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ORIGINAL ARTICLE

Effect of low phosphorus and iron-deficient conditions on phytosiderophore release and mineral nutrition in barley

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Abstract

To investigate the effect of low phosphorus (P) conditions on phytosiderophore (PS) release and mineral nutrient status in graminaceous plants, an experiment was conducted in a phytotron with barley plants grown in iron-deficient (Fe0) nutrient solutions with four P levels (0.5, 5, 50, 500 [control] μmol L^{-1}) at pH 6.5 for 21 days after treatment (DAT). The results showed that the growth and chlorophyll index of plants cultured under low P conditions (0.5, 5 and 50 μmol L^{-1}) were higher than those of the control plants. The accumulation amount (mg or μg/plant) of mineral nutrients in shoots was higher for potassium, iron and copper in plants in the low P treatment than in the control plants. The accumulation of PS in roots and the amount of PS released from the roots at 14 DAT were lower in plants in the Fe0 and low P treatments. These results indicated that low P depressed PS release from roots and PS accumulation in roots in Fe0 barley. This might result from the higher Fe content in shoots and the alleviation of Fe chlorosis with low P treatment of plants. These results showed that low P treatment enhanced the growth, chlorophyll index and Fe mobilization to shoots in Fe0 barley. Low P conditions alleviated Fe-deficiency symptoms, suggesting that P is physiologically competing with Fe in plant tissues.

Key words: chlorophyll index, iron deficiency, low phosphorus, mineral nutrient status, phytosiderophore.

INTRODUCTION

Phosphorus (P) and iron (Fe) are essential mineral elements for both animals and plants. Except for strains of lactobacilli, bacteria found in milk, which are unique organisms capable of living without Fe, neither plants nor animals can grow without P and Fe (Brady and Weil 2002). Phosphorus is the energy currency of the living cell in the form of adenosine triphosphate (ATP) and the seat of genetic inheritance DNA and RNA, which control protein synthesis in both plants and animals (Brady and Weil 2002). Iron is involved in the reduction of O_2, CO_2 and N_2 (Neilands 1994). Plants need Fe for the development of their photosynthetic apparatus. Iron has already been identified as a constituent of blood, and the content of hemoglobin Fe in an adult human is approximately 80 mmol (Neilands 1994).

Iron chlorosis occurs in plants growing in every region of the world (Neilands 1994). The problem of P deficiency in practical agriculture mainly occurs because the P compounds commonly found in soils are mostly unavailable for plant uptake because they are highly insoluble (Brady and Weil 2002). The fixation of P is characterized by the formation of insoluble complexes with Fe, aluminium (Al) and manganese (Mn) under acidic soil conditions and with calcium (Ca) under alkaline soil conditions (Mengel *et al.* 2001). Therefore, combined Fe and P deficiency may potentially occur under both acidic and alkaline conditions, particularly in calcareous soils where, because of high pH values, Fe as well as P is unavailable for plant uptake.

Plant tissues have developed several physiological and biochemical mechanisms to withstand Fe- or P-deficient conditions. For instance, Marschner *et al.* (1986) reported that plants are divided into two groups with respect to their strategies for the uptake of sparingly soluble Fe under Fe-deficient conditions, namely strategy I plants (non-graminaceous monocots and dicots) and strategy

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II plants (graminaceous monocots). The latter group is characterized by the synthesis of the mugineic acid family of phytosiderophores (PS), the release of PS from roots and the absorption of the PS–Fe (III) complex by roots for Fe acquisition in the rhizosphere (Marschner *et al.* 1986, Takagi 1976). Under P-deficient conditions, some crops show a multiplication of roots and root hairs and the secretion and release of organic acids to take up sparingly soluble P in the growth medium (Mengel *et al.* 2001). In *Stylosanthes hamata* grown under P-deficient conditions, shoot growth decreased rapidly, whereas the roots continued to grow not only by retaining most of the P, but also by translocating P from shoots to roots (Smith *et al.* 1990). Rhizosphere acidification has been reported to be a widespread response to P deficiency. Depending on the plant species, acidification was brought about by proton or organic acid release. For example, in P-deficient tomato plants, increased net H^+ efflux occurred as a consequence of depressed nitrate uptake (Heuwinkel *et al.* 1992). A number of plants such as rape (Hoffland *et al.* 1989) and leguminous species (Ohwaki and Hirata 1992) released organic acids, particularly citric acid, under P-deficient conditions (Mengel and Kirkby 2001). In general, the numerous reactions for the synthesis and release of PS and for the absorption of mineral elements by plants are ATP-dependent reactions. The deficiency of P in the growth medium of strategy II plants should potentially affect the activity of roots, including PS release and the absorption of PS or PS-Fe^{3+}, and the mineral nutrition of plants grown under Fe-deficient conditions. The effect of Fe-deficiency on PS production of strategy II plants has been well documented. However, the effect of combined Fe and P deficiency in the growth medium, which is likely to occur under natural conditions, on PS and the growth of these plants remains to be investigated.

Therefore, the objective of the present experiment was to investigate the combined effect of Fe deficiency and low P concentration in the medium on the growth, PS activity and mineral nutrition of barley plants.

MATERIALS AND METHODS

Plant growth

Procedures for culture under Fe0 (without the addition of Fe source) and low P conditions were modified based on the method of Kawai *et al.* (1993). The seeds of barley (*Hordeum vulgare* L. cv. Minorimugi) were surface sterilized with 2% chlorinated lime for 30 min, then rinsed with tap water and kept soaked between two moistened towels covered with plastic wrapping paper at 25°C for 1 day. It is generally recognized that this cultivar releases mugineic acid and 2′-deoxymugineic acid, compounds belonging to PS (Kawai *et al.* 1993). The germinating seeds were transferred to a plastic net covering a bucket filled with a solution of 2 mmol L^{-1} $CaCl_2$. The seed box was covered with wrapping aluminium foil and kept in a phytotron (day/night, 14/10 h; temperature, 17/10°C; light intensity, 280 μmol m^{-2} s^{-1}). The wrapping foil was removed after 2 days and the seedlings were maintained in the solution for 7 days. Then, the growth medium was replaced with a 1/5-strength modified Hoagland–Arnon solution (Hoagland and Arnon 1950) containing 4.0 μmol L^{-1} Fe-Ethylene Diamine Tetraacetic Acid (EDTA). The seedlings were grown in this solution until their second leaf reached approximately 20% of the size of the first leaf. At this growth stage, the seedlings were transferred in bunches of three plants, and 16 bunches were placed in each of the plastic buckets (10 L) filled up with 1/2-strength modified Hoagland–Arnon solution containing 10 μmol L^{-1} Fe-EDTA, whereas the plants were suspended through holes of a plastic lid cover put over the bucket of the nutrient solution. The plants were allowed to grow in this +Fe medium for 48 h.

The roots were carefully washed with deionized water and transferred to the –Fe 1/2-strength modified Hoagland–Arnon solution (Takagi 1993) with the following P levels, 0.5, 5, 50 and 500 μmol L^{-1} (control) supplied as NaH_2PO_4. In the present study, these media were designated as Fe0 media. The plants were allowed to grow for 21 days after transfer (DAT). The pH of the nutrient solution was monitored daily and adjusted to 6.5. The nutrient solution was renewed at 7-day intervals.

Phytosiderophores released from roots

Roots from one bunch in triplicate for each treatment were soaked in 500 mL beakers filled with deionized water just before the onset of light and were allowed to release PS for 4 h at 7, 14 and 21 DAT. Then, the plants were returned to the growth medium. Approximately 10 mg of thymol (Kanto Chemical Company, Tokyo, Japan) was added to each beaker with the root washing solutions to prevent microbial degradation of PS. The root washings were filtered and passed through a column (1.5 cm internal diameter × 13 cm) of Amberlite IR-120 cation exchange resin (Rohm and Hass Company, Philadelphia, USA), and the resin was washed with deionized water. The PS adsorbed to the resin was eluted with 125 mL of 1 mol L^{-1} NH_4OH. The ammonium solution containing PS was condensed under vacuum and kept in a freezer until analysis. The amount of PS released from the roots was determined using the method of Takagi (1976).

Phytosiderophores accumulated in roots

Three bunches of plants were sampled in each treatment just before the onset of light on day 14 (14 DAT) because the PS concentration in roots was highest at that time (Kawai *et al.* 1993). The roots were washed

with deionized water and lyophilized and homogenized in 80% ethanol using a mortar and pestle. The resulting paste was diluted with 80% ethanol, filtered and concentrated using a vacuum evaporator. The condensed solution was diluted to 100 mL with deionized water and introduced to a column of Amberlite cation resin, similar to the method used for the release of PS. The amount of PS extracted from the roots was also determined using the method of Takagi (1976).

Chlorophyll index of leaves

The chlorophyll index, which represents the chlorophyll content of the 4th leaves of three bunches of plants, was measured at 14 DAT using a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan).

Chemical analysis of plant materials

Three bunches of plants were collected at 14 DAT for mineral element analysis, washed with deionized water and oven-dried at 55°C for 24 h continuously. The plants were separated into shoots and roots, weighed and digested in a mixture of nitric-perchloric acid for minerals other than N (Piper and Piper 1950).

For the analysis of N, another group of triplicate samples was collected at 14 DAT, washed carefully with deionized water and lyophilized for 24 h continuously. These samples were also separated into roots and shoots, weighed and digested in a mixture of sulfuric acid–hydrogen peroxide (Jones *et al.* 1991).

Phosphorus content in the plants was determined using a method in which vanadomolybdophosphoric yellow color was measured based on the absorbance at a wavelength of 420 nm using a spectrophotometer (UV-Mini 1240, UV-Vis Spectrophotometer; Shimazu Corp., Kyoto, Japan) (Barton 1948; Jackson 1958). Determination of the N content in the plants was carried out using the Kjeldahl method (Alam *et al.* 1991). Measurement of the other mineral elements was carried out using an atomic absorption spectrophotometer (170-30 Atomic Absorption Spectrophotometer; Hitachi, Ltd, Tokyo, Japan).

Statistical analysis

The experimental design consisted of a completely randomized block with 3 replicates. Data were subjected to an ANOVA (SAS Institute 1988). The means were compared according to the Ryan–Einot–Gabriel–Welsch Multiple Range Test ($P = 0.05$) using the computer "Origin 5" at Iwate University.

RESULTS AND DISCUSSION

Visual symptoms

Iron-deficiency symptoms were observed visually in the control plants (500 μmol L^{-1} P), and started with interveinal chlorosis 4 DAT that developed into whitish young leaves at 14 DAT. Slight interveinal chlorosis of young leaves was also observed in plants in the 50 μmol L^{-1} P treatment. However, no Fe-deficiency symptoms were observed in plants in the lower P treatments (5 and 0.5 μmol L^{-1}), whereas P-deficiency symptoms, characterized by dark green leaves and bronze-violet discoloration of the leaf edges, were observed from the beginning of the growth period. After 14 DAT, the control plants began to wither from damage associated with the severity of the Fe-deficiency symptoms, whereas plants in the low P (50 and 5 μmol L^{-1}) treatments were still healthy at 21 DAT. Plants in the lowest P (0.5 μmol L^{-1}) treatment developed severe P-deficiency symptoms. The symptoms of P-deficiency in the plants in the lower P (5 and 0.5 μmol L^{-1}) treatments progressed to old leaf senescence, starting from the tip of the leaf blade and developing to complete senescence of the oldest leaf.

Iron-deficiency symptoms were considerably reduced in plants in the 50 μmol L^{-1} P treatment and alleviated in plants in the lower P (5 and 0.5 μmol L^{-1}) treatments. This indicates that in barley plants grown in a Fe-deficient nutrient solution with low P, Fe-deficiency symptoms could be alleviated, attenuated or masked by treatments with low levels of P. In other words, it was considered that the expression of Fe-deficiency symptoms in the control plants might be induced by the high P concentration in the growth medium.

Dry matter production

Plants in the Fe0 and low P (50, 5 and 0.5 μmol L^{-1}) treatments displayed a higher dry matter yield in shoots and roots than the control plants (Fig. 1). Among the three low P treatments, the highest dry weight value was obtained when the P concentration of the medium was 50 μmol L^{-1} P. In addition, growth of the plants subjected to 5 and 0.5 μmol L^{-1} P was more vigorous than that of plants in the 500 μmol L^{-1} P treatment, but was less vigorous than that of plants in the 50 μmol L^{-1} P treatment. It was considered that the inferior growth of plants in the 500 μmol L^{-1} P treatment resulted from the high P concentration of the medium, indicating that retardation of growth in the control plants depended on the P concentration in the growth medium. These results suggested that low P supply to barley plants grown under Fe0 conditions alleviated Fe-deficiency symptoms and enhanced growth without being related to the severity of the P-deficiency symptoms.

Chlorophyll index

Plants grown under Fe0 and low P conditions exhibited a higher chlorophyll concentration than the control plants, although no significant differences were detected among plants in the 3 low P treatments (50, 5 and

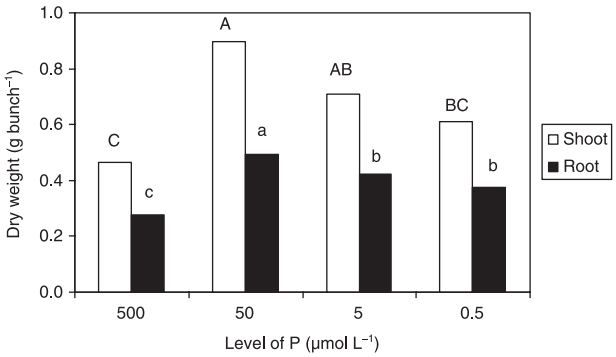

Figure 1 Dry matter weight of shoots and roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus (P) at 14 days after treatment. Different letters at the top of each bar indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

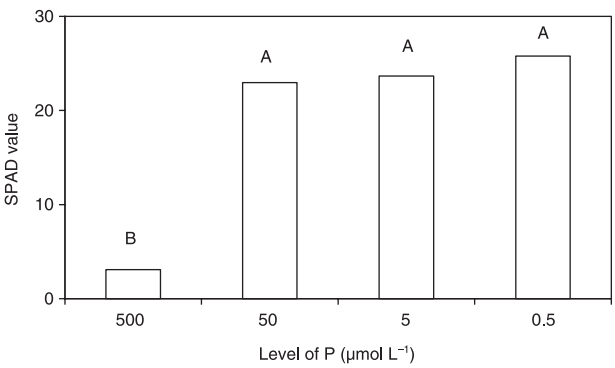

Figure 2 Chlorophyll index (SPAD value) of new leaves of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus (P) at 14 days after treatment. Different letters at the top of each bar indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

0.5 μmol L^{-1}) (Fig. 2). This result indicated that chlorophyll synthesis was enhanced under Fe0 and low P conditions. The chlorophyll indices of the young leaves were not significantly different among the low P levels (50, 5 and 0.5 μmol L^{-1}). These results were consistent with the findings of Omar *et al.* (1971), who observed a relationship between increased phosphate (Pi) content and the degree of Fe chlorosis. The results were also consistent with the report of Dekock *et al.* (1957), who observed that the amount of etherized acid-soluble (0.1 N HCl) "active Fe" (i.e. chemically active Fe fraction required for chlorophyll synthesis) in the leaves was markedly reduced by an increase in P content because a decrease in the P concentration of the Fe0 medium led to higher shoot Fe and chlorophyll contents. The close relationship between the contents of Fe and P in the appearance of chlorosis might be related to the fact that

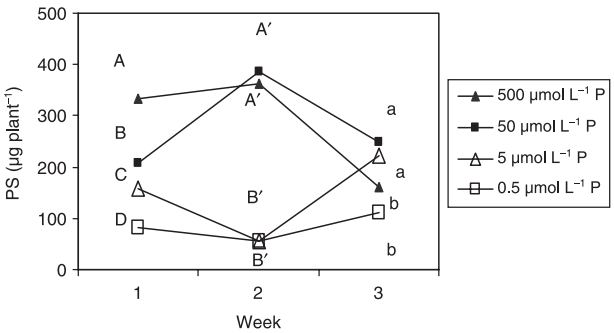

Figure 3 Phytosiderophore (PS) release from roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus (P). Different letters at the top of each drawn line indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

all the Pi in the low P treatment plants was located in the cytoplasm and chloroplasm of the leaves (Foyer and Spencer 1986), and that up to 90% of the plant total Fe was "housed" in the chloroplast (Price 1968).

Phytosiderophore release from roots and accumulation in roots

The amount of PS released and its variation pattern were affected by the low P treatments (Fig. 3). The order of the variations was as follows: 7 DAT: 500(control) > 50 > 5 > 0.5 μmol L^{-1} P. This release pattern indicated that PS release decreased with decreasing P concentration in the growth medium. (14 DAT: 500 = 50 > 5 = 0.5 μmol L^{-1} P). Tthe amount of PS released decreased significantly under lower P (5 and 0.5 μmol L^{-1}) conditions. The amount of PS released in plants in the 50 μmol L^{-1} P treatment was similar to that of the control plants. (21 DAT: 50 = 5 > 500 = 0.5 μmol L^{-1} P). Even in plants in the lowest P (0.5 μmol L^{-1}) treatment, the amount of PS released reached a similar level to that of the control plants.

In plants grown under less severe P-deficient conditions (50 μmol L^{-1}), the amount of PS released at 14 DAT was similar to that of the control plants. These findings are not always consistent with the assumption that the amount of PS released varies depending on the severity of Fe-deficiency (Mori 1994; Gries *et al.* 1995) because the Fe-deficiency symptoms appearing as chlorosis (Fig. 2) were mild or not severe in the plants in the 50 μmol L^{-1} P treatment. These results showed that greening of leaves was not a reliable indicator for predicting the amount of PS released. It has been suggested that the mechanisms of PS release are genetically controlled and may be absent or uncontrollable in special cases (Jolley and Brown 1994). The activity of the production of PS in roots may be responsible for the amount of PS released, which might be physiologically regulated by the P and Fe status in shoots and roots in Gramineae.

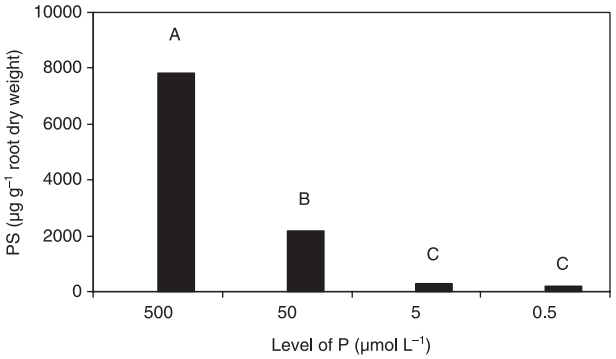

Figure 4 Phytosiderophore (PS) accumulation in roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus (P) at 14 days after treatment. Different letters at the top of each bar indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

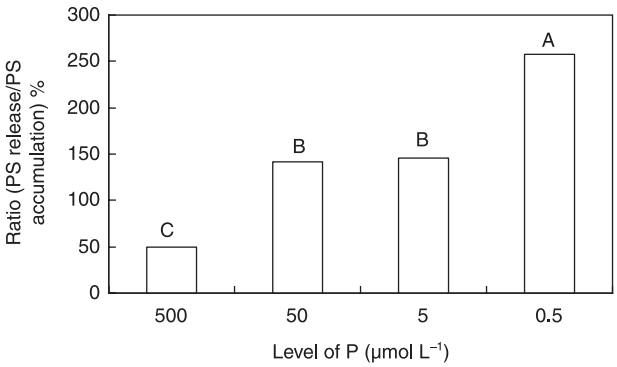

Figure 5 Relative ratio of phytosiderophore (PS) release from roots and PS accumulation in roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus (P) at 14 days after treatment. Different letters at the top of each bar indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

The pattern of PS accumulation in roots at 14 DAT was different from that of PS release from roots (Fig. 4). The amount of accumulated PS decreased with decreasing P level in Fe0 medium. These results indicated that a decrease in P concentration in Fe0 medium decreased PS accumulation in roots. It was apparent that higher P conditions induced higher PS accumulation in roots. However, it is possible that low P conditions damaged the plasma membrane of the roots and enabled the leakage of PS that had accumulated in the roots. This possibility should be examined in future studies.

Relative ratio of phytosiderophore release from roots and accumulation in roots

The relative ratio of PS release from roots and PS accumulation in roots at 14 DAT was higher in plants in the Fe0 and low P treatments compared with the control plants (Fig. 5). The reason why a higher P concentration in the Fe0 media reduced the relative ratio of PS release/PS accumulation has not been determined. High P concentration might affect the site of PS release on the root membrane and decrease PS release. Otherwise, a high P concentration might enhance the re-absorption of released PS.

Furthermore, approximately 50% of the PS accumulated in the roots of the control plants was released (Fig. 5). In plants in the Fe0 and low P treatments the relative ratio of PS exceeded 100%, indicating that the amount of PS released was greater than the accumulated PS under low P conditions. These results were repeatedly obtained. These new phenomena were observed only under low P conditions. Based on these results, the origin of released PS remains to be elucidated. It is possible that potential PS, which was not extracted with 80% ethanol, may occur in the roots of plants grown under Fe0 and low P conditions.

Further investigations on the ratio of PS release from roots and PS accumulation in roots should be conducted to determine the role of P in PS release and accumulation in the roots of graminaceous plants.

Mineral nutrition of plants

The accumulation (mg plant^{-1}) and concentration (mg g^{-1} dry weight) of macronutrients in roots and shoots are shown in Tables 1 and 2. The accumulation of N in shoots and roots in plants in the 50 μmol L^{-1} P treatment was higher compared with the control plants (Table 1). This higher accumulation of N in plants in the 50 μmol L^{-1} P treatment may result from the higher Fe concentration in shoots and to the less severe P deficiency symptoms because Fe and P are necessary for N uptake and metabolism, which require ATP and ferredoxin. Obviously, the accumulation and concentration of P in shoots and roots decreased with decreasing P concentration in the medium.

The accumulation of K in shoots and roots was higher in plants in the low P (50 and 5 μmol L^{-1}) treatments than in the control plants. However, K accumulation in the shoots and roots of plants in the lowest P (0.5 μmol L^{-1}) treatment was lower than that of the control plants. The accumulation of K in shoots and roots was highest in plants in the 50 μmol L^{-1} P treatment. This higher K accumulation may be one of the factors responsible for the vigorous growth of these plants. The concentration of K in shoots and roots was not appreciably affected by the low P treatment compared with the control plants. Therefore, K concentration may not be responsible for the greening of the leaves of plants in the low P treatment.

The accumulation and concentration of Ca in shoots were barely affected by the low P treatment. The

Table 1 Accumulation of nutrients in shoots and roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus at 14 days after treatment

Treatment	mg plant^{-1}					μg plant^{-1}			
P (μmol L^{-1})	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
Shoot accumulation									
500	7.75b	0.439a	13.7c	0.320a	0.410a	3.68c	1.18c	7.17a	5.95b
50	10.7a	0.089b	22.6a	0.437a	0.411a	5.97b	1.48bc	7.51a	6.37b
5	7.01b	0.028b	19.2b	0.294a	0.407a	8.17a	2.33a	8.27a	9.62a
0.5	7.01b	0.025b	23.9a	0.296a	0.401a	8.26a	1.85ab	7.28a	9.30a
Root accumulation									
500	3.33b	0.250a	9.05c	0.059c	0.359b	6.96a	1.45b	1.85b	3.74a
50	4.54a	0.082b	12.8a	0.116a	0.355b	5.84a	1.12b	2.01b	3.29a
5	2.90bc	0.040c	10.8b	0.088b	0.455a	7.61a	4.89a	2.82a	3.91a
0.5	2.19c	0.014c	6.47d	0.064c	0.324b	6.44a	1.18b	1.36b	3.45a

Means followed by different letters in each column are significantly different ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Multiple Range Test.

Table 2 Concentration of nutrients in shoots and roots of barley plants grown in iron-deficient nutrient solutions with different levels of phosphorus at 14 days after treatment

Treatment	mg g^{-1} dry weight					μg g^{-1} dry weight			
P (μmol L^{-1})	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
Shoot concentration									
500	47.4a	2.84a	88.9ba	2.09a	2.65a	23.9b	7.63ba	46.3a	38.5a
50	42.8b	0.299b	63.1b	1.48ba	1.39c	20.3b	4.98b	25.5a	21.6b
5	34.8c	0.120c	83.7b	1.26b	1.74cb	34.8a	9.98a	35.5a	40.9a
0.5	34.6c	0.124c	118.6a	1.45ba	1.97b	40.6a	9.10a	35.8a	45.7a
Root concentration									
500	39.3a	2.73a	82.2a	0.644ba	3.98a	75.8a	15.8b	20.3a	40.6a
50	33.1b	0.499b	74.8a	0.711a	2.15c	35.8c	6.89c	12.3b	20.2b
5	27.1c	0.288c	76.8a	0.626ba	3.23ba	54.1b	34.8a	20.1a	27.8b
0.5	20.4d	0.114d	53.1a	0.513b	2.59bc	51.9cb	9.47c	10.9b	27.8b

Means followed by different letters in each column are significantly different ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Multiple Range Test.

accumulation of Ca in roots was higher in plants in the low P (50 and 5 μmol L^{-1}) treatments. The low P treatments enhanced Ca uptake by the plants and the excess Ca taken up may be accumulated in the roots, which could account for the more vigorous root growth because Ca is a regulator of growth in length and is involved in cell division. The accumulation of Ca in shoots was not affected by the low P treatments.

The accumulation of magnesium (Mg), a component of chlorophyll, in shoots was not affected by the low P treatment. The concentration of Mg in the shoots of plants in the low P treatments (50, 5 and 0.5 μmol L^{-1}) was lower than that of the control plants. The increase in the chlorophyll index in plants in the low P (50, 5 and 0.5 μmol L^{-1}) treatments (Fig. 2) was not accompanied by a high Mg concentration in the shoots of the plants. Therefore, Mg may not be responsible for the greening of the leaves in plants in the low P treatments.

The accumulation (μg plant^{-1}) and the concentration (μg g^{-1} dry weight) of micronutrients in shoots and roots are shown in Tables 1 and 2. The accumulation of Fe in the shoots of plants in the low P (50, 5 and 0.5 μmol L^{-1}) treatments and the concentration of Fe in the shoots of plants in the low P (5 and 0.5 μmol L^{-1}) treatments were higher than the control plants. All the Fe concentrations of the shoots in this experiment were within the range of the critical deficiency level, 30–50 μg g^{-1} (Römheld and Marschner 1991), at which the leaves should show Fe chlorosis. It is interesting to note that Fe chlorosis did not develop in the leaves of plants in the low P treatment despite the low Fe concentration in shoots. It appears that leaf chlorosis is regulated not only by the Fe concentration but also by the P concentration in shoots.

Total accumulation (shoots + roots) of Fe calculated from the data in Tables 1 and 2 was higher in plants in

the low P treatments (50, 5 and 0.5 μmol L^{-1}) than in the control plants. Total accumulation of Fe should be uniform among the treatments because the Fe0 media in all treatments should not contain Fe. This higher Fe accumulation may be derived from marginal contamination with Fe of the Fe0 media, which were prepared with deionized water.

Plants grown under low P conditions were more efficient in the uptake of sparingly contaminated Fe, and it was inferred that low P conditions might facilitate Fe uptake by roots.

Plants in the low P treatments (50, 5 and 0.5 μmol L^{-1}) showed a lower Fe concentration in roots and a higher Fe concentration in shoots than the control plants. This higher Fe concentration in the shoots of plants in the low P treatment may result from the enhancement of internal Fe mobilization within the plant tissues. It appeared that the translocation of Fe from roots to shoots might be enhanced by the low concentration of P in the shoots, although the accumulation of Fe in the roots of the plants in the low P treatment was similar to that in the control plants.

DeKock and Alexander (1955) and Pushnik *et al.* (1984) reported that the severity of Fe chlorosis may be controlled by the Fe/P ratio. The results of the present study showed that the Fe/P ratio in shoots and roots of plants increased with decreasing P concentration in the Fe0 medium (Fig. 6). Our results are consistent with the findings previously reported. The higher concentration of P in the control plants may lead to inactivation of Fe in the plant tissues, appearance of Fe-deficiency symptoms and higher production of PS in roots. However, the mechanism of the inactivation of Fe by high P concentration in plant tissues is yet to be elucidated.

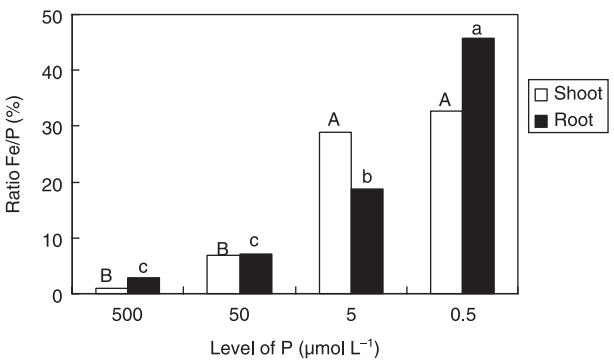

Figure 6 Ratio of iron (Fe) and phosphorus (P) concentrations in shoots and roots of barley plants grown in iron-deficient nutrient solutions with different P levels at 14 days after tratment. Different letters at the top of each bar indicate significant differences ($P < 0.05$) according to the Ryan–Einot–Gabriel–Welsch Mutiple Range Test.

The accumulation of Cu in shoots was higher in plants grown under low P conditions (5 and 0.5 μmol L^{-1}) than in control plants, whereas the accumulation of Cu in roots was not significantly affected. This higher Cu content in shoots is consistent with a report indicating that P deficiency resulted in a slightly higher Cu accumulation than that of plants grown under adequate P conditions in bush bean (Wallace 1984). The concentration of Cu was the highest in the roots of plants in the 5 μmol L^{-1} P treatment. This phenomenon was repeatedly observed in subsequent studies.

The accumulation and the concentration of Mn in shoots were not significantly affected by the low P concentration in the medium. The accumulation of Mn in roots was not significantly affected, except for plants in the 5 μmol L^{-1} P treatment.

The accumulation of zinc (Zn) in shoots was higher in plants in the low P (5 and 0.5 μmol L^{-1}) treatment, indicating a Zn–P antagonistic effect (Marschner and Cakmak 1986) under Fe-deficient conditions. However, Zn accumulation in roots of plants in the low P treatment was similar to control plants. The concentration of Zn in the shoots was lower in plants in the 50 μmol L^{-1} P treatment, but was not affected by the other low P treatments, compared with the control plants. The concentration of Zn in the roots of plants in the low P (50, 5 and 0.5 μmol L^{-1}) treatments was much lower than the control plants.

In conclusion, our results indicated that low P conditions alleviated Fe-deficiency symptoms, such as Fe chlorosis and higher release of PS by the barley roots, despite the low Fe concentration (in the range of critical deficiency level) of shoots. Furthermore, it was found that the chlorophyll index was not reliable for predicting the amount of PS released. The results obtained suggested that P is physiologically competing with Fe in plant tissues. It was also suggested that the depression of chlorophyll synthesis and loss of chlorophyll under Fe-deficient conditions did not result from the low concentration of Mg or Fe, but rather from the high P concentration that may repress the translocation of Fe from roots to shoots. It is considered that the lower ratio of Fe/P in plants grown under 500 μmol L^{-1} P (control) and Fe0 conditions may be a major factor in the induction of Fe-deficiency symptoms. Therefore, further studies focusing on the mechanism of inactivation of Fe by P in plant tissues should be carried out.

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