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Effect of Phosphorus Nutrition on the Exudation of Organic Carbon and Amino Acids from Root

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Summary

This study identified the effect of the phosphorus status of plants on the exudation of organic carbon and amino acids from root of six plant species (*Zea mays* L., *Hordeum vulgare* L., *Glycine max* Merr., *Vigna angularis* L., *Pisum sativum* L. and *Beta vulgaris* L.) at four phosphorus levels (0(P0), 0.4(P1), 2 (P2) and 8(P3) mgP/liter). Plants were grown in water culture in glasshouse and compounds released by 0.5 mM $CaSO_4$ from root were collected. Low phosphorus tolerance appeared in the following orders: pea > soybean > adzuki bean > maize > barley > sugar beer. Sugar beet exuded more organic carbon and amino acid than other plant species. In all plant species except soybean the amount of amimo acids in root exudates were highest at PO level. The amount of amino acids in root exrdates decreased with an increase of phosphorus concentration in nutrient solution. Concentrations of serine, glycine and alanine were higher in root exudates of every plant species. Low phosphorus tolerance of plants were correlated with the rate of increase in root exudation of amino acids in P-deficient plant.

INTRODUCTION

Plant root releases organic and inorganic compounds into rhizosphere soil. These compounds are released as secretion, leakage from root cells and formation of mucilage. However it is difficult to determine these processes separately. Therefore root exudates include all substances that are released as secreteon, leakage and mucilate. These compounds are used for the growth of rhizosphere microorganisms and stimulate nutrients uptake by increasing their availability. The amount and composition of the root exudates vary between species and age of plant.

Root exudates influence nutrient availability and microbial activity in rhizosphere soil. They may influence nutrient solubility and uptake indirectly through their effects on microbial activity, physical properties of rhizosphere and root growth patterns, and directly by acidification, chelation, precipitation and oxidation-reduction reactions[9]. Environmental factors such as soil fertility, light and temperature affect root exudation. When a plant is under some nutrient deficiency, its root exudes some compounds to correct the deficiency. Iron-deficient grasses release nonproteinogenic amino acids[8]. It is also known that pigeon pea releases piscidic acid to increase available phosphors[1]. Water-deficit stressed plants tended to exude more soluble organic carbon[7].

It was reported that exudation of amino acids from roots increased in P-deficient plants[2]. It was also shown that citrate exudation from roots of P-stressed alfalfa was higher than from that of non-stressed plants[3]. Mechanisms of acceleration of root exudation in P-deficient plant are not known. When plants were grown in soil or in a simulated soil-like substrate, roots had to be damaged by mechanical forces. There are no useful methods to collect root exudate of plants grown in soil. Therefore we grew several plant species in water culture and collected root exudate using the method of Schwab et al[6].

Orgnic compounds exudated from root can affect the

growth of rhizosphere microorganisms. Few information is available on amount of organic compounds exuded from root under phosphorus stress. The objective of this research was to determine the effect of P status of plants on root exudation of various plant species.

MATERIALS AND METHODS

Plant growth. The six plant species used were : maize (*Zea mays* L. cv. Pioneer 3352), barley (*Hordeum vulgare* L. cv. Benkei), soybean (*Glycine max* Merr. cv. Sudzuyutaka), adzuki bean (*Vigna angularis* Wight cv. Takara), pea (*Pisum sativum* cv. Kinuzaya) and sugar beet (*Beta vulgaris* L. cv. Monohikari). The seeds were germinated in tap water. Ten days after sowing, the seedlings were trasnplanted to 60 liters of plastic containers (22x40x68cm) containing 54 liters of nutrient solution in glasshouse. Twenty-four seedlings were grown in each container. The nutrient solution at the time of transplanting contained the following concentrations of mineral nutrients (mg per liter) : 40mgN (NH_4NO_3), 20mgN ($NaNO_3$), 60mgK (K_2SO_4), 80mgCa ($CaCl_2$), 40mgMg ($MgSO_4$), 2mgFe ($FeSO_4$), 1mgMn ($MnSO_4$), 0.01mgCu ($CuSO_4$), 0.005mgMo ($(NH_4)_6Mo_7O_{24}$), 0.4mgB (H_3BO_3), 0.2mgZn ($ZnCl_2$). The pH of the solution was adjusted to 5.0 with diluted H_2SO_4 and NaOH. P concentration in the solution was adjusted to 0, 0.4, 2, 8mg P/liter with NaH_2PO_4. All solutions were aerated continuously and were replaced weekly. Maize and pea were grown for 21 days from transplanting, soybean and adzuki bean for 30 days and barley and sugar beet for 34 days in glasshouse. Each treatment was replicated twice.

Collection and analysis of root exudates. Root exudate were collected by the modified method of Schwab et al[6]. $CaSO_4$ (0.5mM) solution was used instead of $CaCl_2$ because the Cl^- ions interfered with the titration in measurement of organic carbon. Roots from each treatment were submerged in 500 ml of an aerated aqueous solution of 0.5 mM $CaSO_4$ containing 0.05 mg/ml rifampicin and 0.025 mg/ml tetracycline to reduce the bacterial contamination of exudates. After 2 hours in the antibiotic solution, roots were rinsed with sterile distilled water containing 0.5 mM $CaSO_4$ for 12 hours, the contents were evaporated to dryness under reduced pressure at 45℃ on a rotary evaporator, and resuspended in 10 ml of distilled water. Samples were stored at − 20℃ until analysis.

The total amount of carbon in root exudate was determined by dichromatic titration[4]. Samples for amino acids analysis were passed through a 10-ml bed volume column of cation exchange resin (Amberlite IR-120B, H^+ form). Neutral and acidic compounds were washed from the resin with 200 ml of 2.0 M HCl. The neutral-acidic mixtures were passed through a 10-ml bed volume column of anion exchange resin (Ambirlite IRA-410, $HCOO^-$ form). Neutral compounds were washed from the resin with 80 ml of 6.0 M formic acid. Each neutral fraction was resuspended in distilled water, each basic fraction in a 0.2 M Na-citrate buffer (pH 2.2), and each acidic fraction in 0.05 M H_2SO_4. Amino acids in the basic fraction were separated and quantified on an amino acid analyzer (ATTO Co. Ltd. Tokyo).

Analysis of plant material. Shoot dry weight was determined after drying at 70℃ for 48 hours. Dry matter was ground and digested with a HNO_3-$HClO_4$-H_2SO_4 mixture. The content of phosphorus in the digested solution was determined colorimetrically using vanado molybdate yellow assay.

RESULTS

Plant growth Table 1 shows the growth of shoots and roots of the six plant species in each P treatment. At the lowest concentration (0mgP/liter), the shoot dry weights of all plant species were lowest. Increasing the phosphorus concentration of nutrient solution to 0.4 mgP/liter caused a marked increase in dry weight. Soybean and adzuki bean showed maximum shoot dry weight at P1 level. Raising the phosphorus concentration to P2 level produced further increase in the shoot dry weight of maize, barley, sugar beet and pea. Maize and barley showed maximum shoot growth at P2 level. Increasing the phosphorus concentration to P3 level increased the shoot dry weight of pea and sugar beet and decreased the dry weight of maize and barley. The dry weights of roots in every plant species showed similar results (Table 1). Maize, soybean and adzuki bean produced maximum root growth at P1 level. Pea, barley and sugar-

Table 1. Growth and P uptake of each plant species

Plant	Plevel mg/liter	Dry weight (g/plant) shoot	root	total	P content (%)
Maize	0	0.815±0.045*	0.590±0.030	1.405±0.075	0.10±0.00
	0.4	3.755±0.245	1.345±0.135	5.100±0.380	0.30±0.02
	2	3.890±0.280	1.235±0.065	5.125±0.345	0.68±0.00
	8	3.070±0.340	0.985±0.185	4.055±0.525	0.83±0.02
Barley	0	0.229±0.026	0.163±0.011	0.392±0.037	0.06±0.00
	0.4	0.840±0.131	0.315±0.036	1.155±0.167	0.49±0.03
	2	1.644±0.100	0.401±0.003	2.044±0.103	0.55±0.00
	8	1.315±0.138	0.303±0.038	1.618±0.175	0.38±0.03
Sugar beet	0	0.034±0.007	0.023±0.002	0.056±0.009	0.05±0.00
	0.4	0.415±0.034	0.153±0.030	0.568±0.064	0.37±0.01
	2	1.114±0.227	0.472±0.126	1.586±0.354	0.39±0.01
	8	1.115±0.072	0.467±0.106	1.582±0.178	0.49±0.02
Soybean	0	0.905±0.005	0.575±0.015	1.480±0.020	0.08±0.00
	0.4	2.325±0.445	0.710±0.180	3.035±0.625	0.28±0.02
	2	2.115±0.075	0.605±0.005	2.720±0.070	0.55±0.00
	8	1.880±0.190	0.510±0.050	2.390±0.240	0.71±0.04
Adzuki bean	0	0.565±0.025	0.265±0.045	0.830±0.070	0.07±0.01
	0.4	2.250±0.240	0.665±0.075	2.915±0.315	0.32±0.03
	2	2.115±0.715	0.540±0.210	2.655±0.925	0.62±0.04
	8	1.880±0.480	0.470±0.110	2.350±0.590	0.80±0.01
Pea	0	0.660±0.130	0.195±0.025	0.855±0.155	0.06±0.01
	0.4	0.740±0.020	0.210±0.050	0.950±0.070	0.19±0.05
	2	0.985±0.105	0.275±0.025	1.260±0.130	0.27±0.02
	8	1.160±0.010	0.215±0.015	1.375±0.005	0.55±0.01

* : standard error of mean

beet produced maximum root growth at P2 level. The increase of total dry weights was also similar to that of shoot dry weight (Table 1).

Phosphorus uptake. Phosphorus content of shoot at PO level was less than 0.1% in all plant species (Table 1). Increasing the phosphorus concentration of the nutrient solution to 0.4mgP/liter caused an increase in the phosphorus content of shoot (percentage shoot dry weight) in all plant species. All plant species except barley exhibited continuous increase in phosphorus content of the shoot to P3 level.

Organic carbon contents in root exudate. Organic carbon contents in root exudates were differed greatly among six plant species (Fig. 1). Sugar beet exuded much organic carbon. Especially at PO level organic carbon content in root exudate was 92 mgC/g dry root/12 hours. Maize exuded less than 1 mg organic carbon at PO level. Barley, adzuki bean and sugarbeet exuded the most organic carbon at PO level. Increasing the phosphorus concentration of the nutrient solution from 0.4 mgP/liter to 8 mg caused a decrease in the exudation of organic carbon in maize, barley, sugar beet and adzuki bean. The exudation of organic carbon from soybean root increased at P3 level. The exudation of organic carbon from maize and pea at P0 level was lower than those at P1 level.

Amino acids contents in root exudate. The exudation of amino acids showed almost same pattern in six plant species (Fig. 2). Sugarbeet exuded the most amino acids among six plant species. Amino acid content in the root exudate at P0 level was highest in all plant species ex-

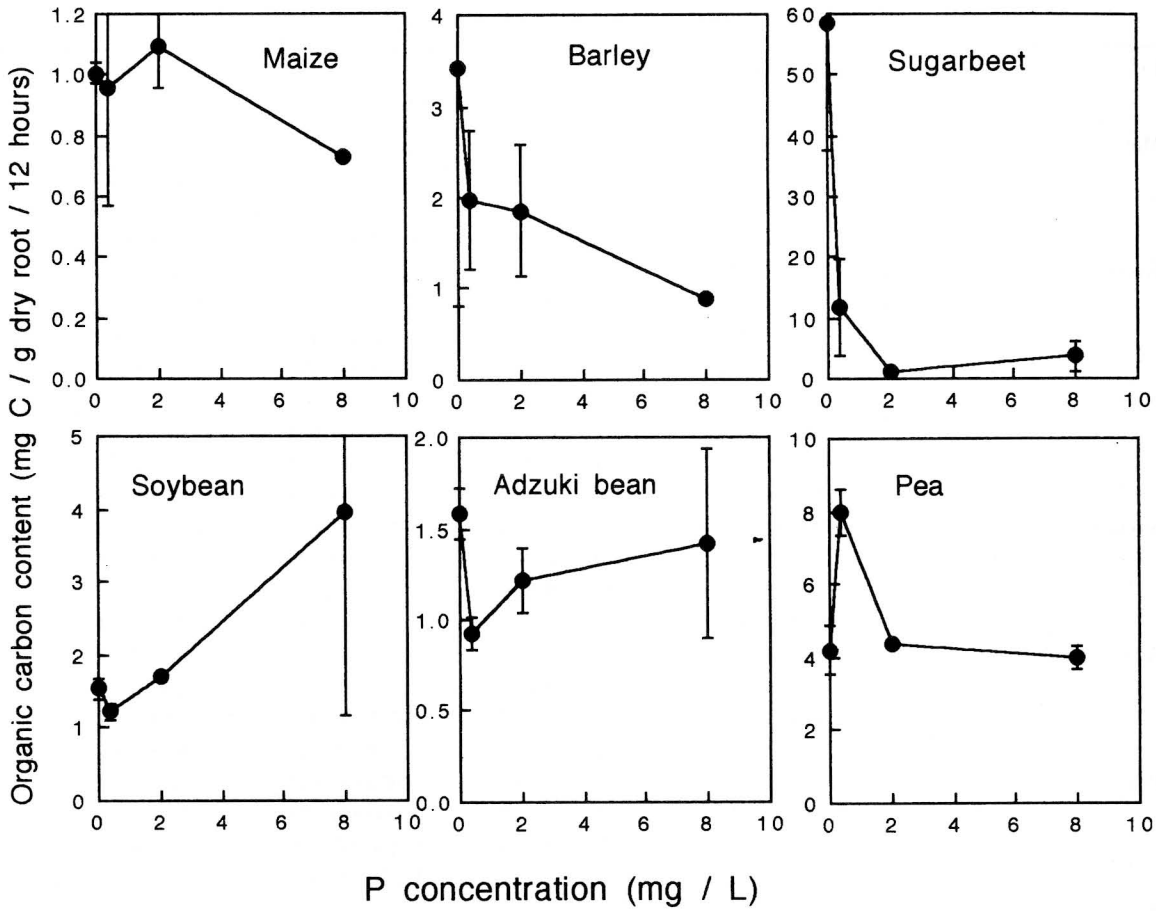

Fig. 1. Organic carbon content in root exudates.

cept soybean. Increasing the phosphorus concentration of nutrient solution decreased the exudation of amino acid in all plant species except soybean. Soybean exuded more amino acid at P3 level than at P2 level.

Concentrations of serine, glycine alanine and asparagine in the root exudate were higher in every plant species (Fig, 3a, 3b) . The concentrations of valine, isoleusine, leusine and histidine at P2 level were higher than those at P0 level in maize and barley. Increasing the phosphorus concentration of nutrient solution to 2 mgP/liter caused a decrease in concentration of amino acids except proline, cysteine, methionine, tyrosine in root exudate of sugar beet. The concentrations of amino acids except glutamic acid, proline, cysteine, valine, methionine, phenylalanine at P0 level in soybean were higher than those at P2 level. The concentrations of asparagine, glycine, valine, isoleucine, leucine and lysine in root exudate of pea were highest at P0 level and decreased with increase in phosphorus. The concentrations of glutamic acid was highest at P0 level in root exudate of adzuki bean.

DISCUSSION

Phosphorus deficiency increased root exudation in every plant species. Every plant species exhibited phosphorus deficiency at P0 level (Table 1). Organic carbon contentin in root exudates was higher in P-deficient plant than P-sufficient plant. Amino acid content in the root exudates was also higher in P-deficient plant than P-sufficient plant. It was reported that decreasing con-

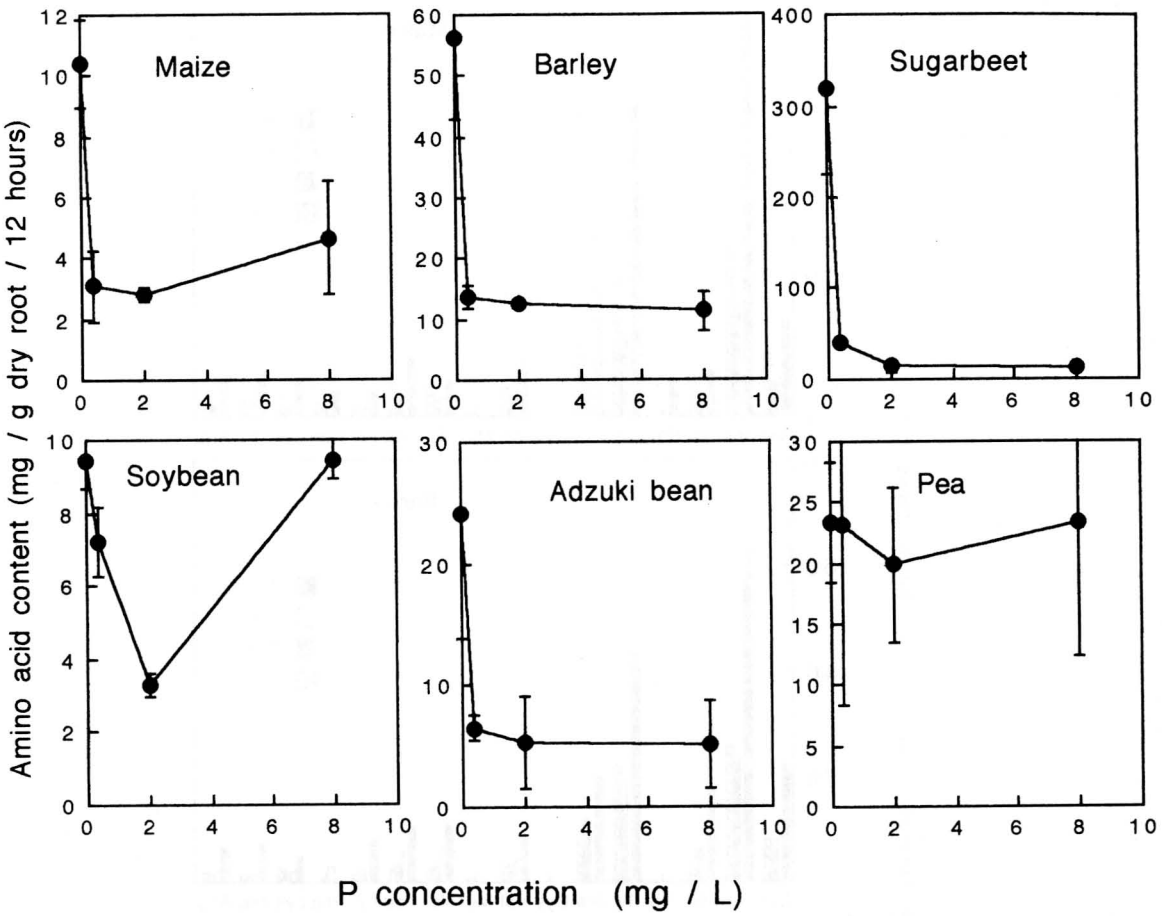

Fig. 2. Amino acid content in root exudate.

centrations of P in plant tissue increased the rate of exudation of soluble amino acid and reducing sugars from the roots of pine[2] and sorghum[6]. The amount of exudation was correlated with a P-induced decrease in phospholipid which could affect the permeability properties of the root plasmamembrane of sour orange and sudangrass[5]. In this experiment we did not determine the phospholipid content in the roots. The phospholipid content in the root of every plant species might be lower if they are grown at P0 level. Though we could not differentiate the secretion and leakage, a greater part of exudates in P-deficient plant was released as leakage. The differences in amino acid composition of the root exudates could be the result of metabolism in the root cell and membrane permeability of each amino acid. Activity of enzyme that is involved in amino acid metabolism could be changed with phosphorus stress. It has not been known what enzyme control the root exudation of each metabolite.

Low phosphorus tolerance of plant varied among plant species. Calculating from the relative shoog dry weight (0ppmP/2ppmP), the low phosphorus tolerance of the plants was in the following order; pea＞soybean＞adzuki bean＞maize＞barley＞sugarbeet. Increase rates of organic carbon and amino acid in P-deficient plant were different among plant species. Result of organic carbon content in the root exudates showed greater variation than that of amino acid. Different pattern of organic carbon exrdation in soybean could be due to that variation. We therefore discussed the relationship between the root

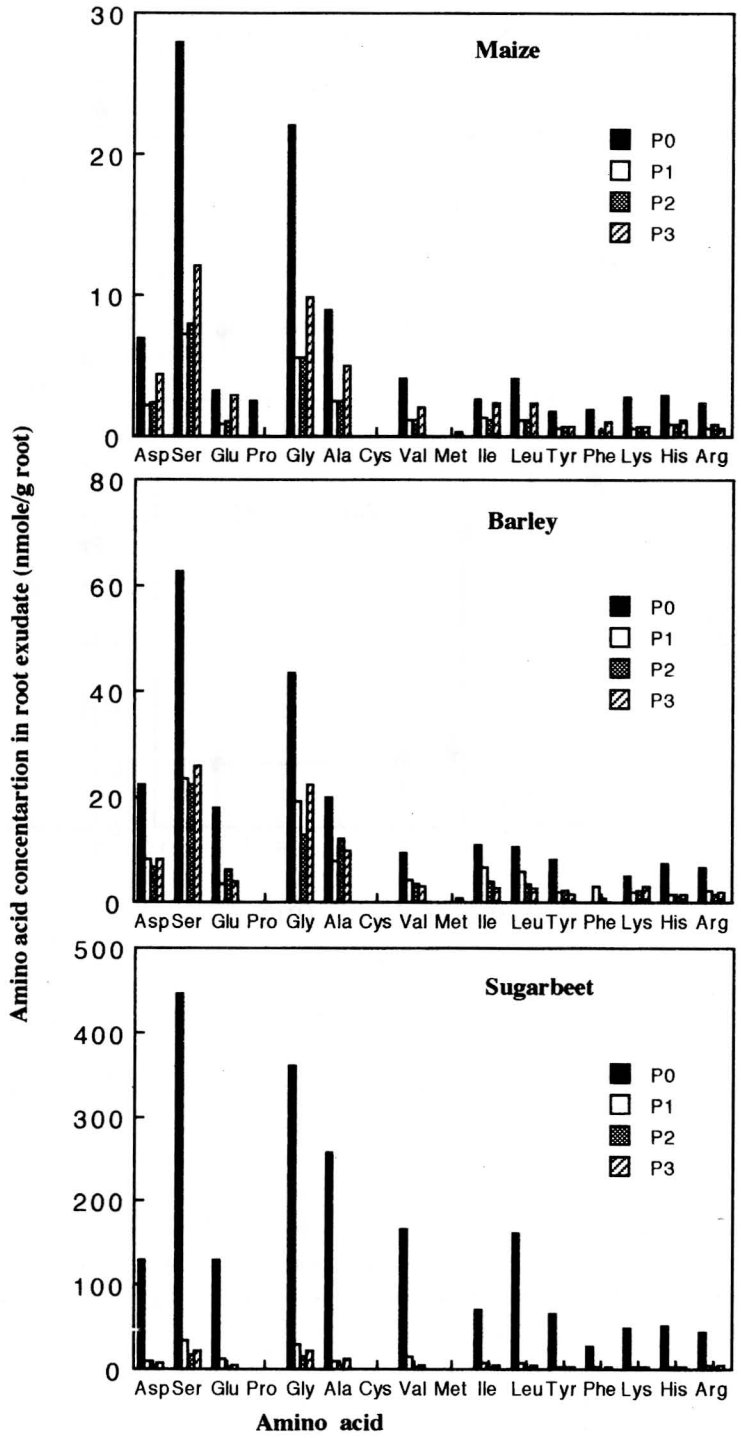

Fig. 3a. Amino acid concentration in root exudate of maize, barley and sugarbeet.

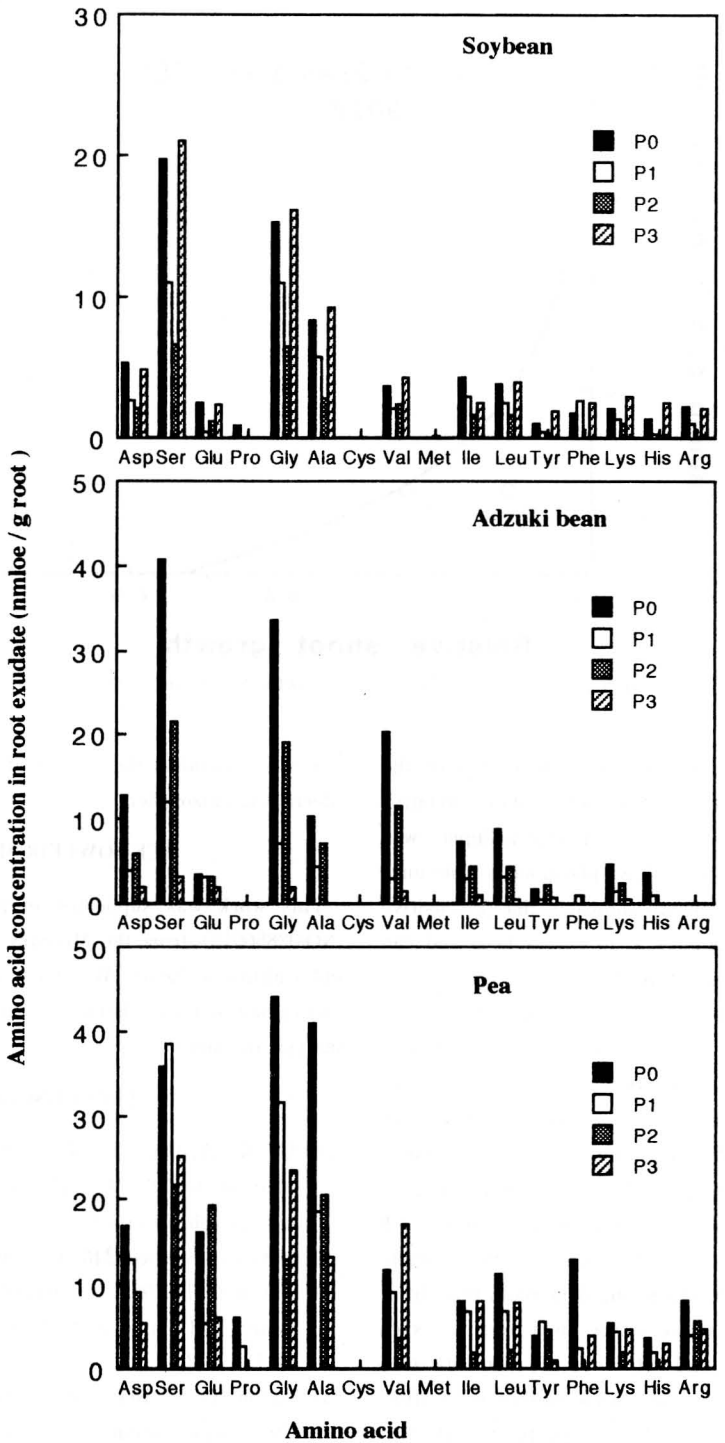

Fig. 3b. Amino acid concentration in root exudate of soybean, adzuki bean and pea.

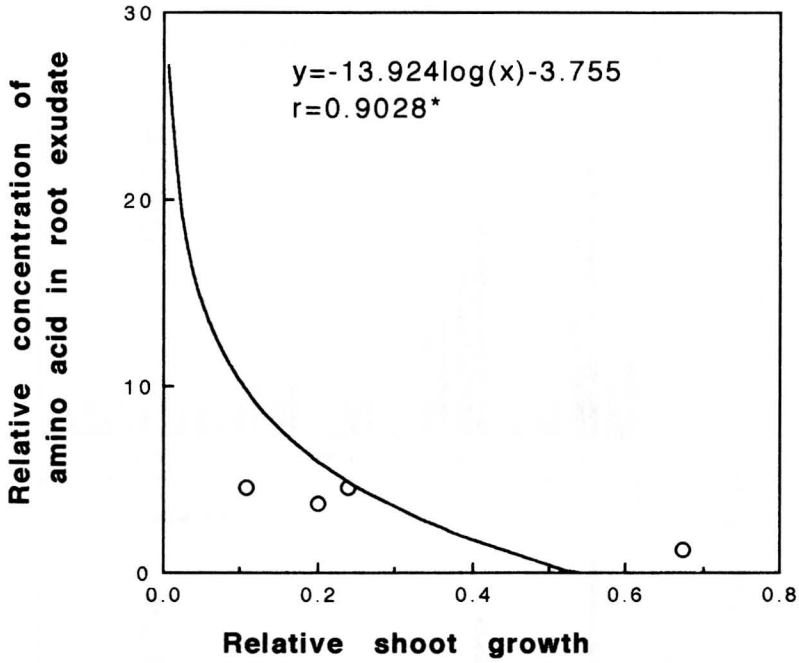

Fig. 4. Relationship between relative shoot growth and relative exudation of amino acid.

exudation and the low phosphorus tolerance with the data of amino acid content in the root exudates. Relative amount of exudation of amino acid (0ppm/2ppm) was negatively correlated with low phosphorus tolerance (Figure 4). This relationship indicates that P-sensitive plants release more amino acid to rhizosphere and can not maintain metabolites within the root.

Mechanisms of low phosphorus tolerance of plants is not known. The relationship between low phosphorus tolerance and root exudation shows that plasmamembrane permeability of root plays an important role in the low phosphorus tolerance. Plant root that is sensitive to low phosphorus could not synthesis phospholipid under phosphorus stress condition and leaked much metabolite that assimilated in the shoot. It is necessary to compare the content of phospholipid in roots that differ in low phosphorus tolerance. Other plant nutrients such as calcium, magnesium and potassium are also related to the function of root plasmamembrane so they must affect root exudation. It is necessary to clarify the effect of these nutrients status on the root exudation. The elucidation of the effect of nutrient status of plants on root exudation is useful in the control of many kinds of fungal infection in rhizosphere.

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植物のリン栄養状態が根からの有機炭素とアミノ酸の浸出におよぼす影響

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摘　　要

トウモロコシ，オオムギ，ダイズ，アズキ，エンドウおよびテンサイの6植物種を4段階のリン濃度(0(P0), 0.4(P1), 2.0(P2), 8.0(P3) mg/liter)に生育させ，植物のリン栄養状態が根からの有機炭素とアミノ酸の浸出におよぼす影響について検討した．地上部の生育からみた低リン耐性はエンドウ＞ダイズ＞アズキ＞トウモロコシ＞オオムギ＞テンサイの順であった．トウモロコシ，オオムギ，テンサイ，アズキの浸出物中の有機物中の有機炭素濃度とダイズ以外の植物の浸出物中のアミノ酸濃度P0区でもっとも高く，培養液のリン濃度の上昇に伴い低下した．いずれの植物も浸出物中のセリン，グリシン，アラニン濃度が高かった．低リン耐性は浸出量と関係があった．