Ecotoxicology and Environmental Safety 125 (2016) 107–115

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Effects of the ionic liquid 1-hexyl-3-methylimidazolium bromide on root gravitropism in *Arabidopsis* seedlings

Liang Zhang [a,b], Tianqi Wang [a], Fengxia Zheng [a], Lingyu Ma [a], Jingyuan Li [a,b,*]

[a] College of Life Science, Henan Normal University, Xinxiang 453007, China
[b] Engineering Laboratory of Green Medicinal Material Biotechnology, Henan Province, Xinxiang 453007, China

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form
27 November 2015
Accepted 30 November 2015
Available online 10 December 2015

Keywords:
Ionic liquid
Root gravitropism
Amyloplast
Auxin
Auxin efflux carrier
Arabidopsis seedling

ABSTRACT

The toxic effects of ionic liquids (ILs) have attracted increasing attention in recent years. However, the knowledge about the toxic effects of ILs on tropism in organisms remains quite limited. In this study, the effects of 1-hexyl-3-methylimidazolium bromide [C_6mim]Br on root gravitropism were evaluated using *Arabidopsis* seedlings. Our results showed that the root growth and gravity response were significantly inhibited with increasing IL concentration. [C_6mim]Br treatment affected the amount and distribution pattern of amyloplasts in root cap compared with controls. The auxin distribution marked with *DR5rev::VENUS* was altered in IL-treated seedlings. The signal intensity and gene expression of auxin efflux carriers PIN2 and PIN3 were obviously decreased by IL stress. Moreover, as consequences in response to gravity stimulus, the asymmetric DR5 signals in control root apex were impaired by IL treatment. The predominant PIN2 signals along the lower flank of root and PIN3 polarization in columella cells were also significantly reduced in seedlings exposed to IL. Our results suggest that the ionic liquid [C_6mim]Br affects the amount and distribution of amyloplasts and disturbs the deployment of PIN2 and PIN3, thus impairing auxin flows in response to gravity stimulus and causing deficient root gravitropism in *Arabidopsis* seedlings.

1. Introduction

Ionic liquids (ILs), a kind of salt-like materials that present liquid phase at room temperature, are composed of large asymmetric organic cations and small organic or inorganic anions (Petkovic et al., 2011). The technical properties of ionic liquids can be finely altered through changing the combinations of different cations and anions (Fatemi and Izadiyan, 2011). Based on the physicochemical properties, such as negligible vapor pressure, non-volatility, high stability, and powerful solvation potential, ILs have been regarded as "green" solvents (Bubalo et al., 2014). The characterization of ILs facilitates its wide application in various fields, including organic synthesis, electrochemistry, catalysis, and extraction and isolation processes (Plechova and Seddon, 2008). Therefore, ILs could make their way into the natural environment and the impact on living organisms must be taken into consideration thoughtfully.

Recently, the toxic effects of several ILs have been intensively investigated in different biological levels, including microorganisms, algae, terrestrial invertebrates, vertebrates (Ranke et al., 2004, Kulacki and Lamberti, 2008; Luo et al., 2008; Pretti et al., 2009; Du et al., 2014), as well as human cell lines (Stepnowski et al., 2004; Wang et al., 2007). Moreover, the toxic effect of ILs on higher plants has received considerable attention. The treatment with ionic liquid ([C_8mim]PF6) seriously inhibited the germination, root and shoot growth in wheat seedlings (Liu et al., 2014). Imidazolium chloride ILs are phytotoxic to rice growth and their photosystem, and the toxicity enhanced as the alkyl chain length increased (Liu, H. et al., 2015; Liu, T. et al., 2015). The ROS level and the activities of antioxidant enzymes, including peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD), were increased in *Lemna minor* exposed to [C_8mim]Br (Zhang et al., 2013). In addition, the ionic liquid ([C_4mim]Cl) showed genotoxicity and cytotoxicity on *Vicia faba* seedlings in soil (Liu, H. et al., 2015; Liu, T. et al., 2015). However, the knowledge about the mechanism underlying the toxic effects of ILs on plant growth is still limited.

In plants, gravitropism is a process that plants can sense gravity and guide their growth as the response to gravity vector. For gravitropism in roots, the response pathway has been considered to be triggered by the sedimentation of starch-filled plastids (amyloplasts) in the columella cells of root tip (Leitz et al., 2009).

Abbreviations: ANOVA, One-way analysis of variance; CAT, catalase; dLRC, distal lateral root cap; IL, ionic liquid; LRC, lateral root cap; PIN, PIN-FORMED; POD, peroxidase; SOD, superoxide dismutase

* Corresponding author at: College of Life Science, Henan Normal University, Xinxiang, Henan 453007, China.
E-mail address: ljy041026@htu.cn (J. Li).

http://dx.doi.org/10.1016/j.ecoenv.2015.11.038
0147-6513/

The reorientation of amyloplasts to the lower side of columella cells promotes asymmetric release of the growth regulator auxin at root apex (Boonsirichai et al., 2003; Ottenschlager et al., 2003). The resulting lateral auxin gradient is transmitted to the elongation zone and accumulates in the lower side of root, thus inhibiting cell elongation on the lower side and causing the root to bend downward (Boonsirichai et al., 2002; Morita, 2010).

The previous reports about the toxic effects of ILs on plants mainly focus on growth parameters, antioxidant system and photosynthetic activity. While, the knowledge about the effects of ILs on gravitropism in plants is still unclear. Given that root gravitropism is a necessary physiological event for plants to anchor themselves into the soil and find resources needed for growth and development, the aim of the present study was to examine the responses of root gravitropism in *Arabidopsis* seedlings to [C_6mim]Br treatment comprehensively from the cell biological and molecular level for the first time. This work provides new information on the toxic effects of ILs on plant growth and also give some information for environmental safety evaluation of ILs.

2. Materials and methods

2.1. Plant materials and growth conditions

The plants used for treatment with ionic liquid [C_6mim]Br were *Arabidopsis thaliana* Columbia-0. The plans harbouring *pDR5rev::3XVENUS-N7*, *pPIN2::PIN2-GFP*, and *pPIN3::PIN3-GFP* were described previously (Xu and Scheres, 2005; Kleine-Vehn et al., 2010; Le et al., 2014). For seedlings grown on agar-containing plates, *Arabidopsis* seeds were pretreated with70% ethanol for 1 min, surface-sterilized in 2.5% bleach for 10 min, and washed with distilled water for four times. The seeds were planted on 1/2 MS medium (Sigma) supplemented with 1% (w/v) sucrose, 1% (w/v) agar (pH 5.8). After vernalization at 4 °C in the dark for 48 h, the plates were placed under a light bar at 23 °C and providing 140 ± 20 μmol m v^{-2} s^{-1} from cool white fluorescent lamps with a 16-h-light/8-h-dark cycle.

2.2. Ionic liquid and treatment

The ionic liquid [C_6mim]Br (99% purity, CAS No. 85100-78-3) was purchased from Monils Chemical Co. Ltd. (Shanghai, China). [C_6mim]Br was dissolved with distilled water at the concentration of 10 g L^{-1}. For treatment with IL in *Arabidopsis* seedlings, the seeds were planted on agar-containing plates supplemented with [C_6mim]Br at final concentrations of 10, 20, 40, 60, 80 mg L^{-1}, respectively. After vernalization, the seedlings were placed under a light bar and vertically grown for 5 days. Then, seedlings were sampled for the analysis of root growth and gravity response.

2.3. Root length measurement

The root length of control and IL-treated seedlings was measured on the fifth day after vernalization. The primary roots of seedlings vertically grown in petri dish were photographed with a digital camera. The length of primary root was measured using Image J software with “Segmented Line” tool after setting scales. 30 individual seedlings were measured for each treatment. All of the data represent the mean of three biologic replicates.

2.4. Root gravitropism assay

The root gravity response was determined using 5-D-old seedlings treated with or without IL. The vertically grown seedlings were rotated 90° and images of the roots were captured at 4, 6, 8, 10 and 12 h after reorientation with a digital camera (Canon 70D). The angles of root curvature were measured with reference to the gravity vector by using image-analysis program Image J (http://rsb.info.nih.gov/ij/). 30 roots were scored for each treatment.

For the analysis of responses of auxin-related markers to gravity stimulus under IL treatment, the seedlings expressing auxin-responsive reporter *DR5rev::VENUS* and auxin efflux carriers PIN2-GFP and PIN3-GFP were rotated 90° for 2 h, 2 h, and 30 min, respectively before the observation by confocal microscope.

2.5. Amyloplast staining and light microscopy observation

Amyloplast starch in columella cells of root cap were visualized with 1% I_2-KI solution in 5-D-old control or IL-treated seedlings vertically grown on 1/2 MS. The roots were stained for 1 min, rinsed with fresh liquid medium, cleared with 50% chloral hydrate for 40 s and photographed under Olympus BX51 equipped with a digital camera. The amount measurement of amyloplasts in columella cells was performed according to the method described by Takahashi et al. (2003).

2.6. Confocal microscopy and quantification of fluorescence intensity

For confocal analysis, seedlings mounted in liquid 1/2 MS were analyzed with Leica SP8 confocal microscope. For imaging of VENUS- or GFP-tagged fluorescent proteins, the roots of 5-D-old seedlings were visualized by excitation with an Argon laser at 488 nm and detected with a 500- to 550-nm emission filter. The images were edited using Image J software and Adobe Photoshop CS2.

For the quantification of fluorescence intensity, confocal images were analyzed with Image J software using tools of oval or polygon selections and ROI Manager. For the quantitative analysis of root cells expressing *DR5rev::VENUS* without gravity stimulus, all the nuclear DR5 signals from the roughly median plane were summed. For the quantitative analysis of the effects of IL on *DR5rev::VENUS* under gravity stimulus, the ratio of nuclear fluorescence intensity from lateral root caps (LRC) of two sides (lower side/upper side) was determined. For the quantitative analysis of PIN2-GFP without gravity stimulus, GFP signals in root epidermal and cortical cells of two sides from the roughly median plane were added. For the quantitative analysis of the effects of IL on PIN2-GFP under gravity stimulus, the ratio of fluorescence intensity from root epidermal and cortical cells of two sides (lower side/upper side) was determined. For the quantitative analysis of PIN3-GFP without gravity stimulus, GFP intensity in root columella cells from the roughly median plane was measured. For the quantitative analysis of the effects of IL on PIN3-GFP under gravity stimulus, serial optical sections were collected for 10 frames at 2-μm intervals along the *z* axis, and the numbers of cells displaying polarized PIN3 were quantified by 3D reconstruction images using Image J software. 20 seedlings were used for each treatment.

2.7. RNA isolation and real-time quantitative RT-PCR

The total RNA from 5-D-old seedlings treated with or without [C_6mim]Br was extracted using trizol reagent (Invitrogen, Carlsbad, CA, USA) for three times independently. The RNA samples were further treated with RNase-free DNase I (Promega) to eliminate DNA contamination. The cDNA synthesis and real-time quantitative RT-PCR were performed according to a method described previously (Qi et al., 2015). Briefly, the first-strand cDNA was synthesized with random hexamers and SuperScript III reverse transcriptase (Invitrogen) according to manufacturer's instructions. Quantitative real-time PCR was performed using SYBR®