Introduction

Root system development is the most important factor in uptake limited resources like nutrient and water from heterogeneous soil. In our previous study, it was found that inosine (20 mg L^{-1}) has positive effects on plant growth, especially on root growth, under both aseptic and non-aseptic conditions (unpublished data). Inosine is a purine nucleotide widely found in plants, animals and other forms of living matter. It is comprised of purine base hypoxanthine and the sugar D-ribose. Boldt and Zrenner (2003) cited that purine nucleotide is one of the essential constituents of cytokinin, which control the plant growth and development. Also, the nucleotides are one of the most important nitrogen compounds in all living organisms. Thus the aim of this work was to clear the mechanism of the inosine-induced enhancement of root growth with emphasis on plant hormone and metabolite profile.

Material and Methods

Plant precultivation- The rice seedlings (*Oryza sativa cv* Nipponbare) were transferred to glass pots with 450 mL of sterilized nutrient solution (changed every 3 days pH 5.4), then covered with a plastic bag resistant at autoclaving. The plastic bag had filter paper to avoid contamination by microorganisms. Plants were grown for 3 weeks (for acclimatization) in a growth chamber with constant temperature (25^{o} C); the photoperiod was16 hours of light and 8 hours of dark. Nutrient solution contained 6 mg L^{-1} N (NH_4NO_3), 0.4 mg L^{-1} P ($NaH_2PO_4 \cdot 2H_2O$), 6 mg L^{-1} K (K_2SO_4), 10 mg L^{-1} Ca ($CaCl_2 \cdot 2H_2O$), 4 mg L^{-1} Mg ($MgSO_4 \cdot 7H_2O$), 0.4 mg L^{-1} Fe ($FeSO_4 \cdot 7H_2O$), 0.1 mg L^{-1} Mn ($MnSO_4 \cdot 5H_2O$), 0.1 mg L^{-1} B (H_3BO_3), 0.04 mg L^{-1} Zn ($ZnSO_4 \cdot 7H_2O$), 0.002 mg L^{-1} Cu ($CuSO_4 \cdot 5H_2O$), 0.001 mg L^{-1} Mo ($(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$).

Treatment for plant hormone analysis - After the precultivation, seedlings were transferred to pots with the follows treatment solutions (0 N + 0 inosine, 0 N + 20 mg L^{-1} inosine, 4 mg L^{-1} N + 0 inosine and 4 mg L^{-1} N + 20 mg L^{-1} inosine). Two weeks after the treatment started, xylem sap was collected by sterilized cotton attached for 12 hrs in cut stem. The ELISA method was used to determine cytokinin and auxin concentration in the xylem sap. Fresh and dry weight of shoot and root, and root length were determined. Total nitrogen and NO_3 concentration were determined, by Kjeldahl method and capillary ion analyzer, respectively.

Treatment for GC-MS analysis- After precultivation, inosine (20 mg L^{-1}) was applied in the same nutrient solution. They were sampled at 0, 1, 2, 24, 72 and 96 hours after the treatment started. Dry weight (shoot and root), and root length and number of lateral root were determined. The samples were lyophilized at -80^{o}C, and milled.

The extraction and derivatization were done before the GC-MS analysis. The metabolite

analysis was carried out according to the method of Okazaki et al. 2008. After extraction and derivatization, a 1 ml aliquot of the sample was injected into a gas chromatograph (Agilent GC 6890) in the splitless mode. Gas chromatography was performed on an Rtx-5Sil MS with an integrated guard column (30 m, 0.25 mm film; Restek GmbH, Bad Homburg, Germany). Metabolites were identified by mass spectral and retention index using AMDIS software (http://chemdata.nist.gov/mass-spc/amdis/). Identified metabolites were quantified using Quant software (JEOL, Tokyo, Japan). Before statistical analysis, the data were normalized using the peak area of ribitol.

Results and Discussion

In the absence of N nutrition the application of inosine did not affect root growth and N concentration significantly (Table 1). In contrast, in the presence of 4 mg L^{-1} of inorganic N, the application of inosine induced significant differences in shoot dry weight, root length, trans-zeatin riboside (t-ZR) and indole-3-acetic acid (IAA) contents (Table 1). It knew that this endogenous plant hormone is capable to promote growth root and shoot. The most abundant cytokinin found in xylem sap is zeatin riboside, which is mostly synthesized in root apical meristems (Taiz and Zeiger, 2006).

Inosine application improves the root length significantly 72 hrs after the application (Figure 1a). When comparing the metabolite profile during the treatment, it was found that allantoin concentration was significantly increased by the inosine application (Figure 1b).

Table 1. Shoot and root dry weight, root/shoot rate, root length, total nitrogen, nitrate and trans-zeatin riboside (t-ZR) and indole-3-acetic acid (IAA) results from rice plant after growing 2 weeks in fallow treatments.

Treatment		Dry weight		Root/ Shoot	Root length	Total Nitrogen		Nitrate	t-ZR	IAA
Inorganic N mg kg^{-1}	Inosine mg kg^{-1}	Shoot ……mg……	Root		…cm…	Shoot ……mg g^{-1}	Root DW……	mg kg^{-1} Shoot DW	pmol mL^{-1} xylem ……. sap …….	
0	0	121 **a**	47 **a**	0.34 **ab**	299 **a**	6.9 **a**	4.7 **a**	4.0 **b**	0.57 **ab**	58.0 **a**
0	20	136 **ab**	48 **a**	0.39 **bc**	404 **ab**	9.1 **a**	5.3 **a**	5.3 **b**	0.57 **ab**	44.5 **a**
4	0	171 **b**	55 **ab**	0.32 **a**	405 **ab**	14.9 **bc**	8.8 **b**	9.5 **c**	0.56 **a**	49.8 **a**
4	20	210 **c**	61 **bc**	0.29 **a**	593 **cd**	16.4 **c**	9.6 **b**	11.4 **c**	0.70 **bc**	90.5 **b**

Within each column, values followed by different letters are significantly different based on Tukey test ($P<0.05$).

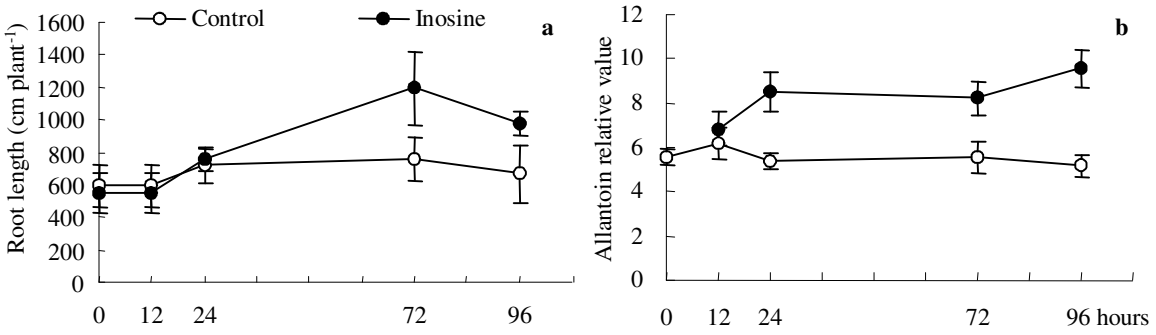

Figure 1. Root length (a) and allantoin relative value (b) from GC-MS. Relative values of abundance were obtained in comparison to ribitol as internal standard. Bar means ±SE.

Thus, it is suggested that inosine application increased concentration of several plant hormones and allantoin, possibly resulting in growth enhancement of plant root. In fact, it has been reported that exogenous allantoin (4 mM) is capable to improve the root length in soybean embryonic axis segments (Bulbul et al. 2008).

Reference

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