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The growth and uptake of Ga and In of rice (*Oryza sative* L.) seedlings as affected by Ga and In concentrations in hydroponic cultures

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ABSTRACT

Limited information is available on the effects of gallium (Ga) and indium (In) on the growth of paddy rice. The Ga and In are emerging contaminants and widely used in high-tech industries nowadays. Understanding the toxicity and accumulation of Ga and In by rice plants is important for reducing the effect on rice production and exposure risk to human by rice consumption. Therefore, this study investigates the effect of Ga and In on the growth of rice seedlings and examines the accumulation and distribution of those elements in plant tissues. Hydroponic cultures were conducted in phytotron glasshouse with controlled temperature and relative humidity conditions, and the rice seedlings were treated with different levels of Ga and In in the nutrient solutions. The growth index and the concentrations of Ga and In in roots and shoots of rice seedlings were measured after harvesting. A significant increase in growth index with increasing Ga concentrations in culture solutions (< 10 mg Ga L^{-1}) was observed. In addition, the uptake of N, K, Mg, Ca, Mn by rice plants was also enhanced by Ga. However, the growth inhibition were observed while the In concentrations higher than 0.08 mg L^{-1}, and the nutrients accumulated in rice plants were also significant decreased after In treatments. Based on the dose-response curve, we observed that the EC_{10} (effective concentration resulting in 10% growth inhibition) value for In treatment was 0.17 mg L^{-1}. The results of plant analysis indicated that the roots were the dominant sink of Ga and In in rice seedlings, and it was also found that the capability of translocation of Ga from roots to shoots were higher than In. In addition, it was also found that the PT_{10} (threshold concentration of phytotoxicity resulting in 10% growth retardation) values based on shoot height and total biomass for In were 15.4 and 10.6 μg $plant^{-1}$, respectively. The beneficial effects on the plant growth of rice seedlings were found by the addition of Ga in culture solutions. In contrast, the In treatments led to growth inhibition of rice seedlings. There were differences in the phytotoxicity, uptake, and translocation of the two emerging contaminants in rice seedlings.

1. Introduction

Gallium (Ga) and indium (In) are regarded as toxic substances to humans based on previous reports (Fowler et al.. 1993; Ivanoff et al.. 2012; Kabata-Pendias and Mukherjee, 2007; Tanaka 2004). In general, Ga and In are produced as byproducts in the production of Al (bauxite), Pb (galena) and Zn (sphalerite), and these two elements are widely used in semiconductor manufacturing and the electro-optical and medical industries (Alfantazi and Moskalyk, 2003; Kabata-Pendias and Mukherjee, 2007; Kabata-Pendias 2011; Yu and Liao, 2011). Nowadays, due to progressively increasing usage of Ga and In, wastewater containing them may be discharged into farmland soils by the irrigation system, further raising the risk of human exposure to Ga and In through the food chain. Chen (2006) reported the high concentrations of Ga (up to 41 μg L^{-1}) and In (up to 20 μg L^{-1}) in groundwater contaminated by wastewater from semiconductor manufacturing area of Taiwan. To date, the information about the contents of Ga and In in soils contaminated by semiconductor manufacturing is limited. However, there were some studies indicated that the contents of Ga and In in soils near area of automobile and Zn-Pb smelting industries were 10.89–22.46 mg kg^{-1} and 0.11–1.92 mg kg^{-1}, respectively (Asami et al., 1990; Yu et al., 2015). Nevertheless, studies about the effects of Ga and In on the growth of edible crops are still scarce. Therefore, understanding the interaction between Ga and In and edible crops is necessary.

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Gallium and indium are grouped among the IIIA group of elements in the periodic table, and the content of Ga and In in worldwide soils ranges from 3 to 70 and 0.11–0.25 mg kg^{-1} respectively (Kabata-Pendias, 2011). In general, the oxidation states of Ga and In are +3, but oxidation states of +1 and +2 may also occur under anaerobic conditions. At 25 °C, the soluble species of Ga and In present in acidic conditions are Ga^{3+}, $Ga(OH)^{2+}$, $Ga(OH)_2^{+}$, and In^{3+}, $In(OH)^{2+}$, $In(OH)_2^{+}$; the soluble species present in alkaline conditions are $Ga(OH)_4^{-}$ and $In(OH)_4^{-}$, and the insoluble species $Ga(OH)_3$ and $In(OH)_3$ are present in conditions of pH 4–6 and pH 5–9 (Kabata-Pendias, 2011; Wood and Samson 2006). These solubility characteristics are similar to those of Al, which suggests that Ga and In are amphoteric elements. Orians and Bruland (1988) showed that the geochemistry and aquatic chemistry properties of Ga are similar to Al, but that the reactivity of Ga is less than that of Al.

Rice is a dietary food for about half of the world's population, and for over 90% of the population in Asia (Meharg et al.. 2009). Because paddy fields may suffer from Ga- and In-associated wastewater contamination, study of the accumulation of Ga and In in rice plants and the effect of these two emerging contaminants on rice plant growth is merited. The chemical properties of Ga and In are similar to those of Al, and several studies have investigated Al toxicity and tolerance in rice plants (Roy and Bhadra, 2014; Silva 2012; Tanaka and Navasero, 1966). However, little information about the effects of Ga and In on the growth of rice plants exists to date. Yu et al.. (2015) reported an associated reduction in relative growth rate, transpiration rate and water use efficiency of rice seedlings grown for 2 days in solution culture of increasing Ga concentration. Yu and Zhang (2015) found that over-accumulation of Ga in plant tissue resulted in cell death and growth inhibition in rice seedlings. Some studies have also investigated the toxic effect and accumulation of Ga and In in other plant species (Berg and Steinnes, 1997; Fergusson 1990; Kopittke et al., 2009; Shacklette et al., 1978). Shacklette et al. (1978) reported that the concentration of Ga in a variety of native species ranged from 3 to 30 mg kg^{-1} in the United States. Berg and Steinnes (1997) indicated that atmospheric deposition may result in the elevation of Ga content by up to 16 mg kg^{-1} in moss growing wild in Norway. Fergusson (1990) found concentrations of In ranging from 80 to 300 μg/kg FW in beets, 0.64–1.8 μg/kg FW in leaves of fruit trees, and 30–710 μg/kg FW in vegetables. Kopittke et al. (2009) reported that soluble Ga and In reduced cowpea root growth and caused cell rupture in hydroponic experiments.

To the best of our knowledge, the uptake and accumulation of Ga and In in rice plants and their effect on rice growth is still unclear. Therefore, our objective in this study was to investigate the effect of Ga and In on rice plant growth and how uptake of these two elements affects rice seedlings grown in solution cultures treated with different concentrations of Ga and In. In particular, Ga and In may impact the uptake of nutrients through competitive uptake by roots or through phytotoxicity; thus we also investigated the effect of Ga and In treatments on the uptake of nutrients by rice plants.

2. Material and methods

2.1. Hydroponic cultures

The hydroponic cultures were performed in a phytotron with a controlled temperature (25/20 °C, day/night) and relative humidity (70–95%) under sunlight. The cultivar of paddy rice (*Oryza sative* L., cv Taikeng 9) was used in this study because it is commonly planted in Taiwan and considered to be of high quality. Rice seeds were sterilized in a solution containing 1% sodium hydrochloride solution and one drop of Tween 20 for 30 min, and then washed with distilled water for 30 min. The seeds were then germinated in Petri dishes containing tissue paper under moist conditions at 37 °C for 2 days. After germination, thirty seedlings were then transferred to an iron mesh set on the surface of the culture solution, contained in a 0.6-L beaker. Seedlings were then raised in half-strength modified Kimura B nutrient solution (pH was adjusted to 4.8–5.0 and the solution was renewed every three days) for 14 days until they reached the three-leaf age. Afterward, the solution was replaced with full-strength nutrient solution and treated with the indicated amount of Ga or In stock solution. The stock solutions of Ga and In were prepared using $GaCl_3$ (99.999%, ultra dry, Alfa Aesar) and $InCl_3$ (99.999%, anhydrous, Alfa Aesar) respectively. The Ga treatment concentrations were 0, 1, 3, 5, 10, 15 mg L^{-1}, and the exposure time was 40 days (growth period: Nov.–Dec., 2014) The In treatments were separated into two parts: (a) For the high-In treatment (preliminary experiment), the In treatment concentrations were 0, 0.1, 1, 3, 5, 10 mg L^{-1}, and the exposure time was 25 days (growth period: Jul.–Aug., 2014); (b) for the low-In treatment, the In treatment concentrations were 0, 0.04, 0.08, 0.1, 0.15, 1, 2 mg L^{-1}, and the exposure time was 40 days (growth period: Nov.–Dec., 2014). Despite the concentrations of Ga and In in wastewater higher than 10 mg L^{-1} were very rare, the concentration ranges of Ga and In used in this study were intended to clarify the toxicity concentrations to rice seedlings such as EC_{10} (effective concentration resulting in 10% growth inhibition) and PT_{10} (threshold concentration of phytotoxicity resulting in 10% growth retardation) values. In order to avoid Ga and In precipitation, the culture solutions were made just before use and renewed every day. We verified that no significant changes in Ga and In concentrations in the culture solutions had occurred at 24 h. Three replicates (pots) for each of the Ga and In treatments. The half-strength modified Kimura B nutrient solution was used in this study, which contains the following compositions: 0.18 mM $(NH_4)_2SO_4$, 0.09 mM KNO_3, 0.27 mM $MgSO_4{\cdot}7H_2O$, 0.09 mM KH_2PO_4, 30.6 μM Fe-citrate, 183 μM $Ca(NO_3)_2{\cdot}4H_20$, 25.1 μM H_3BO_3, 2.01 μM $MnSO_4{\cdot}4H_2O$, 2.02 μM $ZnSO_4{\cdot}7H_2O$, 1.19 μM $CuSO_4{\cdot}5H_2O$ and 0.49 μM MoO_3. The concentrations of micro-elements (B, Mn, Zn, Cu and Mo) in full-strength modified nutrient solution were identical with the half-strength modified Kimura B nutrient solution, and the concentrations of other elements in full-strength modified nutrient solution were two folds higher than half-strength modified Kimura B nutrient solution.

After harvesting, the rice seedlings were separated into root and shoot and rinsed first with tap water and then with deionized water. The biomass and lengths of each root and shoot were measured. In addition, their chlorophyll content was also measured with a chlorophyll meter (SPAD-502, Spectrum Technologies). Briefly, 10 plants per pot were selected and the chlorophyll meter was used to measure three points on the least expanded leaf of each plant (the interval between two points was 5 cm). Iron plaque on the roots was removed using modified cold DCB (dithionite-citrate-bicarbonate) solution (Liu et al., 2004) before performing plant digestion. The procedure of DCB extraction was the same as that described in our previous research (Lee et al., 2013). One gram of fresh roots was extracted for 1 h at ambient temperature (20–25 °C) in a 40 mL solution containing 0.03 M sodium citrate (≥ 99.0%, J.T. Baker) and 0.125 M sodium bicarbonate (≥ 99.7%, J.T. Baker), with the addition of 0.6 g sodium dithionite powder (≥ 82%, Sigma-Aldrich), and then the DCB extracts were discarded. The roots were then washed three times with deionized water, removing the residues of DCB extracts. Afterward, the roots without iron plaque and the shoots were oven dried at 70 °C for 72 h, and then ground to a fine powder and stored in a desiccator cabinet.

2.2. Plant digestion and analysis

The dried root and shoot samples (0.1 g) were digested separately with concentrated HNO_3 (69–70%, J.T. Baker)/H_2O_2 (30%(w/w) in H_2O, Sigma-Aldrich) in heating blocks (Meharg and Rahman,