

Effects of the ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate on the growth of wheat seedlings

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Abstract Ionic liquids (ILs) are called "green" solvents, which are due to their unique physicochemical properties and potential applications in various areas. However, the toxicity of ILs has attracted increasing attention from scientific researchers. The present paper studied the toxic effects of 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8mim]PF_6$) on wheat seedlings at 0, 1, 2, 4, 6, and 8 mg l⁻¹ on days 7, 10 and 13. The present results showed that the growth of wheat seedlings was seriously inhibited when the concentration was higher than 2 mg l⁻¹ and the inhibitory effect enhanced with increasing concentration and time. The EC₅₀ values for germination, shoot length, root length and dry weight were 11.104, 5.187, 4.380 and 6.292 mg l⁻¹, respectively. $[C_8mim]PF_6$ could cause an increase in the production of ROS, which led to the oxidative damage and lipid peroxidation. Furthermore, these toxic effects on wheat seedlings were irreversible.

Keywords Plant · Antioxidase system · Lipid peroxidation · Superoxide anion · Proline · EC₅₀

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Introduction

Ionic liquids (ILs), also named room temperature ILs or room temperature molten salt, are a class of solvents entirely composed of ions at room temperature. ILs are usually made up of large asymmetric organic cations and small inorganic or organic anions (Welton 1999). ILs have many interesting physicochemical properties, such as synthetic flexibility, non-flammable, thermostabilization, among others (Earle et al. 2006; Ranke et al. 2007; Deetlefs and Seddon 2010). ILs as alternatives to organic solvents have been applied in many fields, such as organic synthesis, electrochemical, and separation processes. At present, as a new type of green industrial solvent, ILs have been put into use extensively in industry (Earle and Seddon 2000; Zhao 2006).

However, ILs, as a kind of industrial products, will eventually be released into the environment. Therefore, we must consider the impact of a new chemical on people and environment. With the development of ILs' research and application, the biodegradability and toxicity of ILs have attracted more and more attention from researchers. In 2002, the conference of Green Solvents for Catalysis firstly discussed the potential toxicity of ILs, which marked the start of research on ILs' toxicity. Later, a series of studies showed that the toxicity of ILs may be even higher than that of traditional organic solvents (Matsumoto et al. 2004; Ranke et al. 2004). The toxic effects of ILs on soil microbes, mammal and aquatic organisms have been widely investigated by ILs researchers (Pernak et al. 2003; Yu et al. 2008; Dong et al. 2013). Nonetheless, there are few reports on the toxic effect of ILs on plants, especially on crops (Liu et al. 2008, 2010; Wang et al. 2009).

Accordingly, an investigation about toxic effects of ILs on plants is extremely important for the risk assessment of plants as well as for their living environment. According to Web of

Science, among numerous ILs, imidazolium-based salts are one of the most pervasively used and investigated groups. Liu et al. (2010) reported that wheat is one of main crops in China as well as in the world, and whose cultivation area covers nearly a quarter of the total arable lands in China. During the present study, we used wheat seedlings and 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8mim]PF_6$) as an example to study the toxic effects of ILs on the growth and physiological characteristics of wheat seedlings. The purpose of the present research was to verify whether $[C_8mim]PF_6$ has an inhibitory effect on the growth of wheat seedlings. Moreover, it may provide a theoretical basis for the risk assessment of the environmental safety.

Materials and methods

Materials

The IL $[C_8mim]PF_6$ (99 % purity, CAS No. 304680-36-2) was purchased from Chengjie Chemical Co. Ltd. (Shanghai, China). "Jimai 22" (*Triticum aestivum* L.), which was supplied by College of Agriculture, Shandong Agriculture University, was used in this experiment.

Growth conditions and treatment

$[C_8mim]PF_6$, at concentrations of 0, 1, 2, 4, 6, and 8 mg l⁻¹ (Liu et al. 2010), was dissolved in half-strength Hoagland's nutrient solution (Adesemoye et al. 2009). Uniform wheat seeds were selected, and surface sterilized (30 % sodium hypochlorite solution) for 10 min, then rinsed with distilled water eight times (Lin et al. 2007). Then six beakers were prepared and seeds were soaked in each beaker, which contained different concentrations of Hoagland's nutrient solutions of $[C_8mim]PF_6$. Wheat seeds were put in the dark at room temperature for 12 h and the nutrient solutions were replaced every 4 h. Then the seeds were selected and placed on a layer of filter paper in 15-cm-diameter Petri dishes. One hundred seeds were arranged orderly in each Petri dishes, which have been added 20 ml of Hoagland's nutrient solutions containing $[C_8mim]PF_6$ at different concentrations to keep wet. The nutrient solution was replaced twice each day, at morning and evening, respectively. In order to keep the concentrations of $[C_8mim]PF_6$ stable, the Petri dishes were washed with the same concentrations of nutrient solution three times every time. Wheat seeds were accelerated germination at 25 °C in the dark for 3 days, and the seedlings were then cultivated in a greenhouse with the irradiance of 200 μmol m⁻² s⁻¹, which was provided by white fluorescent light and natural light. In the greenhouse, the temperature was controlled at 22 °C for 14 h/day and 18 °C for 10 h/night, and the relative humidity was controlled at 35–45 %. All of the

treatments were replicated three times. During the culture period, wheat seedlings were randomly sampled on days 7, 10 and 13 for the various analyses.

Germination potential and germination rate

Seeds were considered germinated when both the plumule and radicle were extended to more than 2 mm from their junction (Wang et al. 2009).

$$\text{Germination potential} = \frac{\text{Number of germinated seeds on the 3}^{\text{rd}} \text{ day}}{\text{Seeds number for the test}} \times 100\%$$

$$\text{Germination rate} = \frac{\text{Number of germinated seeds on the 7}^{\text{th}} \text{ day}}{\text{Seeds number for the test}} \times 100\%$$

(Zhao et al. 2012).

Shoot length, root length and dry weight of wheat seedlings

The shoot length and root length were measured as described by Wang et al. (2009). Shoot length can be defined as the length from the tip of the longest leaf to the base of culms, and root length can be defined as the length from the tip of the longest root to the root–shoot junction. The shoot length and root length of 15 wheat seedlings were measured with a ruler, which were randomly selected in each treatment.

The dry weight of wheat seedlings was measured according to the method as described by Lin et al. (2012). Wheat seedlings were oven dried at 105 °C for 15 min and then at 65 °C for 48 h. After that, the dry weight was weighed using the millionth place on an analytical balance.

Determination of pigment content

The method of Makeen et al. (2007) was used to assay the pigment contents of wheat seedlings. Fresh leaves (0.1 g) were put in 15-ml centrifuge tube which contained 10 ml of 80 % acetone. The centrifuge tube was placed in the dark for 40 h, and the pigment was extracted into acetone. The absorbance of the resulting extract was recorded at 470 nm, 646 nm and 663 nm using an ultraviolet–visible spectrophotometer (Shimadzu, UV-2550). The pigment contents were expressed as mg g⁻¹ of fresh weight (FW).

Measurement of lipid peroxidation and proline

Lipid peroxidation level was determined by measuring the amount of malondialdehyde (MDA). The method of Heath and Packer (1968) was used to assay the MDA content. Each parallel was taken 0.3 g of fresh leaf samples and then homogenized on ice bath in 3 ml of 0.1 % trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10,

000×g for 20 min, after which the supernatant (1.5 ml) was added an equal volume of 0.5 % thiobarbituric acid (TBA) which dissolved in 20 % TCA. The sample was heated at 95 °C for 30 min in a water bath, quickly cooled in an ice bath and then centrifuged at 10,000×g for 15 min. The MDA content was determined by measuring the absorbance at 532 and 600 nm. The formation level of MDA was counted by using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

Free proline content was determined according to the method as reported by Ma et al. (2013). Fresh leaf tissues (0.15 g) and 1.5 ml of 3 % sulfosalicylic acid were both added into 10-ml centrifuge tubes, and the sample was then put in a boiling water bath for 10 min. The supernatant fraction (1 ml) was added to an equal volume of glacial acetic acid and 2.5 % acid ninhydrin. The mixture was heated in a boiling water bath for 30 min, and then 4 ml of toluene was added. The proline content was determined by measuring the absorbance of the fraction with toluene aspired from the liquid phase at 520 nm. The proline content was determined using a calibration curve and expressed as μg g⁻¹ FW.

Determination of O₂⁻ generation rate

The generation rate of O₂⁻ was measured according to the method as stated by Elstner and Heupel (1976). Fresh leaves (0.25 g) were homogenized in 2.5 ml of extraction solution including 50 mM ice-cold phosphate buffer (pH 7.8), 1 mM EDTA and 1 % polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000×g for 10 min. The supernatant (0.5 ml) was added into 5-ml centrifuge tubes, which contained 0.5 ml of 50 mM phosphate buffer (pH 7.8) and 1 ml of 1 mM hydroxylamine hydrochloride. The sample was incubated in a water bath at 25 °C for 1 h. After that, 1 ml of 17 mM sulfanilamide and 1 ml of 7 mM α-naphthylamine were added into the sample, and incubated at 25 °C for 20 min. The absorbance of the sample at 530 nm was measured with an ultraviolet–visible spectrophotometer (Shimadzu, UV-2550). Sodium nitrite solution was used to calculate the generation rate of O₂⁻.

Enzyme activity determination

Fresh leaf samples (0.25 g) were homogenized in 2.5 ml of extraction solution containing 50 mM ice-cold phosphate buffer (pH 7.8), 1 mM EDTA and 1 % PVP. The mixture was centrifuged at 15,000×g for 20 min at 4 °C and the supernatant was used for the assay of activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), and ascorbate peroxidase (APX). All operations were carried out at 4 °C.

SOD (EC1.15.1.1) was spectrophotometrically assayed by measuring the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) as stated by Giannopolits and

Ries (1977). The reaction mixture (3 ml) was composed of 50 mM phosphate buffer (pH7.8), 0.1 mM EDTA, 130 mM methionine, 750 μM NBT, 20 μM riboflavin, and 50 μl of the enzyme extract. The reaction mixture was illuminated for 15 min at a light intensity of 5,000 lx. The ultraviolet–visible spectrophotometer was used to record the absorbance at 560 nm, and one unit of SOD activity was taken as the amount of enzyme inhibiting the photochemical reduction of NBT by 50 %.

CAT (EC1.11.1.6) activity was analyzed according to the method described by Miao et al. (2010). The reaction mixture (3 ml) was composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM H₂O₂ and 0.1 ml of the enzyme extract. The enzyme activity was determined by the amount of H₂O₂ decomposed at 240 nm for 1 min.

POD (EC1.11.1.7) activity was determined by monitoring the rate of guaiacol oxidation at 470 nm using spectrophotometry as described by Thongsook and Barrett (2005). And one unit (U) of enzyme activity refers to a 0.01 increment of Δ₄₇₀ in 1 min. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H₂O₂ and 20 μl of the enzyme extract.

APX (EC1.11.1.1) activity was measured by monitoring the decrease in absorbance at 290 nm according to the method as stated by Nakano and Asada (1981). The reaction mixture (3 ml) contained 100 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM H₂O₂, 0.5 mM ascorbate (extinction coefficient 2.8 mM⁻¹ cm⁻¹), and 0.1 ml of the enzyme extract. The reaction was started by adding the enzyme extract or H₂O₂, and the enzyme activity was determined by the decrease in absorbance at 290 nm for 1 min.

Statistical analysis

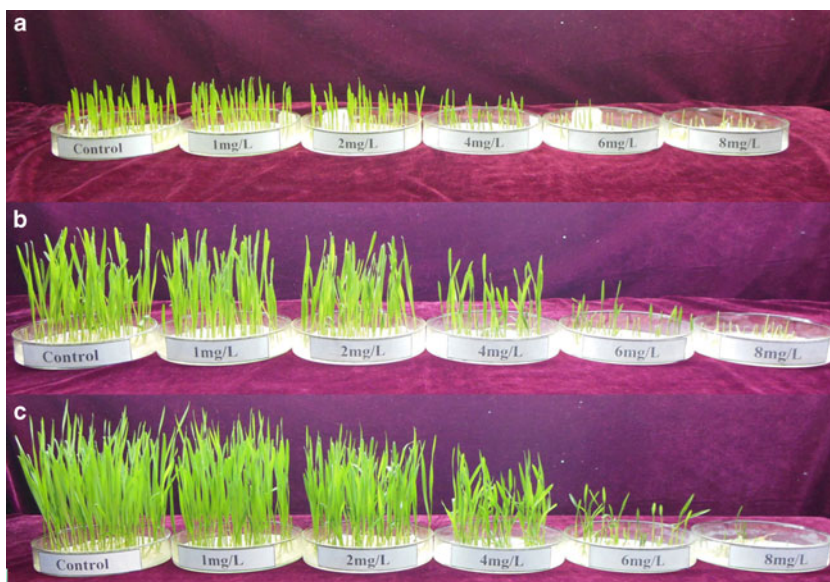
The data were analyzed using the statistical Software Package for Social Science (SPSS) version 17.0, and all of the values were presented as the mean±SD. One-way analysis of variance (ANOVA) was performed on all data, and probit analysis was used to calculate the 50 % effective concentration (EC₅₀). Comparisons were made using the least significant difference (LSD) test, and differences were considered significant at the *p*<0.05 level.

Results

Effect of [C₈mim]PF₆ on the growth index

The growth conditions of different [C₈mim]PF₆-exposed wheat seedlings are listed in Fig. 1. The toxicity of [C₈mim]PF₆ increased with increasing concentration and time. At 1 mg l⁻¹, [C₈mim]PF₆ had little effect on germination on the third day (Fig. 2a). However, the germination rate was

Fig. 1 Growth conditions of wheat seedlings influenced by [C₈mim]PF₆ at concentrations of 0, 1, 2, 4, 6, and 8 mg l⁻¹ on the day 7 (a), day 10 (b) and day 13 (c)



greatly inhibited when the concentration was higher than 4 mg l⁻¹ on the seventh day. At low concentrations, although the growth conditions were hardly affected on day 7, the toxic

effect enhanced on days 10 and 13. The EC₅₀ values for germination, shoot length, root length and dry weight were 11.104, 5.187, 4.380 and 6.292 mg l⁻¹.

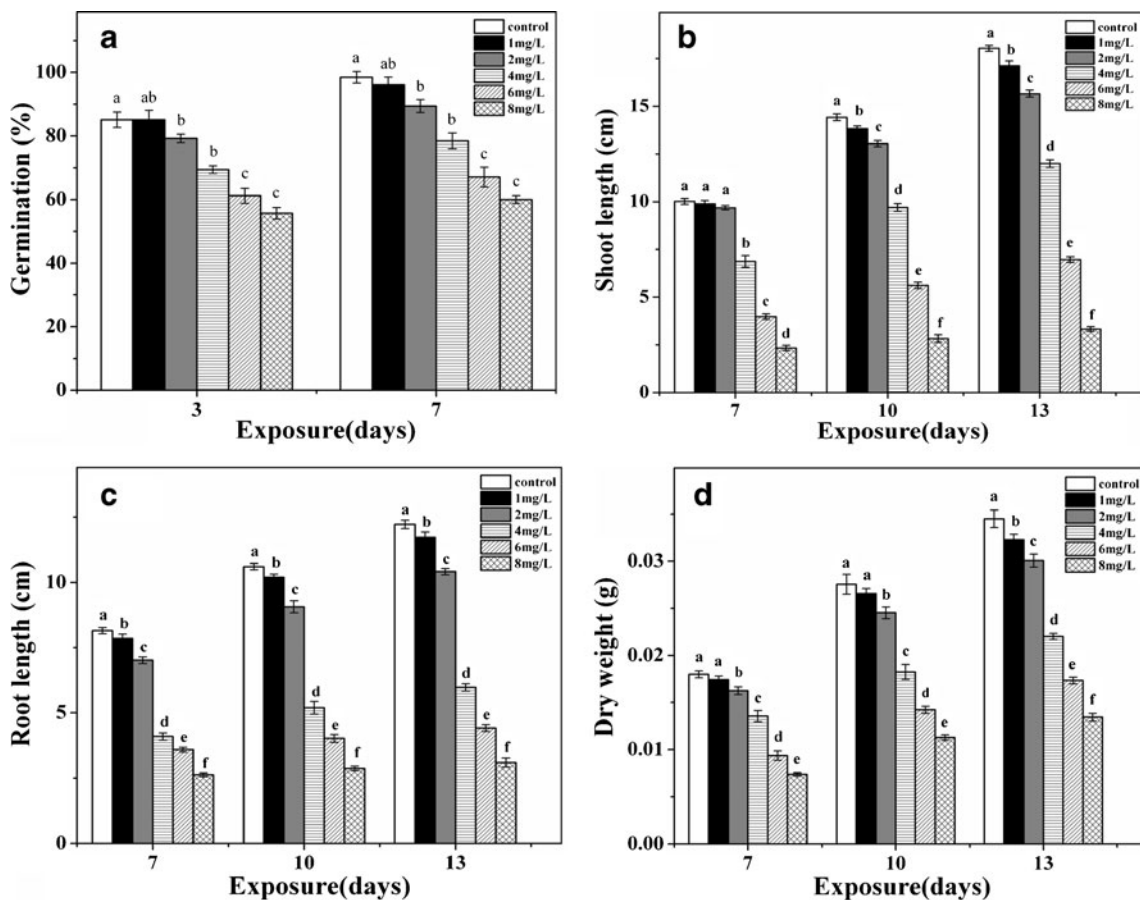


Fig. 2 Effect of [C₈mim]PF₆ on germination (a) of wheat seeds and shoot length (b), root length (c) and dry weight (d) of wheat seedlings. Bars are means±standard error of three replicates. Different letters above

columns indicate significant differences (p < 0.05) between treatments as determined by least significant difference (LSD) test

Effect of $[C_8mim]PF_6$ on pigment content

Pigment contents of wheat seedlings of different $[C_8mim]PF_6$ -exposed treatments are shown in Fig. 3. Contents of pigment in wheat seedlings followed a similar trend to the growth index. Pigment contents were markedly reduced on days 7, 10 and 13 when the concentration of $[C_8mim]PF_6$ was higher than 4 mg l^{-1} . Although 1 mg l^{-1} of $[C_8mim]PF_6$ had a slight promotion effect on chlorophyll a and carotenoids on day 7, this promotion disappeared on days 10 and 13.

Effect of $[C_8mim]PF_6$ on the generation rate of $O_2^{\cdot-}$, lipid peroxidation and proline content

Figure 4a shows that the generation rate of $O_2^{\cdot-}$ increased substantially with increasing concentrations. At low concentrations, the accumulation of $O_2^{\cdot-}$ was not obvious as time increased. When the concentration was higher than 4 mg l^{-1} ,

the $O_2^{\cdot-}$ generation rate increased dramatically with the increasing of time. However, the $O_2^{\cdot-}$ generation rate of the highest concentration (8 mg l^{-1}) showed a downward tendency on the 13th $O_2^{\cdot-}$ day.

The MDA content in wheat seedlings showed a similar trend to the generation rate of $O_2^{\cdot-}$. The MDA content of different $[C_8mim]PF_6$ -exposed wheat seedlings were all higher than that of the control on days 7, 10, and 13, except at 1 mg l^{-1} on day 7. Moreover, the MDA content increased sharply when the concentration of $[C_8mim]PF_6$ was higher than 2 mg l^{-1} . The MDA content of the highest concentration (8 mg l^{-1}) had little change on day 13 compared with that on day 10.

The proline content was the most sensitive indicators in the present study. The content of proline in wheat seedlings was markedly enhanced when the concentration was higher than 2 mg l^{-1} . Compared with the control, the proline content of 4, 6, 8 mg l^{-1} $[C_8mim]PF_6$ -exposed wheat seedlings increased 5, 7, 11 times on the 13th day.

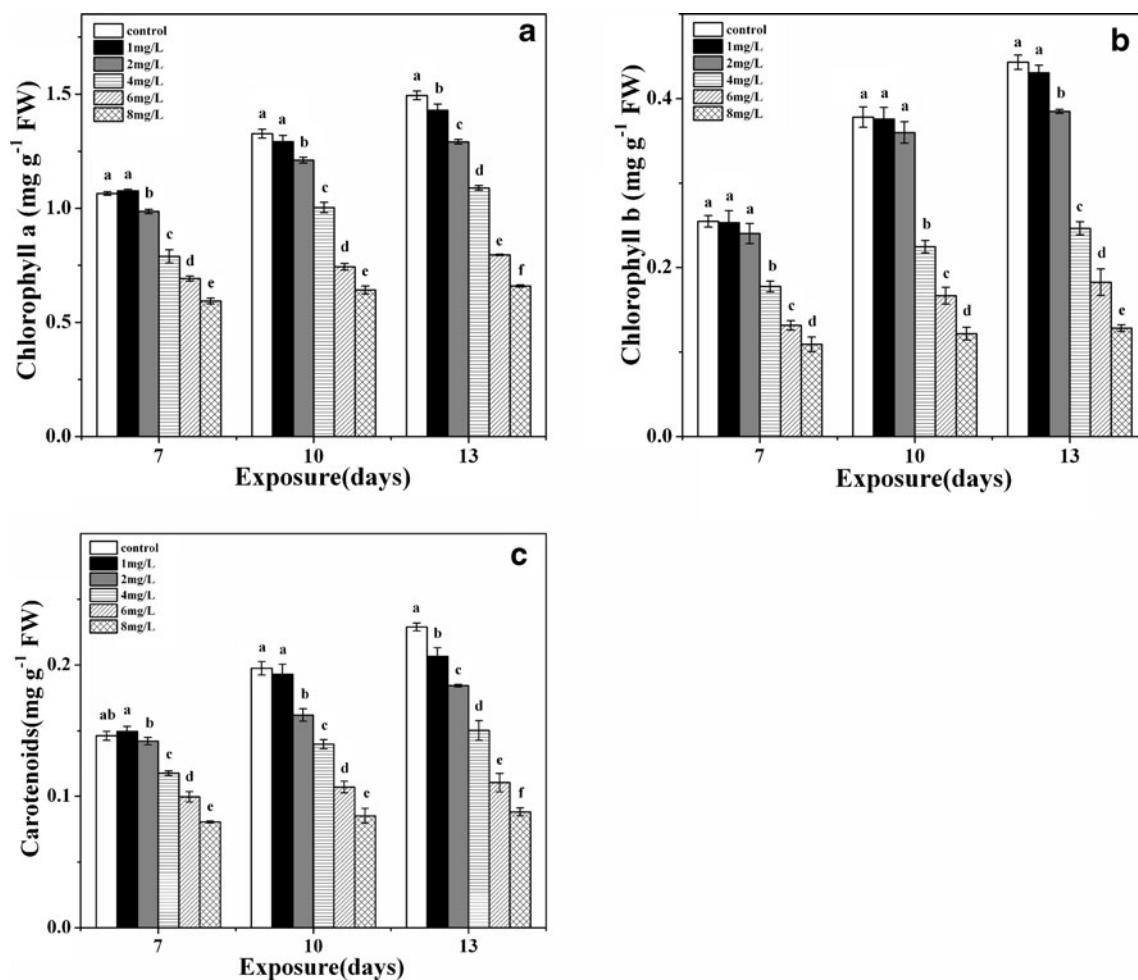


Fig. 3 Effect of $[C_8mim]PF_6$ on the content of chlorophyll a (a), chlorophyll b (b) and carotenoids (c) in wheat seedlings. Bars are means \pm standard error of three replicates. Different letters above columns

indicate significant differences ($p < 0.05$) between treatments as determined by least significant difference (LSD) test

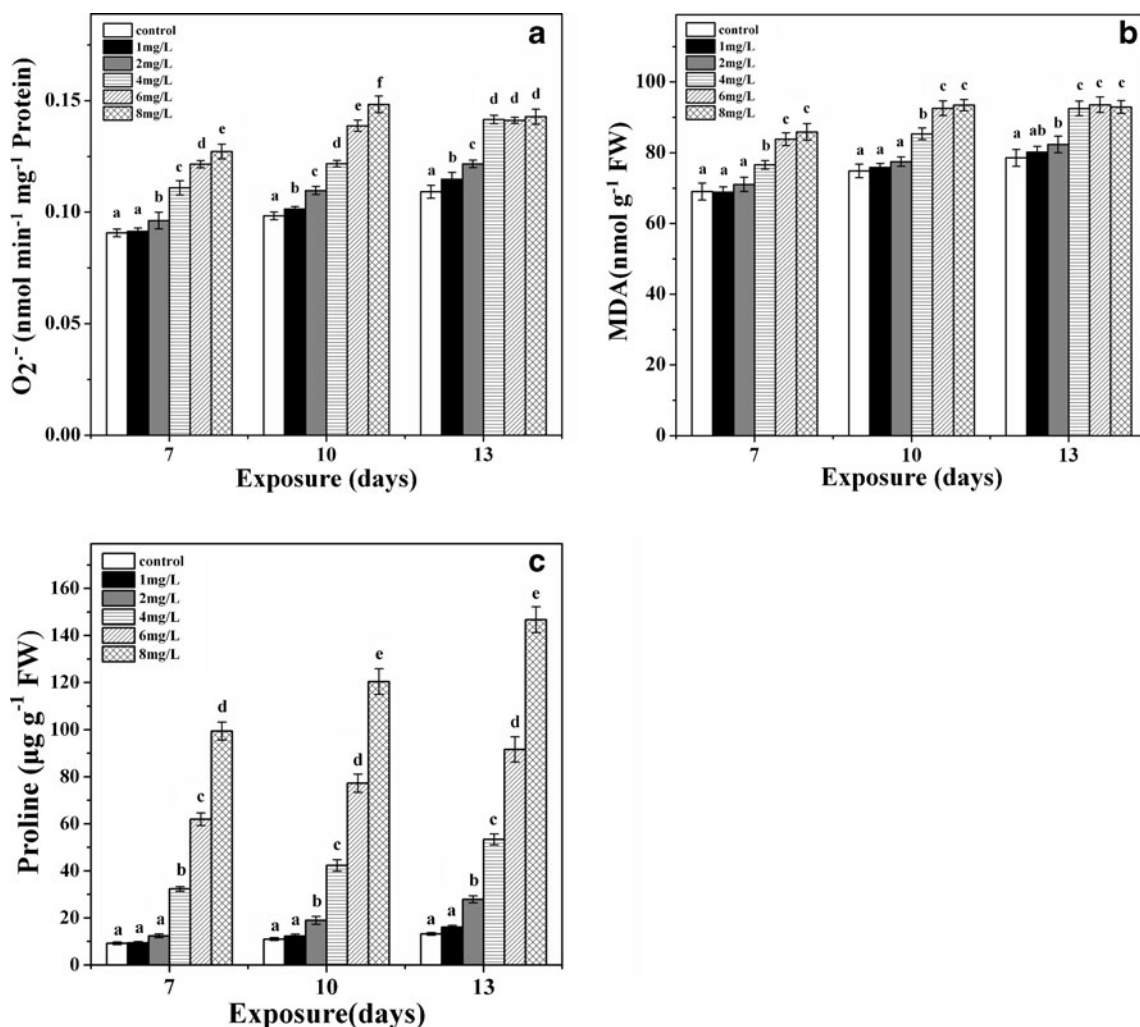


Fig. 4 Effect of [C₈mim]PF₆ on the generation rate of O₂⁻ (a), MDA content (b) and proline content (c) in wheat seedlings. Bars are means± standard error of three replicates. Different letters above columns indicate

significant differences (*p* < 0.05) between treatments as determined by least significant difference (LSD) test

Effect of [C₈mim]PF₆ on the activities of SOD, CAT, POD and APX

As shown in Fig. 5a, the SOD activity increased with increasing concentration on days 7, 10 and 13. There was a significant enhancement of SOD activity when the concentration was higher than 2 mg l⁻¹. However, with the passage of time, the changes of SOD activity on days 7, 10 and 13 were not very obvious.

As shown in Fig. 5b, POD activity was also sensitive to [C₈mim]PF₆. POD activity accumulated with increasing concentration and time. Compared with the control, there was a significant difference in POD activity when the concentration was higher than 2 mg l⁻¹. On day 13, POD activity of the highest concentration (8 mg l⁻¹) increased by 3-fold of the control. On the contrary, POD activity of wheat seedlings treated with 1 and 2 mg l⁻¹ of [C₈mim]PF₆ had little change on days 7, 10 and 13.

Changes in CAT activity are shown in Fig. 5c. In general, CAT activity decreased with increasing concentrations. On

day 7, CAT activity of 4 mg l⁻¹ [C₈mim]PF₆-exposed treatment was higher than that of other groups expect the control. CAT activity of wheat seedlings treated with 2 and 4 mg l⁻¹ of [C₈mim]PF₆ increased on the 10th day. However, they were all return to normal on the 13th day.

In the present study (Fig. 5d), the APX activities in all of the [C₈mim]PF₆-exposed treatments were lower than that of the control, and they decreased with increasing concentrations on day 7 expect at 8 mg l⁻¹. On the 10th and 13th days, the APX activities increased with increasing concentrations, and the APX activity of 6 and 8 mg l⁻¹ [C₈mim]PF₆-exposed treatments were higher than that of the control on the 10th and 13th days.

Discussion

The inhibition of growth is a common response of plants to stress (Munns 2002). In the present study, the growth of wheat

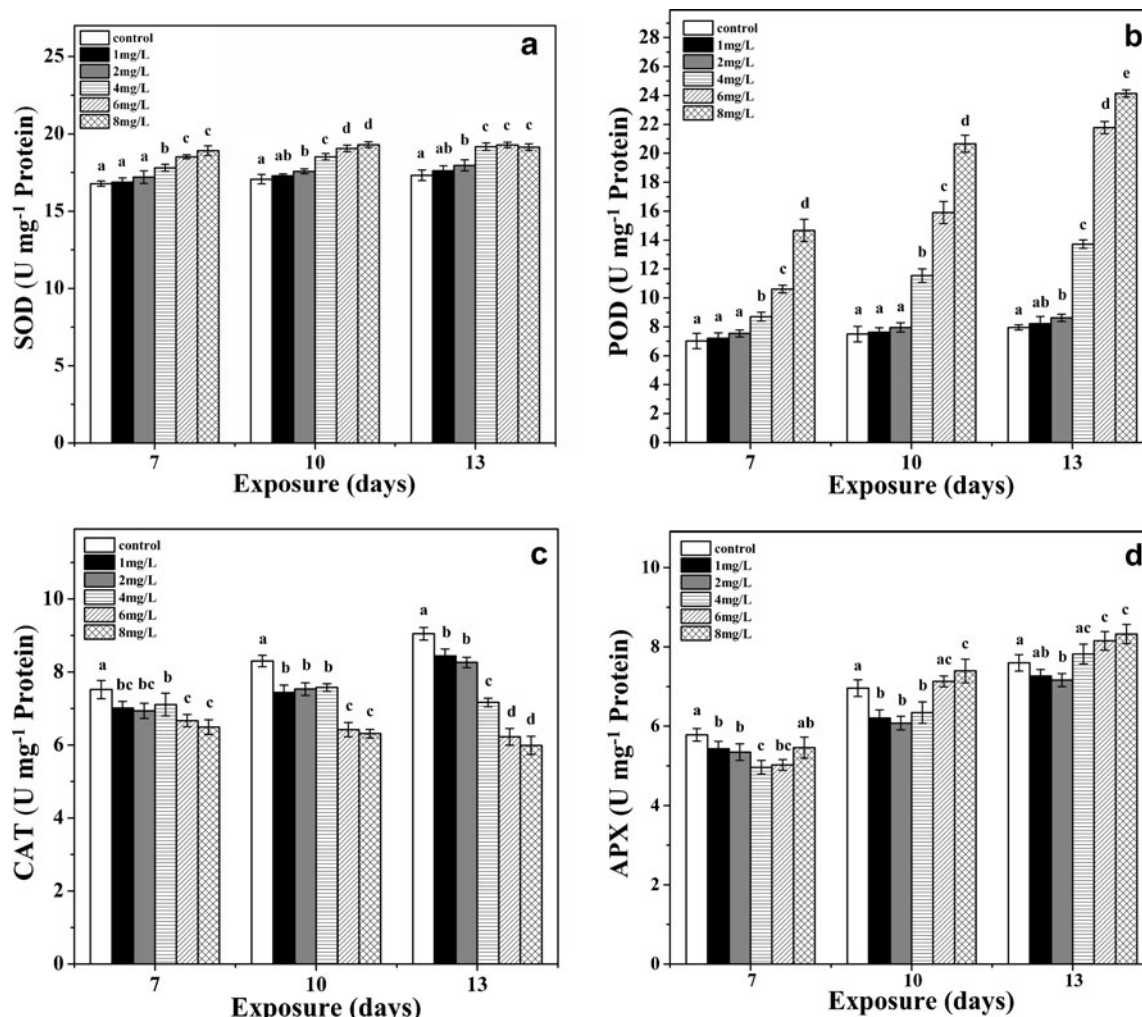


Fig. 5 Effect of $[C_8mim]PF_6$ on the SOD (a), POD (b), CAT (c) and APX (d) activities of wheat seedlings. Bars are means \pm standard error of three replicates. Different letters above columns indicate significant

differences ($p < 0.05$) between treatments as determined by least significant difference (LSD) test

seedlings was inhibited more and more seriously with increasing concentrations and time. At 1 and 2 mg l⁻¹, $[C_8mim]PF_6$ had little effect on the growth of wheat seedlings on the 7th day. However, the toxic effect was enhanced on the 10th and 13th days. It may be because at low concentrations, the behavior of ILs is similar to salts, a little of ILs is absorbed by plants in a short time. Therefore, low doses of ILs could not inhibit the growth of plants; on the contrary, a slight accumulation of ILs could even promote plant growth (Wang et al. 2009). However, with the increase in concentrations and time, the ILs were absorbed more and more by plants which could seriously inhibit the growth of plants, as shown in Figs. 1 and 2. In the present study, the shoot length was more sensitive to $[C_8mim]PF_6$ than germination, root length and dry weight. We suggest that $[C_8mim]PF_6$ is absorbed by the root and transported to the leaf, which results in a higher toxicity. ILs can disrupt phospholipid bilayers, which has a strong impact on the membrane system, and finally results in the increased toxicity (Matzke et al. 2007). The EC₅₀ values for

germination, shoot length and root length were 11.104, 5.187 and 4.380 mg l⁻¹, respectively. Liu et al. (2008) studied the toxic effects of $[C_8mim]Br$ on wheat seedlings. The IC₅₀ values for germination, shoot length and root length were 3.39, 4.98 and 0.64 mg l⁻¹, respectively. The reason for the discrepancy may be that the anion or the variety of wheat seedlings is different. Therefore, we suggest that different anions have different contributions to the toxicity of ILs or different varieties have different response to ILs. Wang et al. (2009) studied the toxic effects of $[C_4mim][BF_4]$ on wheat seedlings. The germination, shoot length and root length were significantly inhibited with increasing concentrations of $[C_4mim][BF_4]$. These results all showed that ILs had toxic effect on the growth of wheat seedlings.

Chlorophyll is a type of very important pigment, with which the plants capture sunlight for photosynthesis; therefore, it is essential to the growth of plants (Masuda et al. 2002). In the present study, the pigment content was significantly inhibited with increasing concentrations of $[C_8mim]PF_6$. Therefore,

photosynthesis of high concentrations $[C_8mim]PF_6$ -exposed wheat seedlings were retarded and the growth of these groups were inhibited. One of the clearest manifestations of that was the reduction in shoot length and root length. The pigment content was one of the most obvious monitors of oxidative damage in plants (Barbara et al. 1999). ILs can accelerate the generation rate of reactive oxygen species (ROS), leading to rapid degradation of photosynthetic pigments (Rai et al. 2004). Therefore, we suggest that the decrease in pigment content may result from both direct and indirect actions of the ILs.

ROS are continuously produced by mitochondria, chloroplasts and peroxisomes in higher plants (Apel and Hirt 2004; Foyer and Noctor 2012, 2013). When plants encounter stress conditions, the balance of production and scavenging of ROS is broken, resulting in oxidative damage and lipid peroxidation (Wu et al. 2006). The generation rate of $O_2^{\cdot-}$ can reflect the level of ROS formation (Liu et al. 2007). In the present study, the generation rate of $O_2^{\cdot-}$ increased with increasing concentrations and time, which indicated that the production rate of ROS had already exceeded their removal rate. The excess ROS inevitably resulted in the increase in membrane permeability and lipid peroxidation, which led to the increase in proline content. MDA is one of the byproducts of lipid peroxidation and its content is routinely used as an indicator of membrane lipid peroxidation (Lacan and Baccou 1998; Qiu et al. 2013). The present results showed that the content of MDA increased with increasing concentrations and time, which followed a similar trend to $O_2^{\cdot-}$ generation. It also confirmed that $[C_8mim]PF_6$ could lead to lipid peroxidation in wheat seedlings. Proline contributes to the stabilization of enzyme and proteins, ROS detoxification, cellular osmotic adjustment, and the protection of membrane integrity (Alia-Saradhi 1991; Gadallah 1999). Proline content will accumulate when plants are subjected to various stress conditions, and proline is considered as one of the most sensitive indicator of plants suffering from stress (Kranteva et al. 2008; Sergio et al. 2012). From Fig. 3c, the proline content markedly enhanced when the concentration was higher than 2 mg l^{-1} . Moreover, the higher the concentration became, the more the proline content enhanced. Liu et al. (2007) reported that MDA content and proline content were all markedly enhanced with increasing concentrations of $[C_8mim]Br$. Previous reports and our results all showed that ILs could cause oxidative damage effect on wheat seedlings, and the toxic effect increased with increasing concentrations of ILs.

Antioxidase system, including SOD, POD, CAT and APX, plays an important role in the metabolism of ROS (Qiu et al. 2013). SOD can catalyze the disproportionation of two $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 , which is an important step in protecting the cell (Sun et al. 2007; Li et al. 2011; Gratao et al. 2012). H_2O_2 also has a toxic effect on plant cell, and it can be converted conversely into H_2O and O_2 by several types of H_2O_2 scavenging enzymes (Wang et al. 2004a, b). In present results, SOD activity increased with increasing

concentrations and time, which showed a similar trend to $O_2^{\cdot-}$ generation. The change of SOD indicated that wheat seedlings started to respond to the damage coming from $[C_8mim]PF_6$. POD is another indicator of oxidative stress in higher plants (Yin et al. 2008). POD has double effects on plant cells: (1) POD is considered as the primary H_2O_2 -scavenging enzymes in the removal of ROS (Foyer et al. 1994; Li et al. 2013); (2) POD can participate in the formation of ROS and the degradation of pigment, as well as cause lipid peroxidation at the later stage of stress (Roychoudhury et al. 2012). Zhang and Kirkham (1994) considered the latter effect was the main function of POD. In our results, POD activity was markedly enhanced with increasing concentrations and time. At the same time, the pigment content was reduced, and the lipid peroxidation level as well as $O_2^{\cdot-}$ generation rate was enhanced. We suggest that these changes may be relevant to the improvement of POD activity to a certain degree. CAT can eliminate H_2O_2 by decomposing it into H_2O and O_2 (Gratao et al. 2005). In our results, CAT activity decreased with increasing concentrations and time, which showed an opposite trend to POD activity. We suggest that CAT activity may be inhibited by $[C_8mim]PF_6$. APX is the main enzyme of the ascorbate–glutathione cycle, and has a higher affinity for H_2O_2 than CAT and POD. Therefore, APX was considered to play the most important role in eliminating ROS and keeping cells safe in higher plants (Sarvajeet and Narendra 2010; Foyer and Noctor 2012, 2013). In the present study, APX activity decreased on day 7, and increased on days 10 and 13, which indicated that APX activity was inhibited at first, then activated. POD, CAT and APX consist of main H_2O_2 scavenging system in plant cells (Zhu et al. 2004; Gratao et al. 2005). The changes of SOD, POD, CAT and APX indicated that different enzymes had different sensitivity to $[C_8mim]PF_6$. We suggest that POD is the most sensitive enzyme to the stress of $[C_8mim]PF_6$, followed by SOD and APX, and CAT is the least. A similar response of antioxidant enzymes was found by Sergio et al. (2012) in wild chicory plants subjected to salt stress. Liu et al. (2007) studied the toxic effect of $[C_8mim]Br$ on the antioxidant system of wheat seedlings. The SOD, POD, CAT and APX activities all decreased with increasing concentrations. The difference between previous research and ours might be due to the different anion and varieties of wheat seedlings, indicating that the anion has a certain influence on the toxicity of ILs and different varieties have different response to ILs. In summary, the abnormal of SOD, POD, CAT and APX reflected that $[C_8mim]PF_6$ had caused oxidative damage to wheat seedlings.

Conclusion

The present study investigated the toxic effects of $[C_8mim]PF_6$ on the growth and physiological characteristics

of wheat seedlings. These indicators were systematically analyzed, and the main conclusions of the present study were as follows:

- (1) At low concentrations ($<2 \text{ mg l}^{-1}$), $[\text{C}_8\text{mim}]\text{PF}_6$ had little effect on germination, shoot length, root length and pigment content. However, $[\text{C}_8\text{mim}]\text{PF}_6$ had an obvious inhibitory effect on the growth of wheat seedlings when the concentration was higher than 4 mg l^{-1} .
- (2) The EC_{50} values for germination, shoot length, root length and dry weight were 11.104, 5.187, 4.380 and 6.292 mg l^{-1} , respectively.
- (3) $[\text{C}_8\text{mim}]\text{PF}_6$ led to irreversible oxidative damage in cells when the concentration of $[\text{C}_8\text{mim}]\text{PF}_6$ was higher than 4 mg l^{-1} , which resulted in the elevation of lipid peroxidation and anomalies in the antioxidase system.
- (4) Shoot length, proline and POD were more sensitive to the toxic effect of $[\text{C}_8\text{mim}]\text{PF}_6$ than other indicators.
- (5) At high concentrations ($>4 \text{ mg l}^{-1}$), $[\text{C}_8\text{mim}]\text{PF}_6$ may have serious impact on higher plants as well as agricultural production, and decreasing IL concentrations is a very important method to reduce IL pollution.

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