Environmental and Experimental Botany 99 (2014) 53–58

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Effect of phenanthrene uptake on membrane potential in roots of soybean, wheat and carrot

Xiaoming Yin[a,1], Xiao Liang[a,1], Guohua Xu[a], Xinhua Zhan[a,b,*]

[a] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province 210095, PR China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province 210008, PR China

ARTICLE INFO

Article history:
Received 6 August 2013
Received in revised form 25 October 2013
Accepted 1 November 2013

Keywords:
Roots
Phenanthrene uptake
Cell membrane potential
Polycyclic aromatic hydrocarbons

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants which are toxic for human due to dietary intake of food crops. However, few studies have been conduced to compare the differences in PAH uptake among various crops. In this paper, the effect of uptake of phenanthrene (PHE), a model compound of PAHs, on membrane potential in roots of soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.) and carrot (*Daucus carota* L.) was conducted and the related physiological mechanism was discussed. The electrical responses in roots triggered by PHE consist of two sequential phases: depolarization followed by repolarization. The depolarization of membrane potential is dependent on PHE concentration within the concentration range of 0–1.2 mg L^{-1}. The magnitude of depolarization in root cell membrane follows the order: soybean > carrot > wheat, which is in good accordance with that of PHE uptake. In the pH range of 4.5–6.5, pH 4.5 promotes the uptake of PHE and causes a stronger depolarization. Soybean produces the largest depolarization in membrane potential, carrot gets the similar degree of depolarization at pH 5.5 and 6.5. Vanadate and 2,4-dinitrophenol inhibit the uptake of PHE and reduce membrane potential depolarization. The activity of plasma membrane (PM) H^+-ATPase in roots of soybean is significantly higher than that of wheat or carrot at PHE concentrations of 0.8–1.2 mg L^{-1}, which agrees with the changes in membrane potential. Nonetheless, wheat roots have the highest activity of H^+-ATPase at PHE concentrations of 0–0.4 mg L^{-1}. It is suggested that the differences in membrane potential responding to PHE uptake among the three crops are due to their different activities of H^+-ATPase and affinities of transporters to PHE. Depolarization of cell membrane potential is a potential indicator in selecting and breeding crops that take up less/more PAHs, which would be useful to enhance food safety and improve PAH-contaminated water or soils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants and potentially carcinogenic, mutagenic and toxic to both human and non-human organisms. It is reported that over 90% of PAHs in the environment occur in soil surface (Wild and Jone, 1995). Crops growing in such polluted soils can become contaminated by PAHs and may have different abilities to take up PAHs. Fismers et al. (2002), for example, have shown that 16 PAHs, listed as priority pollutants by US Environmental Protection Agency, in potato are far more than those in lettuce and carrot. Yin et al. (2005) have observed that phenanthrene (PHE) in Indian lettuce is three times higher than that in cabbage. Thus, it is important to investigate the differences in PAH uptake by roots of different crops for enhanced crop safety and improved phytoremediation of PAH-contaminated soils or water.

Our previous study has shown that the uptake of PHE consists of two sequential phases: a fast accumulation process followed by a slow one (Zhan et al., 2010). Concentration dependent uptake of PHE by roots can be described with Michaelis–Menten equation (Zhan et al., 2012). The capability to take up PHE in roots for the three crops can be arranged as soybean > carrot > wheat, with a K_m of 0.117, 0.124 and 0.540 mg L^{-1}, respectively. Meanwhile, increases in hydroponic solution pH due to PHE uptake are significantly correlated with K_m values (Liang et al., 2012). However, the nature of the transporters and information on the differences among various crop species are not clear.

It has been observed that PAHs are actively transported into the cells via H^+-coupled symporters, and transporters in various crops have different affinities to PAHs (Yang et al., 2009; Zhan et al., 2012). Plasma membrane (PM) H^+-ATPases mediate H^+ and electrical potential difference across the PM, which serves as the motive force for the secondary transporters, such as symporter, antiporter,

* Corresponding author at: College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province, 210095, PR China. Tel.: +86 25 84395210; fax: +86 25 84395210.
E-mail address: xhzhan@njau.edu.cn (X. Zhan).
[1] These authors contributed equally to this work.

0098-8472/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.envexpbot.2013.11.001

and uniporter, to drive their substrates against the concentration gradients (Ueno et al., 2005).

Microelectrode is developed with the study of animal neural electrophysiology. Ion-selective electrode can be used to measure ion activity and the dynamics of intracellular ion activity, continuous and instantaneous change in single cell (Zhang et al., 1990; Wang et al., 2010a). It has been applied in analyzing the characteristics of cell membrane potential during nutrient ion absorption in plant roots, and exploring the mechanisms of nutrient ion transport (Glass et al., 1992; Yin et al., 2006).

In this paper, wheat, soybean, and carrot were the crops of choice for our study, based on the fact that they are common plants used for hydrophobic organic compound uptake (Edwards, 1986; Jones et al., 1989; Chiou et al., 2001; Fryer and Collins, 2003; Li et al., 2005). Our hypothesis is that crops with different capability to take up PAHs have distinct cell membrane potential induced by PAHs, which is dependent on the activity of PM H^+-ATPase and affinity of transporter to PAHs. The aims of this study were to characterize the changes of cell membrane potential during PAH uptake by roots of the three crops and demonstrate the physiological mechanism of the differences in PAH uptake among the three crops.

2. Materials and methods

2.1. Plant materials

Seeds of soybean (*Glycine max* L. cv. Sudou 5), wheat (*Triticum aestivum* L. cv. NAU 9918), and carrot (*Daucus carota* L. cv. Xingyun 3) were surface-sterilized in 10% H_2O_2 for 10 min, then thoroughly rinsed with Millipore water (Milli-Q, Billerica, MA, USA) and germinated on moistened filter paper for 4 days at 25 °C in the dark. The seedlings were transplanted into black plastic pots containing 2500 mL half-strength aerated Hoagland nutrient solution for 5 days and then transferred to full-strength Hoagland solution for a further 5 days under controlled conditions (photoperiod 16 h light/8 h dark, light intensity 400 μmol m^{-2} s^{-1}, day/night temperature of 25/20 °C, relative humidity 60%). The Hoagland solution was prepared with Millipore water. The initial pH of the solution was adjusted to 5.5. After a 10-day growth in Hoagland nutrient solution, the seedlings were immersed in Millipore water for 24 h and then employed in the subsequent electrophysiological experiments and measurements of PM H^+-ATPase activity.

2.2. Measurements of cell membrane potential

New primary roots of intact seedlings were mounted in a Plexiglas chamber attached to the stage of an Olympus compound microscope (Olympus, Center Valley, PA), which was fixed to the surface of a vibration damped table (Kinetic Systems Inc., Boston, MA). The Plexiglas chamber was filled with basic solution (5 mmol L^{-1} 2-(*N*-morpholino) ethanesulfonic acid (MES), 0.5 mmol L^{-1} $CaCl_2$, 0.05 mmol L^{-1} KCl, pH 5.5) at a flow rate of 10 mL min^{-1} for 2 h before the measurements. Micropipettes (tip diameter <0.5 μm) were pulled with a puller (PE-21; Narishige Scientific Instrument Lab, Tokyo, Japan). The micropipettes were filled with 0.1 mol L^{-1} KCl, and the reference was filled with 0.1 mol L^{-1} KCl in 2% agar. They were connected by Ag/AgCl electrodes to an amplifier (FD223; World Precision Instruments; Hertfordshire, UK). The micropipette was inserted into the root cell (1–2 cm from the root apex) with a micromanipulator (Narashige) (McClure et al., 1990; Ullrich and Novacky, 1990). When the resting potential was stable, basic solution was replaced by test solution. The measurements were conducted in the dark at room temperature. Membrane potential (E_m) measurements were expressed as mean values ± SD of 6 replicates of 6 individual roots.

For PHE concentration dependent depolarization experiments, the test solutions were basic solutions plus PHE at concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg L^{-1}, respectively.

For pH dependent depolarization experiments, the pH values of basic solution containing 1.0 mg L^{-1} PHE were adjusted to 4.5, 5.5 and 6.0 with MES, respectively.

For inhibitions of vanadate and 2,4-DNP in depolarization experiments, when the resting membrane potential in basic solution reached a steady state, the test solution was replaced with basic solution plus 1.0 mg L^{-1} PHE and vanadate (0, 0.2, 0.4, and 0.6 mmol L^{-1}, respectively) or 2,4-DNP (0, 0.5, 1.0, and 2.0 mmol L^{-1}, respectively).

2.3. Determination of PM H^+-ATPase activity

PM vesicles were isolated according to Yan et al. (2002). H^+-ATPase activity of PM was determined as described by Zhu et al. (2009) with small modification. The activity of H^+-ATPase was measured under treatments as: reaction solution containing 100 mmol L^{-1} K^+ and 50 mmol L^{-1} Hepes-Tris, at PHE concentrations of 0, 0.1, 0.4, 0.8 and 1.2 mg L^{-1}, and pHs of 7.0, 8.0 and 9.0, respectively. Hydrolytic activity of H^+-ATPase was determined by measuring inorganic P (Pi) after 30 min reaction. The activity is expressed as μmol Pi mg^{-1} protein h^{-1}.

2.4. Extraction and analysis of PHE

PHE in roots of the three crops was extracted and detected using the method of Zhan et al. (2010). After harvest, plant roots were immersed in methanol for 3 min, rinsed with sufficient Millipore water to remove PHE adsorbed on root surfaces, and wiped with tissue paper. Roots and shoots were weighed and ground in a glass homogenizer. Homogenized tissue samples were extracted with acetone/hexane (1:1, v/v) mixture by ultrasonication three times (30 min each time). The combined extracts were passed through an anhydrous Na_2SO_4 column with elution of the 1:1 mixture of acetone and hexane. The eluents were then evaporated to dryness at 35 °C in a rotary evaporator and dissolved in 12 mL hexane. Subsequently, the 12 mL solvent was cleaned in a 2-g silica gel column and eluted with 25 mL hexane/dichloromethane (1:1, v/v) solvents. The eluents were evaporated to dryness again and dissolved in 2 mL methanol. Prior to the analysis of PHE by HPLC, all final extracts were filtered with 0.22 μm filter (Kipopoulou et al., 1999). The average recovery of PHE obtained by spiking plant samples with standards is 95.2% for the entire procedure. None of the data reported here has been corrected for recovery.

The HPLC system employed consists of an automatic injector (Waters 717), a binary high-pressure pump (Waters 1525), a UV detector (Waters 2487), and a fluorescence detector (Waters 2475). Separations were performed with a reverse phase Symmetry C_{18} (ø 4.6 × 150 mm, 5 μm particle) column. The temperature of the HPLC column was kept constant at 30 °C. The mobile phase used was methanol and Millipore water (80:20, v/v), with a flow rate of 1 ml min^{-1}. The injection volume was 10 μL. PHE was quantified at 293.5/395 (excitation/emission wavelength) and 254 nm for fluorescence and UV detector, respectively. Relative standard deviation (n = 5) was less than 2.85% for the method. The method detection limit was 48.5 pg PHE. Analytical standards were measured at the beginning of each series of analyses. Internal standard calibration was performed during the HPLC analyses.

2.5. Statistical analyses

Statistical analyses were performed with SAS software version 9.1.3 (SAS Institute Inc., Cary, NC). Data from different treatments