$C_4$mim]Cl exposure. For carbohydrates and amino acids metabolism, the genes for starch synthesis were down-regulated, while the genes for amino acids and protein degradation were up-regulated. The changes observed in these major metabolic pathways might be an important reason for [$C_4$mim]Cl toxicity.

## 1. Introduction

Ionic liquids (ILs) are a class of organic molten salts composed by organic cations (e.g., alkylimidazolium ions, alkylpyridinium ions, quaternary ammonium salt ions, quaternary phosphonium salt ions) and organic and inorganic anions (e.g., $BF_4^-$, $PF_6^-$, $N(CN)_2^-$, $Cl^-$) (Seddon, 1997). Compared to traditional solvents, ILs have unique physicochemical properties, such as high conductivity, no volatilization, wide temperature range of liquid state, good solubility, and are non-flammable, “designable”, and chemically stable (Izgorodina et al., 2017; Petkovic et al., 2011). In the past few decades, ILs have been widely used in various chemical and catalytic processes, such as biocatalysis, chemical synthesis, clean energy production, pharmaceutics and medicine, and electrochemistry (Egorova et al., 2017; MacFarlane et al., 2014; Qureshi et al., 2014; van Rantwijk and Sheldon, 2007; Vekariya, 2017; Watanabe et al., 2017).

The increasing knowledge on ILs revealed the toxic nature of several kinds of ILs. Many studies have shown that traditional ILs, such as imidazolium-based ILs and pyridine-based ILs, are not really stable in some cases (Wang et al., 2017). The biodegradability and toxicity of ILs on large molecules (e.g., enzymes), microbes, algae, animals, plants were evaluated (Costa et al., 2017; Das and Roy, 2014; Ruokonen et al., 2016; Sun et al., 2017; Tot et al., 2018). As promising solvents, the large-scale industrialization of ILs may also increase the risk of discharging these potential pollutants into the environment. Thus, it is urgent to evaluate the toxic mechanism of ILs on living organisms.

Crop plants are important elements of the land ecosystem. In recent years, several studies concerning the impacts of ILs on crop plants have been performed, mostly considering imidazolium-based ILs, which are widely-used traditional ILs. For example, Xu et al. (2018) found that imidazolium-based ILs negatively affect the elongation of both shoots and roots of wheat seedlings, and a burst of reactive oxygen species

*Abbreviations:* AO, L-ascorbate oxidase; AS, asparagine synthetase; AsA, ascorbate; DEGs, differentially expressed genes; GSH, glutathione; ILs, ionic liquids; NPQ, non-photochemical quenching; PS, photosynthetic; PSI, photosystem I; PSII, photosystem II; QP, photochemical quenching; ROS, reactive oxygen species; RuBisCo, ribulose-1,5-biphosphate carboxylase/oxygenase; γ-ECS, gamma-glutamylcysteine synthetase 1

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(ROS) was also detected. Cvjetko et al. (2014) evaluated the phytotoxicity of imidazolium-based ILs with different alkane chains on barley, and showed that these ILs prominently inhibited seed germination and early growth, and that their toxicity increased with increasing alkane chain length. Liu et al. (2015a) studied the mechanisms of imidazolium-based ILs on the growth of rice seedlings, and found that increasing concentrations of ILs affected the activity of antioxidant enzymes, such as superoxide dismutase, peroxidase, and catalase, which increased initially and then decreased. The content of malondialdehyde also increased in response to increasing concentrations of ILs (Liu et al., 2013). Similar results were also observed in other crop species, such as broad beans (Liu et al., 2015b) and radish (Biczak et al., 2014). Overall, studies on different kinds of crops reported that imidazolium-based ILs could notably inhibit crop growth, and oxidative damage was considered the major mechanism underlying this inhibition.

Photosynthesis is fundamental for plant growth, which plays a decisive role in crop productivity. The photosynthetic apparatus converts sunlight energy into chemical energy through a complex series of photochemical and biochemical reactions. As a central biological process, photosynthesis is highly associated with other major metabolic processes, such as carbohydrates metabolism, amino acids metabolism, and redox balance (Beligni and Lamattina, 2002; Gururani et al., 2015). Carbohydrates are initial products of photosynthesis, and different forms of carbohydrates are involved in the formation of the major structural components and energy sources of plant cells (Reguera et al., 2013). Amino acids are basic materials for building proteins, and photosynthetic activity is closely related to leaf protein levels (Distelfeld et al., 2014). Ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo), which is the central enzyme for the photosynthetic dark reaction, is the largest source of leaf protein (Dong et al., 2017; Masclaux-Daubresse et al., 2010). When plants are under environmental stresses, the imbalance of photosynthetic light and dark reactions may result in the excessive production of ROS, leading to oxidative damage to the photosystems (Das and Roy, 2014; Liu et al., 2015a). The maintenance of normal plant growth requires an optimum coordination between photosynthesis and other major metabolic processes.

Most studies conducted recently focused on growth inhibition and oxidative damages caused by ILs exposure, and little information is available on the metabolic impacts of ILs to plants. RNA sequencing (RNA-seq) provides an effective approach to comprehensively analyse the gene expression patterns involved in different biological pathways. However, studies on gene expression profiles of plants in response to ILs are rare. In the present study, 1-butyl-3-methylimidazolium chloride ([$C_4$mim]Cl) was used as a typical imidazolium-based IL to study the effects of ILs on maize (*Zea mays* L.) seedlings. Chlorophyll fluorescence parameters were measured to evaluate the effect of [$C_4$mim]Cl on maize photosystems, and RNA-seq analysis was performed to explore the systematic transcriptional responses of maize seedlings to IL exposure. Gene expression patterns associated with some major metabolic pathways, such as photosynthesis and carbohydrates and amino acid metabolism are discussed. The results obtained in this study contribute to increase knowledge on the physiological impacts of ILs on the growth of maize, which is one of the major food and feed crops worldwide.

## 2. Materials and methods

### 2.1. Chemicals and plant materials

The 1-butyl-3-methylimidazolium chloride ionic liquid ([$C_4$mim]Cl) (99% purity) was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China. The maize seeds (Zhengdan958, *Zea mays* L.) used in this study were purchased from Doneed Seeds Ltd., Beijing, China.

### 2.2. Maize culture and sampling

Maize seedlings were cultivated in a growth chamber. Seeds were surface sterilized by drowning in 75% ethanol for 5 min, washed in deionized water, and germinated on moistened clean filter paper at 28 °C for 3 days. After this period, uniformly germinated seeds were water cultured in plastic culture boxes (10 seeds per box) in 1/2 Hoagland solution (pH = 6.0) until the first leaf stage. The [$C_4$mim]Cl treatment started after the expansion of the first leaf, in 1/2 Hoagland solutions containing different concentrations of [$C_4$mim]Cl as the culture medium. Based on our pre-experiments (data not show), the [$C_4$mim]Cl concentration gradient was 0 (control, CK), 200, 400, 600, and 800 mg/L. Each treatment was performed using six replicates, and the culture solutions were replaced daily. Growth conditions were 16/8 h light/dark at 25/28 °C night/day temperature under 10,000 lx irradiance. Sampling was performed seven days after the onset of each treatment, when maize seedlings reached the three-leaf stage. For biomass analysis, shoot and root were collected and oven dried (80 °C, 48 h). Fresh tissues (second leaf) of seedlings under the CK and 800 mg/L treatments were collected and stored at − 80 °C for RNA isolation and sequence analysis.

### 2.3. Chlorophyll content and chlorophyll fluorescence analyses

Leaf chlorophyll content and chlorophyll fluorescence were measured in vivo. Chlorophyll content was measured in a SPAD-502 chlorophyll meter (Spectrum Technologies, Japan), and results were displayed in SPAD units (Zobiole et al., 2011). The FluorPen portable device (Photon Systems Instruments, Czech Republic) was used to measure the following leaf chlorophyll fluorescence parameters, according to the manufacturer's guidelines. Using detachable leaf-clips, leaf samples were dark adapted for 20 min and then transferred to actinic light. Photochemical quenching (QP) and non-photochemical quenching (NPQ) dynamics were measured in the first 200 s of exposure to actinic light. Minimum fluorescence intensity ($F_0$), maximum fluorescence intensity ($F_m$), maximum variable fluorescence ($F_v$), maximum quantum yield of photosystem II (PSII; $F_v/F_m$), and the potential efficiency of PSII ($F_v/F_0$) were acquired at the end of the measurement.

### 2.4. RNA preparation, library construction, and Illumina HiSeq. 4000 sequencing

Leaf samples from CK and 800 mg/L [$C_4$mim]Cl-treated plants were used for RNA extraction and cDNA library preparation. Total RNA was extracted from leaf tissues using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA was digested by DNase I (TaKara Bio Inc., Shiga, Japan). The quality of the extracted RNA was verified in a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and RNA was quantified in a ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). Only high-quality RNA samples (A260/280 = 1.8–2.2, A260/230 ≥ 2.0, RNA integrity number ≥ 6.5, 28S:18S ≥ 1.0, > 10 μg) were used to construct the cDNA library. Two replications (each containing leaf tissues from three individual plants) were obtained from each treatment, and four cDNA libraries were constructed and sequenced.

The RNA-seq library was prepared from 5 μg of total RNA using the TruSeq™ RNA sample preparation kit (Illumina, San Diego, CA, USA). Firstly, mRNA was enriched by oligo(dT) beads and fragmented; double-stranded cDNA was then synthesized by the SuperScript double-stranded cDNA synthesis kit (Invitrogen) with random primers (Illumina). The synthesized cDNA was end-repaired, phosphorylated, and 'A' bases were added, according to the Illumina library construction protocol. Libraries were size selected for cDNA fragments of 200–300 bp on agarose gel electrophoresis, after PCR amplification using Phusion DNA polymerase (NEB, Ipswich, MA, USA) for 15 cycles. After quantification using TBS380 (Turner Biosystems, Sunnyvale, CA,