Title
Metabolite profiling of rice root exudate under phosphorus deficiency

Authors
Tawaraya, Keitaro
Horie, Ryota
Shinano, Takuro
et al.

Publication Date
2009-06-18

Peer reviewed

Introduction

Plants are thought to produce about 200,000 natural products (Dixon and Strack 2003). These products are transferred to leaf, stem, seeds and roots. Plant roots exude metabolites as much as 10%, or even more, of photosynthetically fixed carbon into the soil (Werner 1992). Root exudates contain sugars, amino acids, organic acids, flavonoids, enzymes, and nucleotides (Rovira 1969).

A large fraction of soil phosphate (P) is bound in a form which is unavailable for absorption by plant roots. Plants which cannot convert unavailable forms of P into available forms become P deficient and thus their growth is limited. Some leguminous plant species which exude organic acids under P deficiency can use unavailable forms of P. However, the mechanisms of root exudation and the effects of most root exudates in the rhizosphere are still unknown.

Exhaustive analysis of root exudates has been limited in the past due to analytical difficulties in simultaneous determination of various metabolites. Metabolomics is unbiased identification and quantification of all metabolites in a biological material. Capillary electrophoresis / time-of-flight mass spectrometer (CE-TOF MS) is a useful analytical instrument in separation and detection of a wide range of ionic metabolites such as amino acids, organic acids, sugar phosphate, and nucleotides.

The purpose of the present study was to develop a procedure to collect root exudates for metabolite profiling using CE-TOF MS and to clarify the composition of rice root exudates under P deficiency.

Materials and methods

Seeds of rice (*Oryza sativa* cv. Nipponbare) were sown in perlite and grown for 10 days. Twenty-one ten-day old seedlings were transplanted to 1/5000 a Wagner pot with a nutrient solution. Rice plants were grown in a glasshouse for 15 days. The culture solution contained mineral nutrients according to Wagatsuma et al. (1988): 500 µM NH_4NO_3, 323 µM $NaNO_3$, 1.54 mM K_2SO_4, 2 mM $CaCl_2$, 1.67 mM $MgSO_4$, 36 µM $FeSO_4$, 18 µM $MnSO_4$, 0.1 µM Cu $CuSO_4$, 0.05 µM $(NH_4)_6Mo_7O_{24}$, 36 µM H_3BO_3, 3 µM $ZnCl_2$. P concentrations of the solution were adjusted to 0 (P0), 3 (P0.1), 32 (P1) or 258 (P8) µM with NaH_2PO_4. Each P treatment was replicated five times.

Rice plants were separated from urethane foam and were rinsed with sterile deionized water several times. Fifty-one plants were bound with urethane foam and were placed at top of a paper cup containing 100 mL of sterile deionized water. Cups were aerated and placed in a dark room for 12 hours. The root exudate solution was filtered with a filter paper (No. 6 Advantec Toyo, Tokyo). Fifty mL of the solution was transferred to polyethylene tube and stored at -20˚C. Frozen root exudates were freeze-dried. After collection of root exudates, the shoots and roots were washed with deionized water. Subsamples of shoots and roots were prepared and their fresh weight was determined. Subsamples were frozen immediately at –20˚C or dried at 70˚C for 3 days, respectively. Shoot dry weight of subsamples was determined. Pulverized shoot sub-samples were digested in a HNO_3-$HClO_4$-H_2SO_4 solution. The P content in the digested solution was determined colorimetricallly by the vanadomolybate-yellow assay.

Two-hundred mg of frozen shoots and roots were homogenized with Zirconia beads using a Mixer Mill at 27 Hz for 3 min. Six hundred µL of methanol including 8 µM internal standard, Met sulfone and camphor-10-sulfonate those were used for compensation of the peak area after CE-MS analysis, was added to homogenized plant materials, and again the mixture was homogenized at 27 Hz for 1 min. The sample solution was then centrifuged at 20,400g for 3 min at 4°C. The supernatant was transferred to a tube and five hundred µL of chloroform and 200 µL of Milli-Q

water were added. This mixture was vortexed for 3 min and centrifuged at 20,400g for 3 min at 4˚C. Six hundred µL of methanol, 600 µL of chloroform and 200 µL of Milli-Q water were added to the freeze-dried root exudates. The upper layer of each solution was transferred to a 1.5 mL tube and evaporated for 30 min by a centrifugal concentrator and then separated into two layers. The upper layer was centrifugally filtered through a Millipore 5-kD cutoff filter at 9,100g at 4˚C. The filtrate was dried by a centrifugal concentrator. The residue was dissolved into 20 µL of Milli-Q water containing a reference compound (3-aminopyrrolidine and trimesate). The CE-MS system and conditions were as described by Watanabe et al. (2008).

Results

Shoot dry weights and shoot P concentrations of rice plants grown in solution culture at the P0 and P0.1 level were significantly lower than those at the P1 and P8 levels (Table 1). P concentrations in shoot tissues below 1.0 mg P g^{-1} are considered to be deficient whereas concentrations higher than 6.0 g kg^{-1} are sufficient for normal growth (Reuter et al. 1997). Thus, root exudates were collected from phosphorus-deficient and phosphorus-sufficient twenty-five-day-old rice plants.

Fifty-one cationic compounds and 29 anionic compounds were identified in root extracts (Table 2 and Table 3). Thirteen compounds out of 80 compounds showed higher concentration at P0 level, while 17 out of 80 compounds showed lower concentration at P0 level. Thirty-two cationic compounds and fifteen anionic compounds were identified in the root extracts. Thirty-nine out of 47 compounds showed higher concentration at P0 level. Only one out of 47 compounds showed lower concentration at P0 level. More than 83% of the metabolites in the root exudates showed higher concentration in P deficient compared to P sufficient rice. On the other hand, 16% of metabolites in the root extract showed higher concentration in P deficient compared to P sufficient rice. These results suggest that rice roots exude many metabolites in response to P deficiency.

The 39 compounds which had elevated concentration in P deficient rice included organic acids and amino acids. Some organic acids which can solubilize insoluble P in the soil are known to be exuded from the roots of P deficient leguminous plants. Exuded organic acids identified in P deficient rice may in the same way contribute to the solubilization of insoluble P in the soil.

Table 1 Dry weight and phosphorus concentration of rice plants grown in solution culture at 0 (P0), 3 (P0.1), 32 (P1) or 258 (P8) µM P . Means followed by the same letter are not significanly different (P<0.05) by Tukey test.

P level	Dry weight (mg/plant) Shoot		Shoot P (mg P/g)	
P0	39	a	0.93	a
P0.1	40	a	1.02	a
P1	54	b	2.45	b
P8	55	b	6.27	c

Table2. Ratio (P0 toP8) of cationic metabolite in root extract and root exudate of rice grown in solution culture at 0 (P0) and 258 (P8) μM P.

Metabolite	Ratio (P0/P8)	
	Root extract	Root exudate
1,4-Butanediamine	1.57	N.D.
ACC	0.70	N.D.
Acetylserine	0.11***	N.D.
Adenine	0.57	N.D.
Adenosine	1.41	2.30
Agmatine	1.52	N.D.
ß-Alanine	1.06	N.D.
Betaine	2.08	1.26
Carnosine	0.40	2.39
Choline^{+}	0.61*	3.47
Citrulline	1.09	2.10
Creatine	N.D.(P8)	0.98
Cytidine	0.69	N.D.(P8)
Cytosine	0.58**	N.D.
Deoxyadenosine	3.47	N.D.
Deoxycytidine	1.49	N.D.
Deoxyguanosine	1.95*	N.D.
D-Glycosamine	N.D.(P8)	N.D.
GABA	0.70	3.17
Glycine	0.78	3.03
Guanine	0.47**	1.23
Guanosine	0.98	N.D.(P8)
Hydroxyproline	0.78	2.09
Hypoxanthine	0.62	N.D.
L-Alanine	0.56**	3.45
L-Arginine	0.32**	12.02*
L-Asparagine	0.85	1.62
L-Aspartic acid	0.79	4.86*
L-Glutamic acid	0.69	4.14
L-Glutamine	0.54*	4.47
L-Histidine	0.65	3.35
L-Isoleucine	0.87	2.61
L-Leucine	0.82	2.49
L-Lysine	N.D.(P8)	2.65
L-Methionine	0.76	2.27
L-Phenylalanine	0.42	3.68
L-Proline	0.43**	3.27
L-Serine	0.63**	3.11
L-Threonine	0.60*	3.89
L-Tryptophan	1.21	1.74
L-Tyrosine	0.56**	2.13
L-Valine	0.68	5.14*
N-α-Acetylornithine	0.16*	N.D.
Nicotinic acid	0.75*	N.D.(P8)
Ornithine	0.96	2.83
Pyridoxal	0.85	N.D.
Pyridoxine	0.48*	N.D.
SAM	0.60	N.D.
Spermidine	0.71	N.D.
Tryptamine	4.44	N.D.
Tyramine	0.92	N.D.

N.D.: not detected, N.D.(P8); not detected at P8

* and ** indicate significant difference at the P=0.05 and P=0.01 level, respectively.

Table 3. Ratio (P0 toP8) of anionic metabolite in root extract and root exudate of rice grown in solution culture at 0 (P0) and 258 (P8) μM P.

Metabolite	Ratio (P0/P8)	
	Root extract	Root exudate
2,6-Diaminopimelate	2.16**	N.D.
2-Hydroxyisobutyrate	1.47*	2.33
Abscisate	0.00	N.D.
α-Ketoglutarate	0.82	N.D.
Butyrate	3.64*	N.D.
cis-Aconitate	0.77*	N.D.
Citramalic acid	2.01*	2.03
Citrate	1.54	1.21
CMP	0.00	N.D.
cyclicCMP	0.22*	N.D.
D-Galacturonate	0.85	N.D.
D-Glucuronate	0.80	N.D.
Fumarate	1.01	2.40
Gluconate	0.80	N.D.
Glutarate	1.75*	2.16
Glycerate	0.55	N.D.(P8)
Homogentisate	N.D.(P8)	N.D.
Isocitrate	0.99	N.D.(P8)
Lactate	2.72	5.20
Malate	0.93	3.22
Malonatc	1.07	3.70*
Mevalonolactone	N.D.	0.45*
N-Acetyl-Glu	0.99	N.D.(P8)
Pantothenate	1.14	N.D.
p-Coumarate	1.09	N.D.
Pyruvate	1.21	2.98
Quinate	1.16	1.18
Shikimate	1.37	N.D.
Succinate	0.83	2.57

N.D.: not detected, N.D.(P8); not detected at P8

* and ** indicate significant difference at the P=0.05 and P=0.01 level, respectively.

References

Dixon RA, Strack D. Phytochemistry meets genome analysis, and beyond. Phytochemistry 2003; 62: 815-816.

Reuter DJ, Edwards DG, Wilhelm NS. Temperate and tropical crops. In: Reuter DJ, Robinson JB (eds) Plant analysis: an interpretation manual, CSIRO Publishing, Collingwood. 1997; 81-284.

Rovira AD. Plant root exudates. Botanical Review 1969; 35, 35-59.

Watanabe M, Kusano M, Oikawa A, Fukushima A, Noji M, Saito K. Physiological roles of the beta-substituted alanine synthase gene family in arabidopsis. Plant Physiology 2008; 146, 310-320.

Wagatsuma T, Kawashima T, Tawaraya K. Comparative stainability of plant root cells with basic dye (methylene blue) in association with aluminum tolerance. Commun. Soil Sci. Plant Anal. 1988;19, 1207-1215.

Werner D. 1992: Symbiosis of Plants and Microbes, Chapman & Hall, Cambridge.