Introduction

Although iodine is one of the essential elements of animals, it is not essential, but harmful, to plants. “Akagare” disease, a physiological disorder by iodine toxicity, occurs in lowland rice cultivated under flooded condition (Tensho and Yeh, 1970, Watanabe and Tensho, 1970). On the other hand, iodine is an essential microelement for human health. Iodine deficiency disorders are believed to be one of the commonest preventable human health problems (Welch and Graham, 1999, Grusak, 2002, Ramakrishnan, 2002).Therefore, enrichment of iodine in crops is a way to contribute to increasing the intake of iodine for human health. To avoid iodine toxicity in crops and to make iodine-biofortified crops, it is important to study iodine uptake and metabolism in plant depending on its chemical forms (I^-, IO_3^- and I_2).

Generally the forms of iodine found in natural environments are dependent on pH and electrochemical potential (Eh) (Mackowiak *et al*., 2005). I^- should be the most prevalent (Yuita, 1992), while IO_3^- exists under more oxidizing conditions. It is known that plants absorb I^- more readily than IO_3^- so that I^- is more phytotoxic than IO_3^- (Mackowiak and Grossl, 1999; Umaly and Poel, 1971), and IO_3^- is electrochemically reduced to I^- before uptake (Böszörményi and Cseh, 1960; Whitehead, 1975). Also I^- may oxidize to I_2. However, iodine metabolism in higher plants using sensitive analytical techniques to separate I^- and IO_3^- was not conducted. Using the ion chromatography and inductively coupled plasma-mass spectrometry system (IC-ICP-MS) for the determination of I^- and IO_3^-, plant uptake and metabolism of inorganic iodine (I^- and IO_3^-) and the r behavior in the rhizosphere were studied.

Materials and methods

Plant culture

Rice seeds (cv. Koshihikari) were surface sterilized with 70 mL L^{-1} ethanol, and the seeds were germinated and grown for 16 days with tap water. Seedlings were transferred to 3L culture solution containing 4 mM N as NH_4NO_3, 0.5 mM P, 3 mM K, 0.5 mM Ca, 1 mM Mg, 1.5 mM S and standard levels of micronutrients in deionized water. The plants were grown in a growth chamber with 14/10 h photoperiod at 120 μmol photons m^{-2} s^{-1} at 27/25ºC. The media was adjusted to pH 6 and replaced every 3 days.

Experiment 1

After 28 days of growing in culture media, I^- or IO_3^- as sodium salts was added to the media at 100 or 1000 μg L^{-1}, respectively. Plants were harvested and separated into shoot and roots, and they were homogenized with 5 times-fold of deionised water after 24 h. of iodine treatments. Also xylem sap was collected with cutting at the basal shoot. I^-, IO_3^- and total iodine concentration in the extraction of plant tissues, xylem sap and culture media were analyzed after pre-purification with a membrane filter (0.2μm).

Experiment 2

Detached roots (500mg FW) sterilized with 700 mL L^{-1} ethanol for 2 min and rinsed with sterile deionized water were obtained from 22-d old rice seedlings grown in the culture media. They were immersed in 10 mL of autoclaved Tris-HCl buffer (50mM, pH 8.0) containing I^- or IO_3^- (20 μg L^{-1} as I).After 1,6 and 24 h. of immersion in the dark at 20 ºC, I^- and IO_3^- concentration in the buffer solution were analyzed.

Iodine analysis

The ion chromatography-inductively coupled plasma-mass spectrometry system (IC-ICP-MS)

(Yoshida *et al*., 2007) was used for the determination of I^- and IO_3^- in solution samples. Detection limit is 0.1 μg L^{-1} (as I). The analysis of total iodine developed by Muramatsu and Yoshida (1998) was applied. Powdered samples were mixed with V_2O_5 in a small ceramic boat and placed in a quartz combustion tube. The end of the quartz tube was connected with a ball-joint to a trap containing H_2O, with tetramethyl ammonium hydroxide (TMAH), and Na_2SO_3 solution. A wet oxygen flow was passed through the tube during the heating at 1000 ºC for 15 min. Sample solutions containing trapped iodine were adjusted to 10 mL by adding H_2O, and iodine content was determined by ICP-MS. Detection limit of total iodine in dry samples is 1 μg kg $(DW)^{-1}$.

Results and discussion

The most of iodine in rice plants was the fraction of the others that would be composed of water soluble organic iodine compounds and molecular iodine (I_2). I^- was detected 24% of total-I in shoots and 22% in roots by IO_3^- treatment, but IO_3^- was not detected in I^--treated plants. Furthermore, xylem sap of IO_3^--treated plants did not contain IO_3^- and 70% of total-I in xylem sap was I^-, and the others. Similarly in the culture media, 11% of supplied IO_3^- was reduced to I^- for 24 h, but I^- was not changed to IO_3^-. Ratio of total-I concentration in shoot and roots to I^- concentration in the culture media of IO_3^- treatment after 24 h (12 in shoot, 42 in roots) was the same level as that of I^- treatment (14 in shoot, 51 in roots), but the ratio to total-I concentration in culture media of IO_3^- treatment (1.4 in shoot, 4.8 in roots) was quite different from that of I^- treatment (12 in shoot, 43 in roots), implying that total-I concentration in IO_3^--treated plants depended on I^- concentration changing from IO_3^-in the culture media. IO_3^- would be reduced to I^- by roots or in the rhizoshere readily, and rice plants would absorb the reduced I^-. In Experiment 2, pH and Eh of the buffer solution were pH 8.1-7.9 and 140-40 mV, respectively. It was indicated that IO_3^- reduction in rice roots could also be performed under alkaline and oxidized conditions.

It is known that phytoplankton can reduce IO_3^- to I^- (Wang *et al*., 2002, Chance *et al*., 2007) as well as nitrate, sulfate or Fe (III) reducing bacteria (Councell *et al*., 1997, Farrenkopf *et al*., 1997, Tsunogai and Sase, 1969). In addition to results shown here, IO_3^- reduction was confirmed in soybean roots and culture cells derived from them. Not only microorganisms but also higher plants would have the ability of IO_3^- reduction. Mechanisms and meanings of IO_3^- reduction and absorption of the reduced I^-, despite the fact that iodine is not essential for higher plants, remain to be examined.

References

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