NUTRITIONAL AND PHYSIOLOGICAL PROPERTIES OF GRAMINEAE IN RELATION WITH PHOSPHORUS AND MICRONUTRIENTS

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Antoine Ladouceur

Laboratory of Plant Nutrition and Physiology

The Course of the Science of Biotic Environment

Faculty of Agriculture

(Iwate University)

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DEDICATED

To

My beloved daughter

Marie Sheltonie Ladouceur

And

The memory of my beloved grand mother

Mrs. Andrélia Ridoré Colin

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CHAPTER 1

GENERAL INTRODUCTION

Chapter 1. General Introduction

1.1. Phosphorus in Plant Physiology

1.1.1. Plant absorption of phosphorus

Phosphorus (P) is one of the essential mineral elements for plant growth. It is a nonmetal nutrient that plant absorbs as monovalent orthophosphate anion ($H_2PO_4^-$) when soil pH is less than 6.8, and at a lower level as divalent orthophosphate anion (HPO_4^{2-}) in soil pH range from 6.8 to 7.2 with a lesser availability. At soil pH higher than 7.2, the predominant form of P is the trivalent PO_4^{3-} virtually unavailable for uptake by plant (Hopkins, 1995). After uptake, reduction of phosphate (Pi) anion does not occur in plants but remains in its highly oxidized form as inorganic Pi or is esterified through hydroxyl group of a carbon chain as simple phosphate-ester (such as sugar-phosphate) or attaches to another Pi by the energy-rich pyrophosphate bond in ATP (Marschner, 1995). In a few minutes after absorption by roots, Pi is incorporated into organic compounds, but is released thereafter as Pi into the xylem (Marschner, 1995). The P requirement for optimal growth varies from 0.2 to 0.5 % of plant dry matter during the vegetative stage of growth (Epstein, 1972; Marschner, 1995). The probability of P toxicity increases at contents higher than 1% of the plant dry matter (Marschner, 1995). However, P toxicity may occur at concentrations of 0.3 % in pigeon pea (Cajanus cajan) or 0.6% in black gram (Vigna mungo) of the shoot dry matter (Bell et al., 1990).

1.1.2. Phosphorus function in plant physiology

Phosphorus is an integral component of important compounds of plant cells, including the sugar-phosphate intermediates of respiration and photosynthesis, the phospholipids of the membranes, the phytic acid, and the coenzymes (Taiz and Zeiger, 2002). It is the

energy currency of the living cell in the form of ATP (Adenosine triphosphate) and the seat of genetic inheritance DNA (deoxyribonucleic acid), RNA (ribonucleic acid), which direct protein synthesis in plant (Brady and Weil, 2002). In the structures of macromolecules such DNA and RNA, Pi forms a bridge between ribonucleoside units and is responsible for the acidic nature, and the high cation concentration of the nucleic acids (Marschner, 1995).

1.1.3. Phosphorus partition and function in plant cell

In most enzymatic reactions, Pi is either a substrate or an end product. The compartment of Pi is essential for the regulation of metabolic pathways in the cytoplasm and chloroplast in plant cells. The vacuole acts as a storage pool (nonmetabolic pool) of Pi in vacuolated cells of higher plants, whereas up to 95% of the total Pi is located in the vacuoles in condition of adequate P supply to the plants (Bieleski and Ferguson, 1983). For instance, in fruit tissue of tomato, the Pi released from the vacuoles into the cytoplasm can stimulate phosphofructokinase activity (Woodrow and Rowan, 1979) which is the key enzyme in the regulation of substrate flux into the glycolytic pathway. The Pi can modulate enzyme activities through phosphorylation. Phospho-enol pyruvate (PEP) carboxylase is one of the key enzymes regulated by phosphorylation in C_3 and C_4 plants. In C_4 plants and in CAM plants, phosphorylation increases the activity of the PEP carboxylase and simultaneously it becomes less sensitive to negative feedback control by high malate concentrations (Budde and Chollet, 1988). A variation by a factor of 20 of the total P content in leaves may occur without affecting photosynthesis because the Pi concentration in the cytoplasm is regulated in a narrow range by an effective Pi homeostasis in which the Pi in the vacuole acts as buffer (Mimura et al., 1990). Though, photosynthesis and carbon partitioning in leaves in the light-dark cycle

are strongly affected by the Pi concentration in the stroma of chloroplasts and the compartmentation between chloroplasts and cytosol (Walker, 1980). A high Pi concentration in the stroma induced the depletion of triosephosphates which are required for the regeneration of ribulose-bis-phosphate (RuBP) by excessive export (Walker, 1980). The RuBP is the CO_2 acceptor in the CO_2 fixation in the Calvin cycle process. It was also shown in isolated chloroplasts that high external Pi inhibit CO_2 fixation (Flügge et al., 1980).

1.1.4. A typical storage form of phosphorus in plant organ

In grains and seeds, the typical storage form of P is the phytate which is synthesized from the cyclic alcohol, myo-inositol, by esterification of the hydroxyl groups with Pi groups. Phytic acid forms sparingly soluble salt with Ca and Mg called phytin. It has also high affinity for Zn and Fe. In legume seeds and cereal grains, the K-Mg salts are the main phytates (Ogawa et al., 1979; Prattley and Stanley, 1982). The percentage of total P in the form of phytate may be 50% in legume seeds, 60-70% in cereal grains and about 86% in wheat mill bran (Lolas et al., 1976). The phytate of the cereal grains is mainly located in the aleurone layer, while that of the legume seeds is found in the cotyledons and embryo axes (Lott and Buttrose, 1978; Welch, 1986).

1.2. Phosphate Mines and Phosphate fertilizer Demand in Agriculture

The total P content of soils is usually about 4-10 times or 20 times lower than that of N or K, respectively. The total P concentration either in surface soil or in subsoil may vary from a few mg kg^{-1} to over 1g kg^{-1} (Brady and Weil, 2002). The quantity of P present in soil solution even at fairly high level of available Pi, ranges between 0.3 and 3 kg P ha^{-1}. An adequately growing crop takes up to 1 kg P ha^{-1} day^{-1} in the form of Pi. Therefore, the Pi of the soil solution must be replenished by mobilization of Pi from the labile pool

(Mengel and Kirkby, 2001). Phosphorus is after N the second most limited nutrients in soils for vegetative growth (Vance et al., 2003), and the most immobile of the major plant nutrients in soils (Mengel and Kirkby, 2001). The deficiency of P in soils is mainly due to the fact that the P compounds commonly found in soils are often insoluble with low availability for plant uptake (Brady and Weil, 2002). Most of the Pi of the soils is adsorbed into insoluble complexes with Fe, Al, and Mn in acidic conditions and with Ca and Mg in calcareous or alkaline conditions (Mengel and Kirkby, 2001; Brady and Weil, 2002). It was reported that less than 15% of the Pi fertilizer applied to soils with high P-fixing capacity was usually uptaken by the crop immediately grown after the application (Greenwood, 1981). Large amount of Pi fertilizer must be supplied to the soils to meet the P demand of the growing crop to overcome the fixation of P and make larger amount of Pi available to plants. The application rate of P to crops grown in arable soils ranges from 20 to 80 kg P ha^{-1} depending on crop species and soil available Pi. In soils with high Pi adsorption capacity P is supplied at higher rates from 100 to 200 kg P ha^{-1} (Jama et al., 1998). In some industrialized and developed countries through intensive agriculture, Pi fertilizer has been applied for years and soils are sometimes enriched in available Pi (Mengel and Kirkby, 2001). It was reported that about 2/3 of the soils in the Midwest of USA did not respond to Pi fertilizer application (Mallarino, 1995). The excess of Pi compounds of the surface soils can leak to the ground water and lead to the phenomenon termed eutrophication. Eutrophication is characterized by excessive growth of the algae and aquatic weeds in the aquatic systems that make ponds, lakes, streams or rivers unsatisfactory environments for fish. In extreme case, massive fish die in sensitive aquatic system (Brady and Weil, 2002). In developing countries in the Sub-Saharan Africa, for instance, the per capita food production continues to

decrease because of the continuous loss of fertility of the soils of the smallholders and the negative balance of plant nutrients, including Pi (Sanchez and Leakey, 1997). These soils respond promptly to P supply as reported Jama et al. (1997) in the increased maize grain yield in acidic soils in Kenya.

By contrast to N, which constitutes 79% of the earth's atmosphere, Pi is present on earth as mineral deposits that are nonrenewable natural resource. The high energy and global costs for mining the Pi rocks available only in few countries, the high price of Pi fertilizers particularly in countries with few or no Pi rock deposits make Pi management complicated with rising concern in agriculture for a large number of countries.

According to Mengel and Kirkby (2001), most of the Pi fertilizers are produced from Pi mined (Rock phosphate) and about 90% of the mined Pi is used for fertilizer production. It was reported based on the world annual amounts of Pi fertilizer consumption that the actual Pi mining reserves can last only another century (Mengel, 1997). In view of the fact that calcareous soils cover more than 30% of the earth's surface (Chen and Barak, 1982) in addition to acidic and alkaline soils which are potentially P-deficient. The developing countries are expected to use much Pi fertilizer in their agriculture to improve crop yields. It is necessary to research and develop more efficient methods for the use of Pi compounds of soils, for improving plant utilization of sparingly soluble P of the soils, for monitoring the rates and fractions of Pi fertilizers applied to crops in relation with the rhythm of plant utilization and the balance of P with other mineral nutrients in plant tissues. Many researchers reported the positive effect of pH correction through liming on increasing Pi availability to plants in soils (Sims and Ellis, 1983; Sturm and Isermann, 1978; Jungk et al., 1993), other researchers reported the positive effect of mycorrhizal infection of plant roots on increasing P availability to

plants (Tawaraya et al., 2003; Turjaman et al., 2005). Research works regarding varying rates of Pi fertilizers in balance with other mineral nutrients are few (DeKock and Alexander, 1955; Pushnik et al., 1984). A deeper understanding of the balance of P with other mineral nutrients in relation with P utilization efficiency of the plants, and the response of plants to P-deficiency combined with other nutrient deficiency in the environment is required for building up Pi fertilizer and soil Pi management programs for a sustainable agriculture.

1.3. Phosphorus Deficiency and Plant Growth

The release of Pi from insoluble Pi compounds is very slow in the soil solution and P is always limited in acidic, neutral or calcareous soils for plant growth. The Pi bound into organic forms in soils are not always available for plant uptake. Organic P must first be converted into inorganic form by the microorganisms of the soils to be made available for plant. The growing crops must compete with the soil microflora for the small amounts of available P or develop a beneficial association with some micro-organisms such as the plant-mycorrhizae association where the fungus enhances the P uptake of plant under low P condition. In natural ecosystems P rather than N is often limited to the plants.

1.3.1. Phosphorus deficiency and plant shoot

The most characteristic manifestation of P deficiency is an intense green coloration of the leaves. In the extreme, the leaves may become malformed and exhibit necrotic spots (Hopkins, 1995). Phosphorus-deficient plants develop a dark greenish color of leaf blades and a purplish color of the leaf edges. Due to the high mobility of P in plant, its deficiency leads to the rapid senescence and death of older leaves by translocation of their P to new leaves. The stems of the plants are usually shortened and made slender,

and the yield of fruits or seeds is markedly reduced (Barry and Miller, 1989). The accumulation of anthocyanin in shoots is known as typical response of plants to P deficiency (Jain et al., 2007), being accompanied with low leaf photosynthetic activity (Rao et al., 1989). The growth of the shoots decreases rapidly due to retranslocation of P from shoots to roots (Smith et al., 1990) and leads to the decrease of the shoot-root ratio of the dry weight (Fredeen et al., 1989).

Phosphorus stressed condition of the plant shoot is accompanied with the reduction of the number of leaves (Lynch et al., 1991), the reduction of leaf surface area and leaf expansion (Fredeen et al., 1989), and may cause a low P content in the epidermal cells of the leaves (Treeby et al., 1987). However, the content of chlorophyll (Fredeen et al., 1989) and that of protein (Rao and Terry, 1989) per unit area of the leaf are not much affected, while the photosynthetic efficiency per unit of chlorophyll was lower in leaves of P-starved plants (Lauer et al., 1989). In leaves of P-deficient plants, Pi is virtually localized in cytoplasm and chloroplasts considered metabolic pools (Foyer and Spencer, 1986). The cytoplasmic concentrations of Pi in P-deficient leaves may drop from 5 mM to less than 0.2 mM, simultaneously the levels of ATP drop to 20-30% of the original level (Theodorou and Plaxton, 1993). The Pi concentration in leaves of P-deficient plants (without vacuolar buffer) may drop to 50% following the dark-light transition (Sicher and Kremer, 1988)

1.3.2. Phosphorus deficiency and plant roots

It is well known that plant roots act as the dominant sink for photosynthate under P deficiency (Fredeen et al., 1989). The increase in the partition of carbohydrate, particularly sucrose, in plant towards the roots was reported in P-deficient plants (Khamis et al., 1990; Fredeen et al., 1989). The growth of the roots is progressed by

retaining most of the plant P and by the importation of P from shoot (Smith et al., 1990). Phosphorus deficiency stress triggers progressive loss of meristematic cells in the primary roots and thereby causes determinate growth (Sànchez-Calderón et al., 2005). The elongation rate of individual root cells and of the roots might be enhanced by P deficiency (Anuradha and Narayanan, 1991). The total respiration in roots was not much affected by the P deficiency according to Rychter and Mikulska, (1990). The cytoplasmic Pi concentration is maintained constant in the range of 6 mM for maize roots and 4.2 mM for pea roots under P-deficient condition, unless the vacuolar pool is depleted (Lee et al., 1990). Some crops multiply roots and root hairs, and release organic acids to uptake sparingly soluble Pi of the medium (Mengel and Kirkby, 2001). The acidification of the rhizosphere of some crops by the release of protons or organic acids was reported. For instance, in tomato plant the net H^+ efflux increased as a consequence of decreased nitrate uptake caused by P deficiency (Heuwinkel et al., 1992). Other plants, such as rape (Hoffland et al., 1989) and leguminous species (Ohwaki and Hirata, 1992), released organic acids, particularly citric acid (Mengel and Kirkby, 2001). The decrease of the hydraulic conductivity of the roots may also occur under P-deficient condition (Radin, 1990).

1.4. Iron in Plant Physiology

Iron is an essential metal micronutrient involving in key metabolic functions of the plants. It is the best known trace elements with regards to its biological functions. Of all the micronutrients, Fe is required by plants in the largest amounts. Plant takes up Fe either as the ferric (Fe^{3+}) or the ferrous (Fe^{2+}) ion. The uptake of the latter cation is more common among crop species due to its greater solubility. The importance of Fe is related to two important functions in plants. It is a part of the catalytic group for many

redox enzymes and it is required for the synthesis of chlorophyll. The concentration of 100 parts per million (ppm) was suggested as adequate tissue level of Fe for growing crops (Epstein, 1972) and the critical deficiency level from 30-50 μg g^{-1} dry weight (DW) (Römheld and Marschner, 1991). However, it was also reported that Fe concentration below 65 μg g^{-1} DW was inadequate for chlorophyll synthesis and subsequent plant growth (Tang et al., 1990). Iron is a constituent of the cytochromes and the nonheme iron proteins which are involved in photosynthesis, N_2 fixation, and respiration in plants (Taiz and Zeiger, 2002). It is also present in several heme containing enzymes, such as the peroxidase which catalyzes the oxidation of various organic substances by peroxides, the catalase which catalyzes the decomposition of hydrogen peroxide, and the cytochrome oxidase which catalyzes the reduction of molecular oxygen to water by electrons coming from nutrient molecules.

The Fe-S enzymes are another important class of Fe containing enzymes without heme, which also function in electron-transferring reactions not only in plant, but also in animal and bacterial cells. An example of this group is the ferredoxin of chloroplasts which functions to carry electrons from light-excited chlorophyll to various electron acceptors. Other Fe-S enzymes function in electron transfer reactions of mitochondria. During the course of electron transfer Fe is reversibly reduced from the Fe^{3+} to the Fe^{2+} state. Ferredoxin is involved in the oxidation-reduction reactions, such as the reduction of nitrate (NO_3^-), sulfate (SO_4^-), and the fixation of N in plants.

1.4.1. Iron deficiency and plant growth

The earth's crust comprises 5% Fe either a total concentration of 50 g kg^{-1}. The total Fe concentration in soils varies from less than 1% to more than 20% with a median concentration of approximately 3.2% (Murad and Fisher, 1988). Despite of the large

amounts of Fe in soils, Fe availability for growing plants in aerated systems within the biological pH range remains very low, because Fe forms insoluble oxides and oxyhydroxides resulting in concentrations of Fe^{3+} and Fe^{2+} as low as 10^{-11} M (available forms for plant uptake) in the soil solution, which is far below that required for optimal growth of plants (Lindsay, 1974). The deficiency of Fe in plant nutrition is worldwide in soils of the arid regions, in alkaline and calcareous soils and can also occur even in acid soils (Welch et al., 1991). The total alkaline and calcareous area was worldwide estimated for one-third of the planet earth's land (Brown, 1961; Vose, 1982). As a consequence, Fe deficiency causes potential economic loss in many crops (Wallace and Lunt, 1960) and limits crop selection for many years (Clark, 1982). The early visual symptom of Fe deficiency in plant is the interveinal chlorosis of the young leaves because of the low mobility of Fe in plant resulting in the non importation of Fe from the older leaves. The chlorosis may progress to the veins, and if the deficiency is severe enough, the leaves may actually turn pale yellow and finally white. Iron deficiencies invariably lead to a simultaneously loss of chlorophyll and degeneration of chloroplast structure.

Iron chlorosis is defined as the yellowish coloration of the leaves that can be overcome by effective Fe application (Brown, 1961). It was also defined as the yellowing of the young leaves caused by the inhibition of chlorophyll synthesis in chloroplast, in consequence of low Fe nutritional status of the plant (Marschner, 1995). It is known that Fe chlorosis in plant is not due to Fe scarcity in the environment, but to the factors that inhibit Fe absorption and translocation or impair its utilization in metabolic processes (Brown, 1961; Welch et al., 1991). However, there is no report on the mechanism for Fe absorption and translocation in plants under P-deficient condition.

1.4.2. Specific Mechanisms for iron acquisition in plants

In a well aerated field with neutral or calcareous soils deficient in Fe, many different plant species are grown. It is always visible that some species develop Fe chlorosis while others do not. Plant species with Fe chlorosis in these soils may have a limited capacity to exhibit the mechanisms to solubilize Fe at the rhizosphere. The mechanisms or the efficiency of a given mechanism for Fe solubilization in the rhizosphere may be different among plant species. The importance of Fe in plant nutrition is highlighted by the numerous mechanisms that plants have developed for Fe uptake under Fe stress conditions. Marschner and Römheld (1986) divided the plant species into two groups depending on their Fe solubilizing mechanisms termed Strategy I plant species (dicots and nongraminaceous monocots) and Strategy II plant species (the gramineae family). The Strategy I plants uptake Fe from soils poor in available Fe by operating the following mechanisms:

1) The reduction of Fe^{3+} to Fe^{2+} at the root surface (Ambler et al., 1971; Chaney et al., 1972)
2) The release of H^+ from roots (Landsberg, 1981; Römheld and Marschner, 1981)
3) The release of reducing compounds from roots (Brown, 1978; Brown et al., 1971)
4) The increase of root contents of organic acids particularly citric acid (Brown and Chaney, 1971; Landsberg, 1986)
5) The induction of transfer cells, the increased formation of root hairs in the epidermal and hypodermal cell layers of the roots (Kramer et al., 1980; Landsberg, 1982)

All of these mechanisms listed above are not exhibited in Strategy I plants. A given species of this group may lack certain responses such as the release of H^+ or the formation of transfer cells or exhibit response that differs from some of those listed. The mechanism which appears to be exhibited on a constant basis in all the Strategy I plants is the reduction of Fe^{3+} to Fe^{2+} at the root surface.

The Strategy II plants, which are monocotyledonous plants of the family of gramineae (barley, maize, oats, rice wheat and other grasses), uptake Fe from soils poor in available Fe by operating the following mechanisms:

1) The synthesis of phytosiderophores (PS) at the apical zones of the roots (Römheld and Marschner, 1986)
2) The realease of PS from the root apex (Marschner et al., 1987)
3) The solubilization of sparingly soluble inorganic Fe^{3+} by chelation with PS (Takagi et al., 1984; Römheld and Marschner, 1986)
4) The uptake of the complex PS-Fe^{3+} by roots (Takagi et al., 1984; Marschner et al., 1987)

Once inside the root, Fe^{3+} is presumably reduced to Fe^{2+} for use by the plant cell but the fate of the PS is unknown.

These mechanisms of the Strategy II plants in regards with PS are operating mostly in specific area of the roots, the apical root zones and the root apex. Any factor that inhibits root growth or any mechanical barrier in the soil-root interface may impair the efficiency or the operation of these mechanisms and reduce Fe acquisition and consequently induce Fe chlorosis in plants. Therefore, an environmental soil condition for root growth must be beneficial for plants in practical agriculture with regards to PS activity and Fe nutrition in the plants of this Strategy.

Some amphoteric Fe^{3+}-chelating compounds were discovered by Takagi (1976) in the root washings of oat and rice grown hydroponically. However, the activity of PS does not appear to be a specific mechanism operating in plants only under Fe-deficient condition but also under the deficiencies of other metal micronutrients, such as Cu deficiency (Gries et al., 1998) or Zn deficiency (Zhang et al., 1991a). Treeby et al. (1989) reported that the PS released from roots of Fe-deficient barley was effective in mobilizing Mn, Zn, and Cu from calcareous soils with a mobilization efficiency range being Mn> Zn> Cu. However, the mobilization of Mn from calcareous soils by PS was also reported to be little or absent (Takagi et al., 1988; Zhang, 1993)

1.5. Zinc in Plant Physiology and Growth

The solubility of inorganic Zn, similarly to inorganic Fe decreases with increasing pH. Within the pH range 5.5 – 7.0, the equilibrium concentration of Zn may decrease 30 to 45 times for each unit of increase in soil pH (Moraghan and Mascagni Jr, 1991). Plant absorbs Zn as the divalent cation Zn^{2+}, which does not undergo valence changes, but forms more stable complexes of low molecular weights in plant cells. The greater part of Zn in leaves is associated with complexes with low molecular weight, storage metalloproteins, free ions, and insoluble forms associated with the cell wall (Brown et al., 1993). Concentrations of Zn of 20 ppm (Epstein, 1972) or 0.3 mmol kg^{-1} DW (Hopkins, 1995) were reported as adequate in tissue levels in plants. The critical deficient level and the critical toxicity level in sorghum for Zn were 10 and 64 $\mu g\ g^{-1}$ DW, respectively (Ohki, 1984). Zinc can be inactivated in the cell either by ligand formation (Leece, 1978) or by complexation with P (Olsen, 1972). Depending on plant species, 58% to 91% of Zn may be soluble in plant tissues (Welch et al., 1976). Most of the soluble Zn in the plant cell is associated with anionic complexes with low molecular

weight, with a small percentage of free ions. This water-soluble Zn fraction considered as physiologically active fraction is regarded as a better indicator of Zn status than the total Zn content of the plant (Cakmak and Marschner, 1987)

Zinc is present in several enzymes such as dehydrogenase, proteinase, and peptidase. Zinc plays a role in protein synthesis, in the formation of some growth hormones, and in the reproductive process of certain plants by promoting seed maturation and production (Brady and Weil, 2002). It is activator of a large number of enzymes including alcohol dehydrogenase catalyser of the reduction of acetaldehyde into ethanol, carbonic anhydrase catalyser of the hydration of carbon dioxide to bicarbonate. Zinc is a constituent of the Cu-containing superoxide dismutase and glutamate dehydrogenase enzymes (Evans and Sorger, 1966). Marschner (1986) reported supporting evidence that Zn is required for the synthesis of tryptophan which is the hormone precursor of auxin (Marschner, 1995).

Zinc-deficiency symptoms are characterized by shortened internodes of the stems and smaller leaves. Loneragan et al. (1979) observed that plant tops with Zn concentrations lower than 16 $\mu g\ g^{-1}$ had necrotic symptoms in addition to bronzing and chlorosis of old leaves and smaller young leaves. There is general agreement that disorders associated with Zn deficiency reflect disturbances in the metabolism of the auxin, indole-3 acetic acid.

1.6. Relation of Phosphorus with Iron and Zinc in Plant

Chlorosis due to Fe-deficiency limits plant growth worldwide especially in calcareous soils with high pH (Neilands, 1994) where P (Brady and Weil, 2002) and probably Zn are also deficient. The deficiency of available P in soils is due to its fixation into insoluble compounds by Fe, Al, Mn in acidic conditions or by Ca in calcareous or

alkaline conditions (Mengel and Kirkby, 2001). Potentially, the soil environmental conditions that limit the availability of P, such as a high pH, reduce also the availability of most of the micronutrients. The simultaneous deficiencies of P and micronutrients, such as Fe, Zn, Mn, or Cu, was reported from high pH soils of India (Srinivasarao et al., 2006).

The ratio Fe/P in plant tissues was reported as one of the factors regulating the expression of Fe chlorosis, and the higher the ratio was, the lower the chlorosis in leaves was expressed (DeKock and Alexander, 1955; Pushnik et al., 1984; Ladouceur et al., 2006). An equilibrium balance between P and Fe concentrations appeared to be a regulating factor for chlorophyll synthesis in leaves of macadamia grown in alkaline soils. The leaves with P content of about 0.2% and Fe concentration less than 30 mg kg^{-1} showed Fe chlorosis, while those with P content of about 0.1% and Fe concentration 20 mg kg^{-1} were normal (North and Wallace, 1959).

Olsen (1972) observed that increased levels of P fertilizers or salts induced or accentuated symptoms similar to those of Zn deficiency in plants grown in soil or culture media deficient in available Zn. His finding led to the discovery of the plant disorder known as "P-induced Zn-deficiency". The responses of plant to Zn nutritional status are affected by P in a numerous and complex phenomena which may operate separately or simultaneously depending on plant species and environmental conditions. Loneragan et al. (1979) identified three distinct mechanisms in related with P in plants grown on soils with low Zn availability: 1) dilution of Zn in plant tissues by enhanced plant growth by Pi fertilizer application (Boawn et al., 1954); 2) inhibition of plant Zn absorption by the cations added with Pi fertilizers in the soil (Chaudhry and Loneragan, 1972); 3) phosphorus enhancement of Zn adsorption by oxides and hydroxides of Fe

and Al in the soil (Stanton and Burger, 1967; Stanton and Burger, 1970; Bolland et al., 1977) resulting in decreased absorption of Zn by plant roots (Stanton and Burger, 1967). Other researchers observed enhanced Zn-deficiency symptoms by P treatment with no variation on Zn concentration in plant tops. These symptoms correlated with the ratio of P/Zn concentrations but not with Zn concentration itself (Millikan, 1963; Boawn and Leggett, 1964; Watanabe et al., 1965; Boawn and Brown, 1968; Millikan et al., 1968). Similar pattern was reported where available Zn was decreased by high P content while shoot Zn concentration remained unchanged in shoot (Cakmak and Marschner, 1986; Cakmak and Marschner, 1987). Those reports suggested that increasing P concentrations in plant tissues induced a higher physiological requirement for Zn. On the other side, Zn-deficiency also enhanced P toxicity at the middle and high levels of P application apparently by limiting plant growth and concentrating P in plant tissues (Loneragan et al., 1979). The Zn-P interaction may not always occur in plants (Pasricha et al., 1987)

The reports about Zn-P antagonistic interactions were mostly derived from work conducted with plants grown either under P toxicity and/or under Zn-deficient conditions. The reports are numerous and sometimes controversial. The Zn-P relationship in soils and in plants needs further investigation. The relation of P with Zn in plant in related with varied Fe status also needs to be investigated.

1.7. Phytosiderophores System in Iron Stress Plants

The plants of the family of Gramineae include not only the staple food for human being but also that for animals which are the providers of meat and milk for the world population. The decreased crop yields, as consequence of lack of available Fe in most agricultural soils, has been a puzzling problem for more than several decades (Brown,

1961). The increased demand for cereals due to continuous increase in inhabitants of developing countries and increased demand for livestock products lead to the emphasis on researches towards the ways to increased yield or reduced yield crop loss in agriculture. Moreover, some human and animal diseases have been developed due to lack of Fe in the bodies. The concentrations of Fe in graminaceous plants including in the grasses are important for the consumers. Along with this insight, phytosiderophores were discovered (Takagi, 1976).

Phytosiderophores (PS) are non-proteinogenic and secondary amino-acids with low molecular weight, approximately 320. The PS function with their amino-, hydroxyl-, and carboxyl-groups as a multidentate cation chelators (Sugiura et al., 1981). Several types of PS have already been identified (Fig. 1.1). The amounts and kinds of released PS vary among the graminaceous species. For instance, 2'-deoxymugineic acid (DMA) is the first PS in the PS biosynthetic pathway from methionine (Mori and Nishizawa, 1987; Kawai et al., 1988b). The other PS, including mugineic acid (MA), 3-hydroxymugineic acid (HMA), 3- epi-hydroxymugineic acid (epi-HMA), avenic acid (AVA), and distichonic acid (DA), are synthesized by the subsequent hydration of DMA (Marschner, 1995). Barley and rye release various kinds of PS including DMA, MA, HMA, epi-HMA, and AVA (Takagi, 1993). By contrast, DMA only was detected in root washings of wheat (Singh et al., 2000).

In roots of barley the synthesis of PS is greatly enhanced with the onset of Fe deficiency (Kawai et al., 1993). The amount of synthesized PS reached often 20 mg PS $day^{-1}g^{-1}$ root DW (Takagi, 1993). The synthesis of PS is under the control of some feedback mechanism because the resumption of Fe supply causes immediate and rapid decrease of PS synthesis (Takagi et al., 1984).

The amount of PS released from roots of Fe-deficient barley exceeds very often 10 mg PS $day^{-1}g^{-1}$ root DW (Takagi et al., 1984) and was estimated as 10 or 100 times higher than those of Fe-deficient-corn or -sorghum, respectively (Kawai et al., 1988a). The amount of PS release is roughly consistent with plant ability to tolerate Fe stress and graminaceous plants are generally classified in the following sequence: barley > wheat = rye > oat > corn > sorghum > rice (Marschner et al., 1986; Kawai et al., 1988a).

In Fe starved plants, the release of PS follows a distinct diurnal rhythm with maximum release rates usually 3-4 hours after the onset of light (Takagi et al., 1984). The release of PS is inhibited by the presence of KCN and DCCD and is therefore highly dependent on metabolic energy (Takagi, 1990). The plant uptake of the PS-Fe^{3+} is also inhibited by the presence of metabolic inhibitors (Takagi et al., 1984). It was observed in barley that the root uptake rate of the PS-Fe^{3+} complex is 100-1000 times faster than those of the synthetic Fe chelators or the microbial Fe siderophores (Römheld and Marschner, 1986). This faster uptake rate of the complex can be considered as a strategy to avoid microbial decomposition of PS in the rhizosphere.

The solubilization of Fe is one of the steps for plant Fe acquisition through PS chelation process of the sparingly soluble Fe in soils. Nomoto et al. (1981) suggested that the chelation results in the form of 1:1 complex for PS and Fe^{3+} and for PS and Cu^{2+}. It has been reported that PS form stable chelates with metal micronutrients, the stability constants of different metal micronutrients are shown in Table 1.1.

The ability of PS to solubilize metal micronutrient in rhizosphere was reported not only for Fe but also for Zn, Cu, and Mn from calcareous soils (Treeby et al., 1989; Singh et al., 1992). In the soil conditions with low available micronutrients, P as macronutrient may be also deficient and there is not report about the role of P itself in

the activity of PS except a few reports on the role of ATP. The schematic presentation of the PS-based models for metal micronutrients is shown in Figure 1.2.

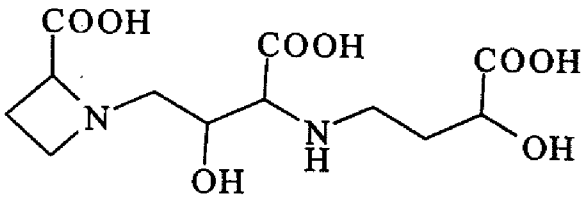
1.8. Rationale of Our Research Work

The interactions between nutrients in the plant growth medium and the specific beneficial balance to reduce adverse effect of mineral deficiency to growing crop have not been sufficiently investigated, though it appears to be one of the potential area of research for increasing plant tolerance to adverse conditions. With an insight of these above mentioned considerations from the literature review, my research work was consisted with hydroponical experiments conducted on the relationship with P and micronutrients such as Fe and Zn in the nutrition and the physiology of barley plants in Fe-deficient and Fe-sufficient rhizospheres. The objectives were to examine:

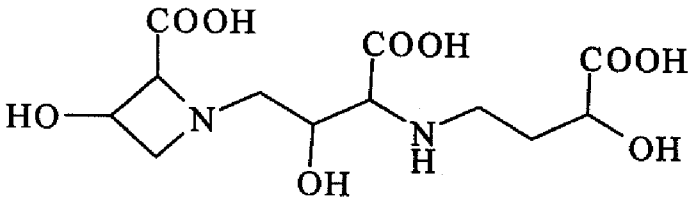
1) The effect of low P on PS release from roots, PS accumulation in plant roots, and on the chelation and transport abilities of PS in Fe-deficient plants.

2) The effect of low P on growth and mineral nutrition of Fe-deficient plants.

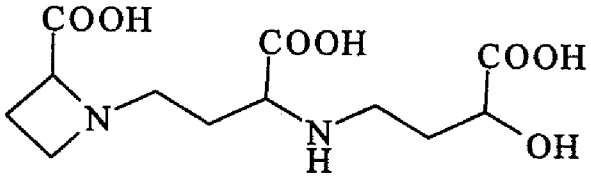
3) The effect of supply PS on Fe and Zn of plants in low P condition.



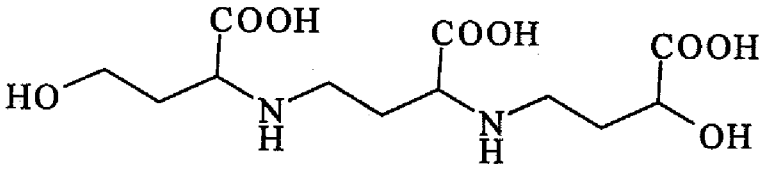
Mugineic acid (MA)



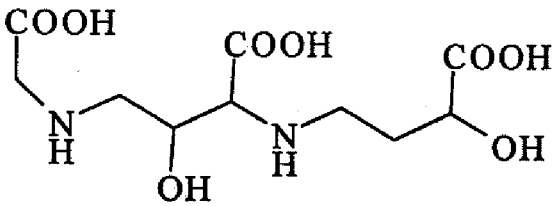
3-Hydroxymugineic acid (HMA)
3-epi-Hydroxymugineic acid (epi-HMA)



2'-Deoxymugineic acid (DMA)



Avenic acid A (AVA)



Distichonic acid (DA)

Figure 1.1. Chemical structures of phytosiderophores (PS)

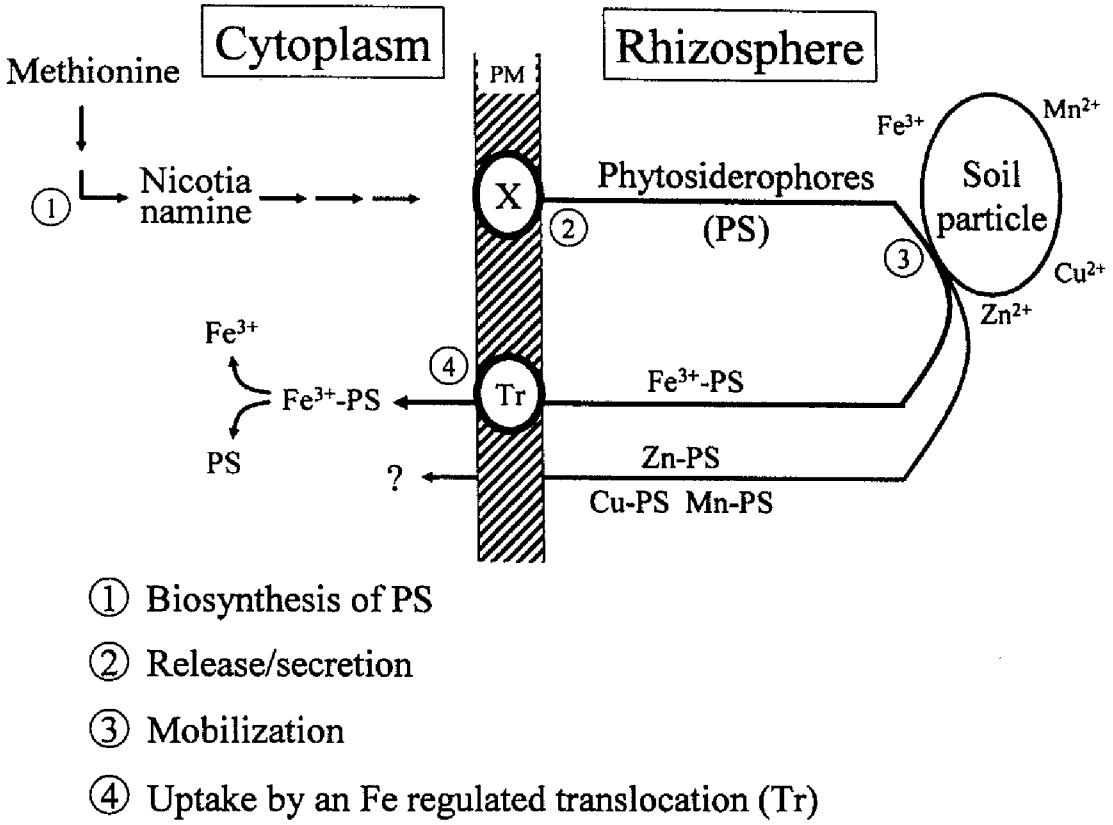


Figure 1.2. Schematic presentation of the PS system of Graminaceae (Römheld, 1991).

Table 1.1. Stability constants of phytosiderophores with metal micronutrients (Murakami et al., 1989; Sugiura et al., 1981).

	Phytosiderophores		
Metal micronutrients	Epi-HMA	MA	DMA
Cu^{2+}	17.9	18.1	17.8
Fe^{3+}	-	18.1	-
Zn^{2+}	12.4	12.7	12.8
Fe^{2+}	10.0	10.1	10.5
Mn^{2+}	8.0	8.3	8.3

CHAPTER 2

GENERAL MATERIAL AND METHODS

Chapter 2. General Materials and Methods

2.1. Biological Material

Barley (*Hordeum vulgare L.* cv. Minorimugi) is the Strategy II (graminaceous) plants which was used as test crop in this research work. Barley is ranked as the fourth most important cereal crop with worldwide distribution (Poehlman, 1985) and moderately tolerant to low levels of available iron in soil (Mortvedt, 1980).

2.2. Mineral Composition of the Nutrient Solutions

The plant seedlings were first precultured in 1/5-strength and the experimental plants cultivated in ½-strength modified Hoagland-Arnon nutrient solutions (Kawai et al., 1993).

Table 2.1. Composition of full-strength modified Hoagland-Arnon nutrient solution.

Salt	Nutrient	Plus Fe medium (+Fe)	Minus Fe medium (-Fe)
KNO_3	N, K	6.0 mM	6.0 mM
$Ca(NO_3)_2$	N, Ca	4.0 mM	4.0 mM
$NH_4H_2PO_4$	N, P	1.0 mM	.....
NaH_2PO_4	P	.....	1.0 mM
$MgSO_4$	Mg, S	2.0 mM	2.0 mM
H_3BO_3	B	3.0 µM	3.0 µM
$MnSO_4$	Mn	0.5 µM	0.5 µM
$CuSO_4$	Cu	0.2 µM	0.2 µM
$ZnSO_4$	Zn	0.4 µM	0.4 µM
H_2MoO_4	Mo	0.05 µM	0.05 µM
Fe-EDTA	Fe	20 µM	.....

The pH of the nutrient solution was adjusted to 5.5 in +Fe condition and to 6.5 in –Fe condition by the addition of 1M HCl or 1M NaOH.

2.3. Environmental Condition of Plant Cultivation

Plants were grown hydroponically in the phytotron (day/night, 14/10 h; temperature, 17/10 °C; light intensity, 280 μmol m^{-2} s^{-1}) or in the greenhouse at the Faculty of Agriculture, Iwate University in spring or summer season under natural sunlight.

2.4. Plant Growth

The seeds of barley were surface sterilized with 2% chlorinated lime for 30 minutes, then rinsed with tap water for 1 h continuously and kept soaked between two moistened towels covered with plastic wrapping paper at 25 °C for 1 day. The germinating seeds were carefully transferred to a plastic net covering a bucket filled up with a solution containing 2 mM $CaCl_2$ 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 two days and the seedling were maintained in the solution for 7 days. Then, the growth medium was replaced with a 1/5-strength modified Hoagland-Arnon solution containing 4.0 μM Fe-EDTA. The seedlings were grown in this solution until their second leaf reached a growth level corresponding in size to 20 % of approximately that of their first leaf. At this growth stage, the seedlings were transplanted as bunch of plants (3 plants were wrapped with sponge rubber to make one bunch) and 16 or 55 bunches were placed over each of the plastic buckets (capacity 10 or 35 liters) filled up with 1/2-strength modified Hoagland-Arnon solution containing 10 μM Fe-EDTA, whereas the roots were suspended through holes of a plastic lid cover so that they reached fully the nutrient solution. The plants were allowed to grow in this plus-Fe (+Fe) medium for 48 hours, then, their roots were thoroughly

and carefully washed with deionized water and transferred to the 1/2-strength modified Hoagland–Arnon solutions (Takagi, 1993) containing phosphorus (P) levels as 500 (control), 50, 5, and 0.5 μMP supplied as NaH_2PO_4 (equimolar amount) in Minus–Fe (–Fe) medium at pH 6.5 or as $NH_4H_2PO_4$ (equimolar amount) in +Fe medium at pH 5.5 with 10 μM Fe-EDTA. The –Fe medium of Takagi (1993) was prepared by removing Fe-EDTA and replacing $NH_4H_2PO_4$ by NaH_2PO_4 in the 1/2-strength modified Hoagland–Arnon solution and adjusting the pH to 6.5.

In the experiments with short term low P treatment in –Fe condition, plants were grown in –Fe and 500 μMP medium for 7 days at pH 6.5 as pretreatment. Then, the plants were transferred to minus-P and –Fe (–P) medium (pH 6.5) or continuously grown in –Fe and 500 μMP (+P) medium. The starting day of the short term low P treatment of the plants was referred to as day 0 (0 d).

The pH of the nutrient solutions was daily monitored and adjusted with the addition of 1M HCl and/or 1M NaOH during the experiments with a digital pH meter (HANNA Instruments, Padoba, Italy. The nutrient solutions were continuously aerated and renewed at 7 day intervals. The solution level was maintained by addition of deionized water.

2.5. Determination of Phytosiderophores Release by Roots and Phytosiderophores Content in Roots

2.5.1. Collection and treatment of root washing solutions

Roots of three bunches of plants from each replicate were soaked into 500 mL beakers filled with deionized water for 4 hours from 8 a.m. when the PS release starts (Takagi, 1976) to 12 a.m. of the sampling day. The root washing solutions were collected and about 10 mg of the thymol (Kanto Chemical Co., Japan) was added to each beaker for

preventing the microbial degradation of PS after returning the bunches of plants to the growth medium. The root washing solutions were filtered and passed through a column of an Amberlite IR-120 cation exchange resin. Subsequently, the resin was washed with deionized water. The PS adsorbed to the resin particles were eluted with 125 mL 1N NH_4OH. The ammonium solution containing PS was condensed under vacuum and kept in a freezer (-20°C) until analysis for the measurement.

2.5.2. Collection and treatment of roots

Three bunches of plants from each replicate of the treatments were collected at 8 a.m. when PS content in roots is maximum (Kawai, 1993) at the sampling day, then washed with deionized water. After that, the whole plants were lyophilized using the EYELA, FREEZE DRYER FDU-506, and separated into shoots and roots before the homogenization of the roots in a mortar and pestle in 80% ethanol. The resulting paste was diluted with 80% ethanol, filtered and concentrated under vacuum. The condensed solution was diluted to 100 mL with deionized water and introduced in an Amberlite cation resin column to collect the PS as described above.

2.5.3. Measurement of phytosiderophore

The amount of PS released from roots or accumulated in roots was determined by Fe solubilizing assay of Takagi (1976) which consists of the formation of PS-Fe^{3+} complex in the solution containing PS through incubation with Fe hydro-oxide suspension. The amount of PS is measured via the absorbance of Fe in the PS-Fe^{3+} solution through spectrophotometer (UV-mini 1240, UV-Vis Spectrophotometer; Shimadzu Corp., Kyoto, Japan) at wavelength 508 nm.

2.6. Collection of Xylem Sap

Plant shoots were removed using stainless-steel razor blades at approximately 2 cm above the roots at 13:00 h, time when PS release from roots does not occur according to Takagi et al. (1984). After discarding the first drop of the flow, the xylem sap was continuously collected for 3 h using a glass capillary equipped with a rubber tube. The collection unit was consisted of 16 bunches of plants in duplicate. Sap samples were frozen at -20°C until analysis.

2.7. Xylem Sap Analysis for Phytosiderophore and Organic Solutes

An aliquot sample of the xylem sap was introduced into a cation exchange resin (Dowex 50W x 4 resin) column of 1.5 cm I.D. x 2 cm long. The effluent from the cation exchange resin column was collected in an anion exchange resin (Amberlite IRA-400 resin) column of similar size. The PS containing substances adsorbed to the cationic resin and the organic solutes containing substances adsorbed to the anionic resin were eluted by 1 M ammonia (NH_4OH) or 1 M formate (HCOOH) solutions, respectively. The eluted solutions were condensed under vacuum. The concentrations of PS and the amino-acids concentrations in the effluent of the cationic resin (cationic fraction) were measured by HPLC (Ishida et al., 1981; Kawai et al., 1987). The compounds in the effluent of the anionic resin (anionic fraction) such as citrate, malate and succinate were also measured by HPLC.

2.8. Analytical conditions of High Performance Liquid Chromatography (HPLC)

The organic acids were measured by HPLC (Shimadzu LC-10 AT, Liquid Chromatograph, Japan) under the following selected conditions:

Column	Shimadzu SCR-120H (600 mm in length)
Temperature	65°C
Flow rate	0.25 mL min^{-1}

Eluting solvent	aqueous H_3PO_4 (pH adjusted to 1.8)
Detector	ultraviolet spectrophotometer
Wavelength	210 nm

The analysis of PS and amino-acids by HPLC (Shimadzu LC-10 AT, Liquid Chromatograph, Japan) was performed in the following selected conditions:

Column	Shodex CX pak P421S (SHOWA DENKO K.K)
Column temperature	38°C
Buffer	A) 0.15 N lithium citrate in 7% ethanol pH 2.65 adjusted with $HClO_4$ B) 0.2 M H_3BO_3 + 0.3 N lithium citrate pH 10 adjusted with LiOH C) 0.2 N LiOH
Buffer flow	0.41 mL min^{-1}
Hypo solution	0.06% NaClO in buffer solution
OPA solution	0.4 g o-phthalaldehyde (OPA) dissolved in 7 mL ethanol + 0.5 g N-acetyl-cysteine + 2 mL Brig-35 solution (10%)
Hypo and OPA flow	0.17 mL min^{-1}
Derivatization temperature	45°C
Wavelength	excitation 348 nm, emission 450 nm

2.9. Xylem Sap Nutrient Measurement

The concentration of the mineral nutrients in the xylem sap was determined through PIXE (Particle-Induced X-ray Emission) analysis. The samples were evaporated before the measurement of the concentration of the nutrients.

2.9.1. Determination of the amount and density of xylem sap

The amount of xylem sap was determined by calculation. An aliquot of 40 μL of xylem sap was weighed and the density was calculated via the formula:

Density = Weight / Volume

2.10. Measurement of Chlorophyll Index of Leaf

Chlorophyll index in new and fully developed leaf was carefully measured with a non-destructive portable chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Japan).

2.11. Determination of Dry Matter Biomass

Plants were harvested at 7 or 14 or 21 DAT depending on the experiment, washed carefully with deionized water and oven dried at 55 ˚C for 48 hours or lyophilized for 24 hours continuously, then separated into shoots and roots. Subsequently, the plants were weighed for obtaining the dry matter weight. For the experiment related to distribution of mineral element in old and new leaves, the harvested plants were divided into roots, new leaves (e.g. the two newest leaves) and old leaves (the remaining leaves with their sheath after taking up the new leaves) prior to oven drying or lyophilization. The dry matter weight of the plant samples was measured.

2.12. Chemical Analysis of Plant Material

2.12.1. Preparation of samples of barley plant

Plant samples for mineral element analysis were collected, washed carefully with deionized water and oven dried at 55 ˚C for 24 hours. Then the plant samples were

separated into shoots and roots, weighed, and digested in a mixture of nitric-perchloric acid for minerals other than N. For the analysis of N, another group of samples were collected in the same DAT, washed carefully with deionized water, and lyophilized for 24 hours continuously. The samples were also separated into roots and shoots, weighed, and digested in a mixture of sulfuric acid - hydrogen peroxide. The barley plants were divided into roots, new leaves (e.g. the two newest leaves) and old leaves (the remaining leaves with their sheath after taking up the new leaves) prior to oven drying the plant samples.

2.12.2. Measurement of Mineral Elements

Mineral elements of barley grown hydroponically were measured as follows:

Phosphorus content in plants was measured through vanadomolybdophosphoric yellow colour method and absorbance spectrophotometer (UV-Mini 1240, UV-VIS SPECTROPHOTOMETER, SHIMADZU) at wavelength 420 nm, N content in plants determination was carried out through the micro Kjeldahl method (Didar-Ul-Alam et al., 1991). The measurement of the other mineral elements was performed using atomic absorption spectrophotometer (HITACHI, 170-30 Atomic Absorption Spectrophotometer).

2.13. Statistical Analysis

The experimental designs were all completely randomized blocks with three, four, or six replications depending on the experiment. The data of each experiment were analyzed through analysis of variance. The means were compared according to either Duncan or Ryan- Einot- Gabriel- Welsch Multiple Range Test ($p < 0.05$), by the origin 5 of the computer in Iwate University.

CHAPTER 3

MINERAL NUTRITION AND GROWTH OF BARLEY UNDER LOW PHOSPHORUS CONDITION

3. Mineral nutrition and Growth of barley under Low Phosphorus Condition

3.1. INTRODUCTION

The fixation of P is characterized by formation of insoluble complexes with Fe, Al, Mn in acidic soil conditions and with Ca in calcareous and alkaline soil conditions (Mengel and kirkby, 2001). In high pH soils the P deficiency may potentially be accompanied with micronutrient deficiency. Srinivasarao et al. (2006) reported the simultaneous deficiencies of P, Fe, Zn, and Mn in soils of the chickpea-growing regions of India. The effects of Pi treatment on growth and P concentration in plants were reported (Asher and Loneragan, 1967; Russell and Martin, 1953). The sites (Hagen et al., 1957) and the ionic species of Pi absorbed (Hagen and Hopkins, 1955) by barley roots were reported. The kinetic rates of Pi absorption by excised roots was also reported from short-term experiments using barley and other crops in relation with the Pi concentration in medium (Noggle and Fried, 1960). Plant species express large differences in regard to the level of P required for optimal growth. Loneragan and Asher (1967) showed that as P concentration in a medium 24 μM was optimal for the growth of 2 plant species, toxic for 3 other plant species, and low for the remaining other species from 8 different plant species investigated. The highest plant DW per unit of P was produced at the lowest Pi concentration in the medium (0.04 μMP) for all of the above 8 species. The interaction between the uptake P and that of other cations was suggested in plants (von Wirén et al., 1996). But the effect of P on the absorption of the other minerals in plants is not known. This experiment was conducted to examine the relationship of medium P concentration and plant nutrient concentrations and growth in barley.

3.2. MATERIAL AND METHODS

The experiment was conducted in a phytotron where barley plants were grown in the 1/2 strength modified Hoagland and Arnon nutrient solution with 4 P levels (500 (control), 50, 5, 0.5 μM) supplied as $NH_4H_2PO_4$. The procedures for the preculture of the seeds and the growth of the seedlings in 10-L buckets containing 1/2-strength modified Hoagland and Arnon solution were described above in chapter 2, The plants were grown on the nutrient solution at pH 5.5 up to 14 DAT (days after treatment). The pH of the nutrient solutions was daily monitored and adjusted. The nutrient solutions were continuously aerated and weekly renewed. The culture solution level of the buckets was maintained by the addition of deionized water when necessary along the experiment. Plants were harvested on 14 DAT. Chlorophyll index of leaves, plant growth, and mineral nutrient contents in plant material were measured by the methods and procedures described in Chapter 2.

3.3. RESULTS AND DISCUSSION

3.3.1. Visual symptoms of the Plants

Shoot and roots of the plants are shown in Photograph 3.1, respectively. The shoots of the low P plants (5 and 0.5 μMP) had senescent old leaves as severe deficiency symptoms of P. The leaves were first darkish green, then brown, and thereafter completely dead. The young leaves and the basal part of stem of the low P plants (5 and 0.5 μMP) have developed a purplish coloration of the edges. The purple color might be due to the formation of anthocyanins, since the accumulation of anthocyanin in shoots was reported as typical response of plants to P deficiency (Jain et al., 2007). The leaves of the plants grown in 50 μMP medium were intact similarly to those of control plants. The low P plants (50, 5, and 0.5 μMP) had apparently larger root system with longer

and slender roots as compared to control plants (500 μMP) and shown in Photograph 3.1. These visual symptoms of the low P plant roots are consistent with the longer and more slender roots reported by Anghinoni and Barber (1980) and the increase in number and length of root hairs (Föhse and Jungk, 1983) in P-deficient plants. It was indicated that in low P condition, the responses of plant roots are similar regardless of the P deficiency level.

3.3.2. Plant Growth

Root DW was higher in the low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants (Figure 3.1). The shoot DW was lower in low P (5 and 0.5 μMP) plants than in control plants or in 50 μMP plants. This is consistent with the report that shoot growth decreased rapidly due to retranslocation of P from shoots to roots (Smith et al., 1990) and led to the decrease of the shoot-root ratio of the dry weight (Fredeen et al., 1989) under P-deficient condition.

It was indicated that plant could maintain its growth level under a medium with the P concentration ranging between 50 and 500 μMP. Phosphorus deficiency symptom was occurred in plants grown at the lowest P level (5 μMP). The whole plant growth came to be higher in 50 μMP with the higher root growth. The Pi level in the cytosol of plant cell is maintained at fairly constant concentration in the range of 5 to 8 mol/m^3 (Lauer et al., 1989) regardless of the external P concentration of the medium except under severe P deficient condition (Lee and Ratcliff, 1993). The higher root growth of the low P plants is consistent with the report that in P-deficient condition plant roots act as the dominant sink for photosynthates (Fredeen et al., 1989). It is known that P-deficiency stress triggers progressive loss of meristematic cells in the primary roots and thereby causes determinate root growth (Sànchez-Calderón et al., 2005).

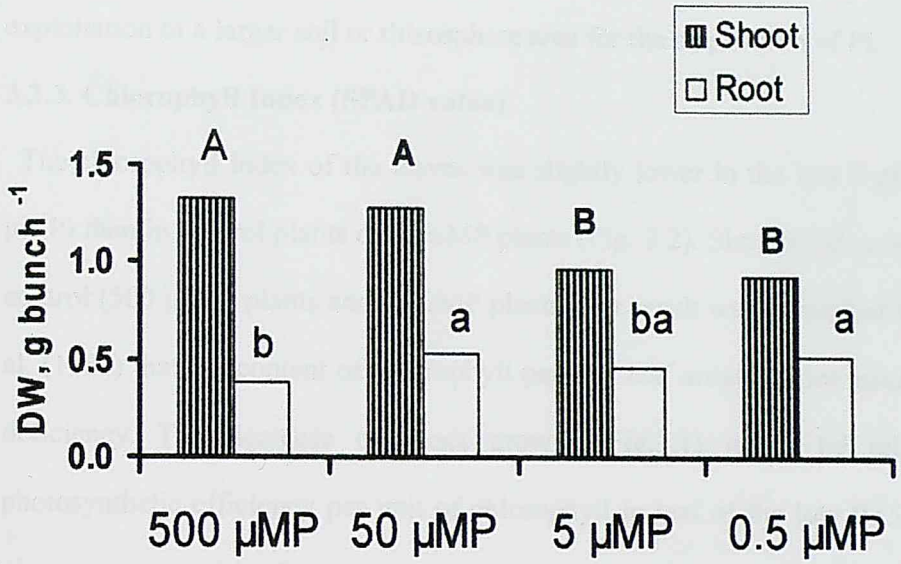
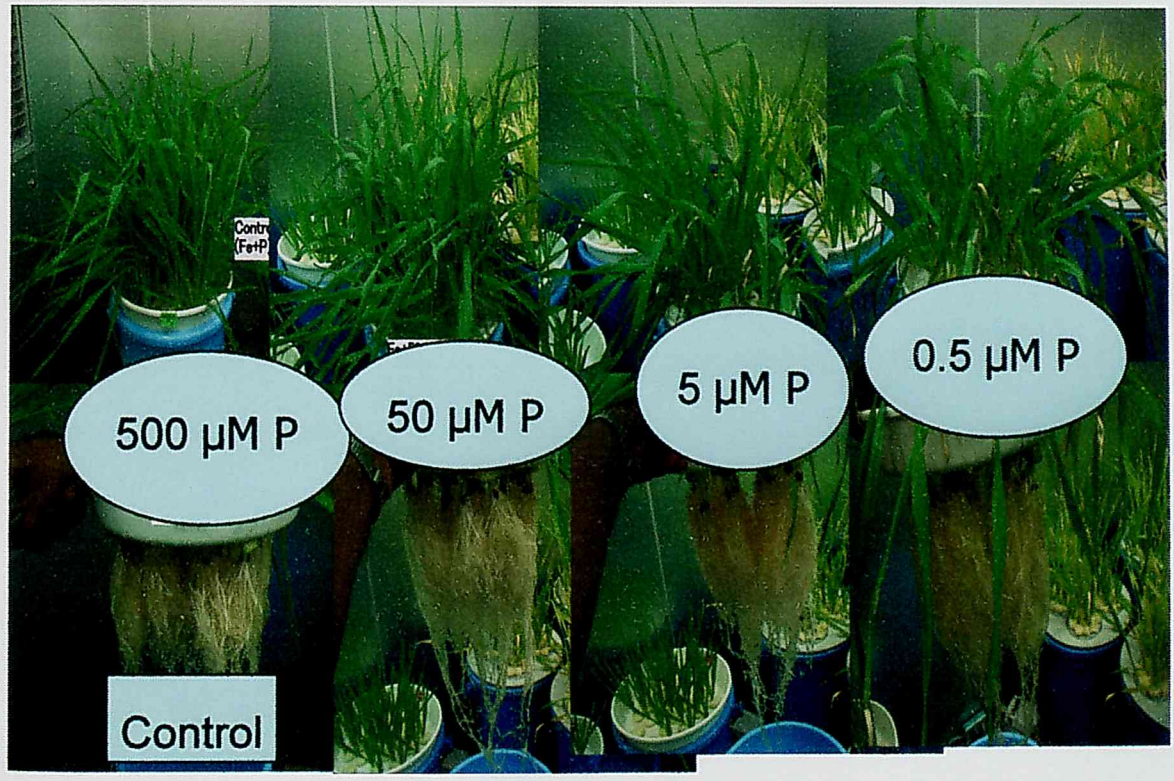


Figure 3.1. Shoot and roots dry matter of barley plants as affected by different P levels at 14 DAT. Different letters at the top of each bar indicate significant differences ($P<0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.



Photograph 3.1. Barley plants as affected by different P levels (14 DAT).

The larger root system of P-deficient plant is considered as a strategy for the exploitation of a larger soil or rhizosphere area for the acquisition of Pi.

3.3.3. Chlorophyll Index (SPAD value)

The chlorophyll index of the leaves was slightly lower in the low P plants (5 and 0.5 μMP) than in control plants or 50 μMP plants (Fig. 3.2). Similar index was obtained for control (500 μMP) plants and 50 μMP plants. Our result was consistent with Fredeen et al. (1989) that the content of chlorophyll per unit leaf area was not much affected by P deficiency. The decrease of shoot growth (Fig.3.1) might be related to lower photosynthetic efficiency per unit of chlorophyll in leaf of the low P (5 and 0.5 μMP) plants as reported by Lauer et al. (1989). The total P content in leaves may vary by a factor of 20 without affecting photosynthesis because the Pi concentration of the cytoplasm is regulated by a Pi homeostasis in which the vacuolar Pi acts as buffer (Mimura et al., 1990). The slight decrease of the index in the low P plants might be related to higher anthocyanin in shoots, which was reported as typical response of plants to P deficiency stress (Jain et al., 2007). The amount of anthocyanin was not measured in this experiment, but the purple color of the leaf edge and of the basal part of plant stem was observed.

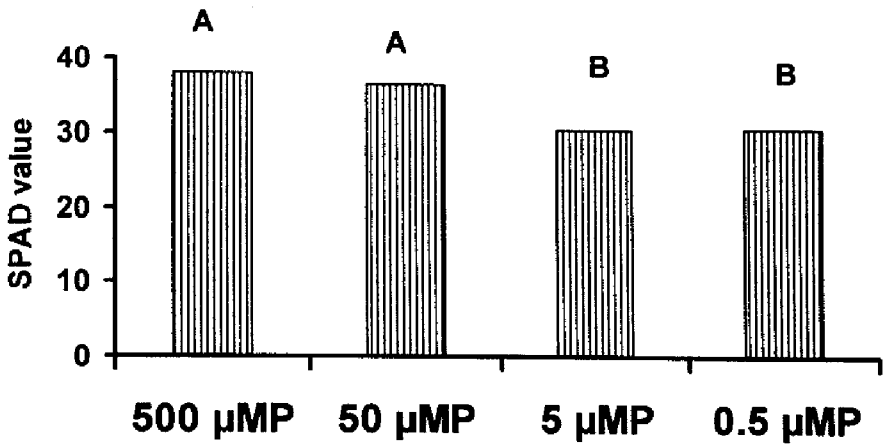


Figure 3. 2. Chlorophyll index (SPAD value) of leaves of barley plants as affected by different P levels at 14 DAT. Different letters at the top of each bar indicate significant differences ($P<0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

3.3.4. Mineral Element Content of Plant Materials

The shoot accumulation of Ca and Mg was lower in the low P (50, 5, 0.5 µMP) plants than in control (500 µMP) plants similarly to that of P (Table 3.1). The accumulation of K in plant shoot was not much affected by the low P treatment. This low Ca and Mg contents in shoots of the low P (5 and 0.5 µMP) plants may be one of the responsible factors for their low growth and slightly low chlorophyll index of their new leaves. It is well known that Ca is involved in cell division and Mg is a constituent of chlorophyll. It is well known that Mg is required by many enzymes involved in Pi transfer, the low P status of the low P plants might reduce plant requirement for Mg. It was noticeable that the plants grown in 50 µMP medium could have similar dry matter to that of control plants in spite of their lower accumulation of P, Ca, and Mg which are 73%, 23%, and 25% less than that of control plants. The accumulation of K and Mg in roots was similar among the plants with low P treatments and control plants (Table 3.1). The

accumulation of P of root decreased with decreasing medium P concentration similarly to the shoots. The roots of the low P plants accumulated higher amounts of Ca than that of the control plants. This high root Ca content in root may contribute to the growth of the roots of low P plants. The accumulation of Mn and Zn in shoot was not affected by the low P concentration of the medium. The well known antagonistic interaction between Zn and P (Loneragan et al., 1979; Olsen, 1972) was not observed, which was consistent with Pasricha et al. (1987) who reported that this interaction may not always occur in plants. The shoot accumulation of Fe and Cu was lower in 5 and 0.5 μMP plants than in control or in 50 μMP plants. The low accumulations of Fe and Cu in shoot of 5 and 0.5 μMP plants may be of the consequences of P stress leading to the low chlorophyll index and low growth, since Fe and Cu are involved in chlorophyll formation and in photosynthetic activities.

The accumulation of Mn and Zn in roots was higher in 5 and 0.5 μMP plants than in control plants. The accumulation of Cu in roots was higher in 50, 5, and 0.5 μMP plants than in control plants. The root accumulation of Fe was higher than that of control plants only at the lowest P level (0.5 μMP). The low shoot growth of the 5 and 0.5 μMP plants may be directly ascribed to low P status and indirectly ascribed to the resulting decreased shoot accumulation of Ca, Mg, Fe, and Cu. The higher root growth of the 0.5 μMP plants may be directly ascribed to low P status leading consequently to high Ca and high micronutrient such as Fe, Cu, Mn, and Zn accumulation. This higher Zn accumulation in roots of 0.5 μMP plants is consistent with the antagonistic Zn/P interaction (Loneragan et al., 1979).

As shown in Table 3.2., the concentration of the mineral elements, except for P, in shoot was not affected by the low P treatments of the plants. Phosphorus concentrations

of shoot and roots of the plants decreased with decreasing the medium P concentration. The concentrations of Ca, Mg, K, Zn, and Cu of roots were not significantly affected by low P treatment of the plants as compared to control plants.

Table 3.1. Accumulation of mineral nutrients in shoots and roots of barley plants grown with different P levels.

Treatment	mg plant $^{-1}$				μg plant $^{-1}$			
P(μM)	P	K	Ca	Mg	Fe	Cu	Mn	Zn
				Shoot accumulation				
500	2.98a	19.9a	2.88a	0.812a	30.2a	2.69a	7.09a	7.85a
50	0.792b	17.5b	2.22b	0.610b	32.2a	2.42ba	6.94a	7.81a
5	0.341c	18.3ab	1.46c	0.465c	22.3b	2.02b	6.53a	7.25a
0.5	0.287c	17.8ab	1.47c	0.474c	24.3b	2.02b	7.19a	7.89a
				Root accumulation				
500	1.112a	7.18a	0.259b	0.678a	37.8b	1.03b	2.53c	2.14b
50	0.378b	9.12a	0.328a	0.725a	34.4b	1.23a	3.23cb	2.33b
5	0.221c	8.46a	0.368a	0.580a	36.5b	1.19a	4.12b	2.86a
0.5	0.202c	9.15a	0.383a	0.619a	65.5a	1.23a	6.52a	3.18a

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test

The Mn concentration in roots was higher in the lowest P plants (0.5 μMP) than control plants. The concentration of Fe was lower in 50 and 5 μMP plants and higher in 0.5 μMP plants than control plants.

The ratio Fe/P in shoot and roots was increased according to the decrease of P concentration of the medium (Fig.3.3). This ratio increased in roots also with decreasing P concentration of the medium. It was suggested that P-deficiency induced high Fe/P

ratio in shoots and roots might be one of the causes of the decreased growth and chlorophyll index of 0.5 and 5 μMP plants. The interaction between P and Fe in relation with the expression of Fe chlorosis in plants grown under Fe-deficient conditions has received much attention (Cumbus et al., 1977; DeKock and Alexander, 1955; Mengel et al., 1984b; Pushnik et al., 1984). However, this interaction needs to be investigated to clarify the mechanism by which P deficiency affect Fe nutritional status of plants.

Table 3.2. Concentration of mineral nutrients in shoots and roots of barley plants grown with different P levels.

Treatment	mg g^{-1}				μg g^{-1}			
P(μM)	P	K	Ca	Mg	Fe	Cu	Mn	Zn
				Shoot concentration				
500	6.76a	45.3ba	6.54a	1.84a	68.3a	6.11a	16.1a	17.8a
50	1.85b	41.4b	5.26a	1.45a	75.6a	5.72a	16.3a	18.6a
5	1.08c	57.1a	4.62a	1.45a	69.9a	6.37a	20.8a	23.1a
0.5	0.92c	56.9a	4.68a	1.52a	77.7a	6.34a	23.1a	25.3a
				Root concentration				
500	8.73a	56.4a	2.04a	5.33a	297.1b	8.08a	19.9b	16.8a
50	2.19b	52.8a	1.87a	4.17a	196.8c	7.05a	18.3b	13.5a
5	1.45b	55.5a	2.42a	3.81a	239.8c	7.81a	26.9ba	18.8a
0.5	1.17b	53.4a	2.23a	3.62a	378.3a	7.13a	38.1a	18.5a

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test.

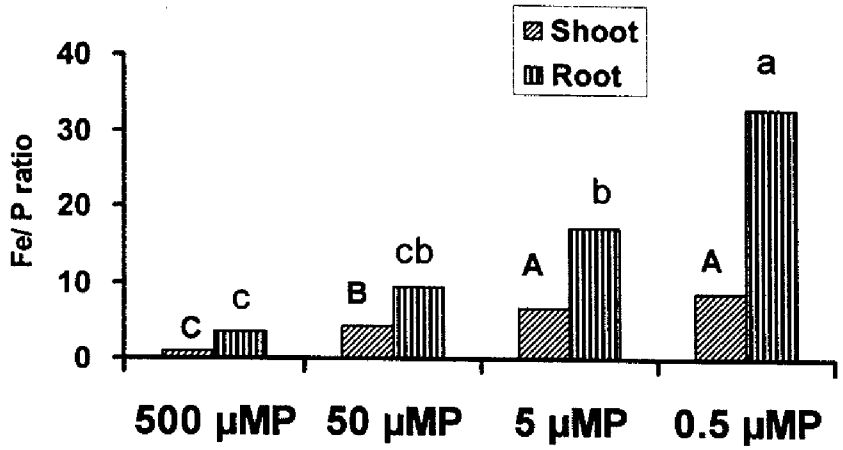


Figure 3.3. Ratio Fe/ P concentrations in shoots and roots of barley plants grown in Fe-sufficient nutrient solutions with different P levels 14 DAT. Different letters at the top of each bar indicate significant differences ($p<0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

3.4. SUMMARY

The results of this experiment showed that a concentration of 50 µMP in the medium may be beneficial in increasing root growth allowing a larger root growth. The supply of 500 µMP to the plants increased P concentration of shoot and roots, but did not increase growth and chlorophyll index as compared to 50 µMP plants. The deficiency of P shown in 5 and 0.5 µMP plants decreased growth, chlorophyll index of the plants. The concentrations of mineral nutrients, such as K, Ca, Mg, Cu, and Zn, in shoot and roots and those of Fe and Mn in shoot were not affected by low P condition (50, 5, and 0.5 µMP). The concentrations of Mn and Fe in plant roots were higher in lowest P level (0.5 µMP). The Fe/P ratio of shoot and roots was increased in plants according to the decrease of P level in the medium, suggesting that the P-deficiency-induced high Fe/P

ratio in plant organs and high root Mn concentration may be one the direct consequences of the detrimental effects of P deficiency in barley plants.

CHAPTER 4
EFFECT OF LOW PHOSPHORUS AND IRON-DEFICIENT CONDITIONS ON PHYTOSIDEROPHORE RELEASE AND MINERAL NUTRITION IN BARLEY

4. Effect of low phosphorus and Fe-deficient conditions on phytosiderophore release and mineral nutrition in barley

4. 1. INTRODUCTION

Phosphorus (P) and iron (Fe) are essential mineral elements for both animals and plants. Except for the strains of lactobacilli, bacteria found in milk, that 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 ATP (adenosine triphosphate) and the seat of genetic inheritance DNA (deoxyribonucleic acid), and RNA (ribonucleic acid) 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 had already been identified as a constituent of blood and in the composition of hemoglobin Fe in the adult human is about 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 is mainly due to the fact that 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, Al and Mn under acidic soil conditions and with Ca under alkaline soil conditions (Mengel et al., 2001). Therefore, combined Fe- and P-deficiency may potentially occur under both acidic and alkaline conditions, especially in calcareous soils where, due to high pH values, Fe as well as P is unavailable for plant uptake.

It has been reported that 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 in their strategies to take up sparingly soluble Fe under Fe-deficient conditions, namely strategy I plants (non graminaceous monocots and dicots) and strategy 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 showed a multiplication of roots and root hairs and a secretion and release of organic acids in order 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, while the roots continued to grow not only by retaining most 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 release of proton or organic acids. 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). The numerous reactions for the synthesis and release of PS and for the absorption of mineral elements by plants are mostly ATP-dependent reactions. The effect of Fe-deficiency on PS production of strategy II plants has been well documented. However, the effect of the combined Fe-

and P-deficiency in the growth medium, which is likely to occur under natural conditions, on PS and growth of these plants remained to be investigated.

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

4. 2. MATERIALS AND METHODS

4.2.1 Plant Growth

The experiment was conducted in phytotron with barley plants. It is generally recognized that the cv. Minorimugi used in this experiment releases mugineic acid and 2'-deoxymugineic acid, the compounds of PS (Kawai et al., 1993). The procedures for the culture under –Fe (without addition of Fe source) and low P conditions were modified based on the method of Kawai et al. (1993). The procedures for the preculture of the seeds and the growth of the seedlings in 10-L buckets containing 1/2-strength modified Hoagland and Arnon solution were described above in chapter 2. The seedlings were grown in the preculture solution until their second leaf reached a size about 20% of that of the first leaf. At this growth stage, the seedlings were transferred as bunches of 3 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 μM Fe-EDTA. The plants were allowed to grow in this +Fe medium for 48 hours. Subsequently, 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, 500 (control), 50, 5, and 0.5 μMP supplied as NaH_2PO_4. In this chapter, these media without addition of Fe source were designated as –Fe media. The plants were allowed to grow up to 21 days after transfer (DAT). The pH of the nutrient

solution was monitored daily and adjusted to 6.5. The nutrient solution was continuously aerated and renewed at 7-day intervals. The level of nutrient solution in the buckets was maintained by the addition of deionized water when necessary during the experiment.

4.2.2. Measurement of Phytosiderophore Released from Roots.

Roots of the bunch in triplicate for each treatment were soaked in 500 mL beakers filled up with deionized water at the onset of light and were allowed to release PS for 4 hours at 7, 14 and 21 DAT. After the collection of PS, the plants were returned to the growth medium. About 10 mg of thymol (Kanto Chemical Co., Japan) was added to each beaker with the root washing solutions for preventing microbial degradation of PS. The method and procedures for the collection of PS from the root washings are described above in chapter 2.

4.2.3. Measurement of Phytosiderophore Accumulated in Roots.

Three bunches of plants were sampled in each treatment at the onset of light at the sampling day (14 DAT), since PS concentration in roots was highest at that time (Kawai et al., 1993). The harvested roots were washed with deionized water. The roots were lyophilized and homogenized in 80% ethanol using a mortar and pestle. The resulting paste was diluted with 80% ethanol, filtered, and concentrated with a vacuum evaporator. The details of the method and procedures for the determination of the amount of PS accumulated in roots of the plants are described above in chapter 2.

4.2.4. Measurement of other plant parameters

The methods and procedures for the measurement of chlorophyll index of leaves, plant growth, and mineral nutrient contents in plant material are described in Chapter 2.

4.2.5. Statistical Analysis

The experimental design consisted of a completely randomized block with 3 replications. The data of the experiment were subjected to an analysis of variance (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" in Iwate University.

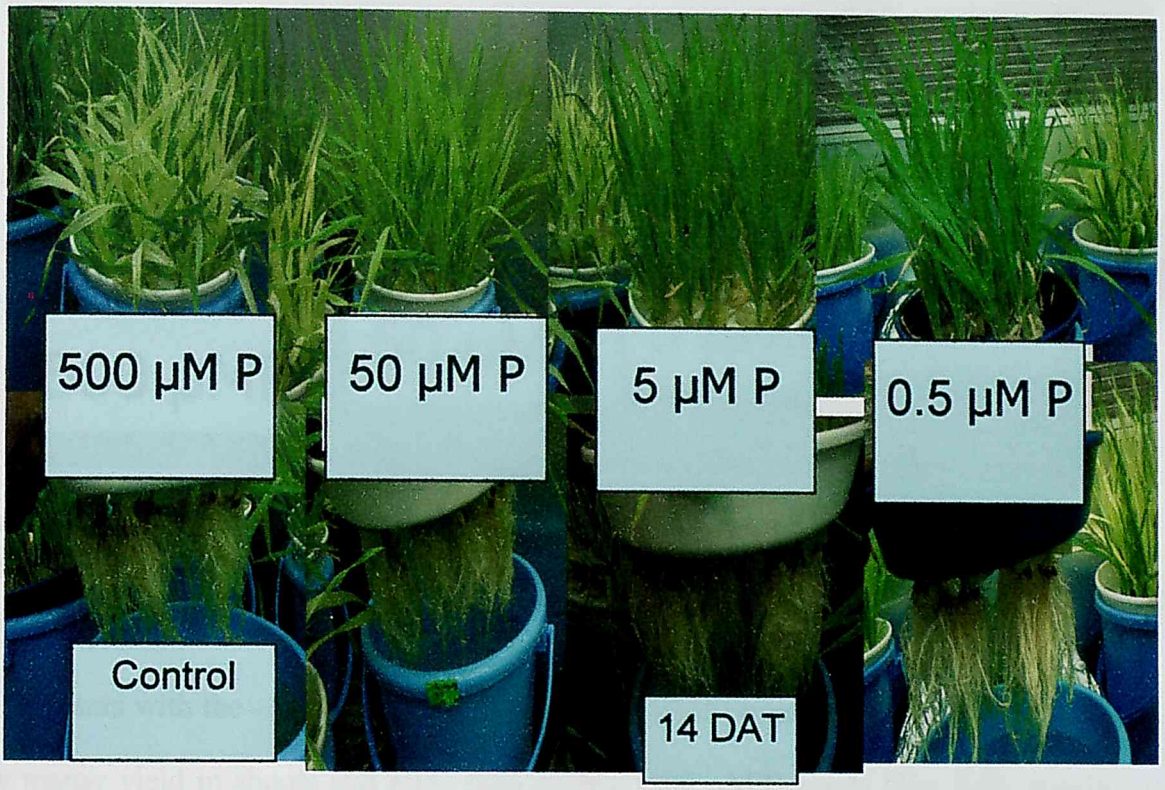
4.3. RESULTS AND DISCUSSION

4.3.1. Visual Symptoms

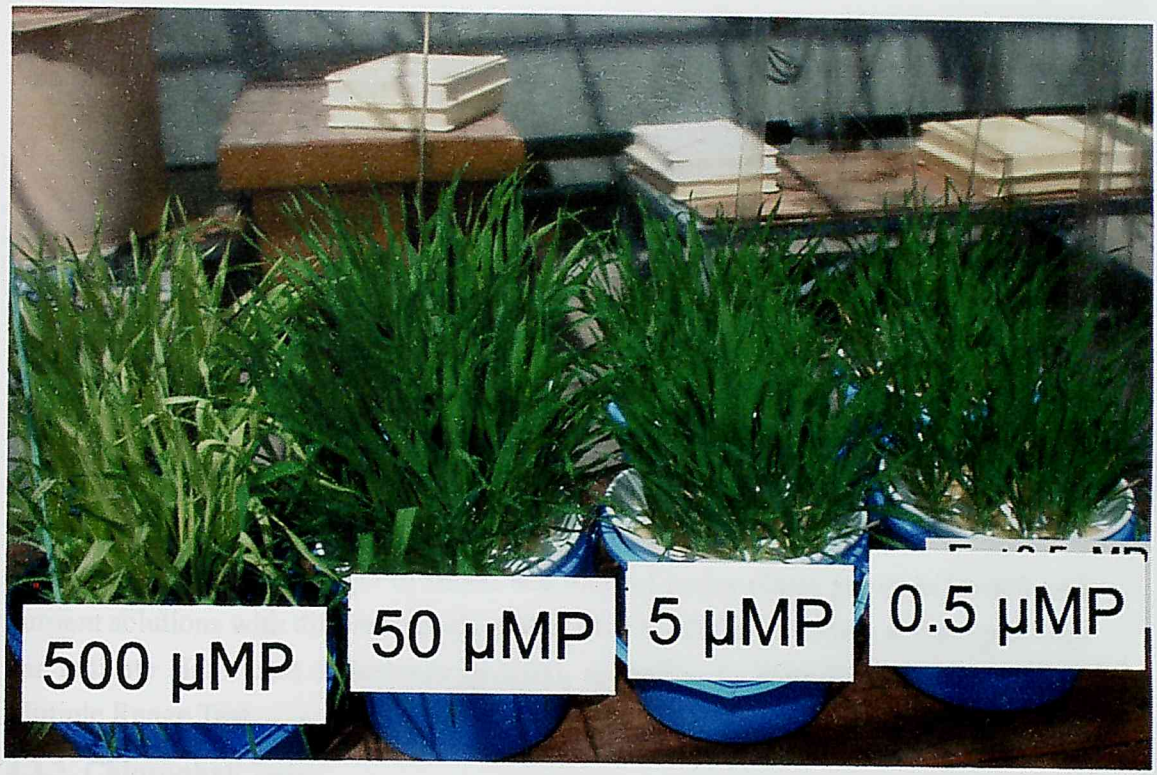
Iron-deficiency symptoms were observed visually in the control plants (500 μMP), starting by interveinal chlorosis at 4 DAT and developing to whitish young leaves at 14 DAT (Photograph 4.1). Slight interveinal chlorosis of the young leaves was also observed in the plants with 50 μMP treatment. However, no Fe-deficiency symptom was observed in the plants grown under the lower P level (5 and 0.5 μMP), 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 due to the damage of Fe-deficiency, while 50 and 5 μMP plants were still healthy at 21 DAT. The 0.5 μMP plants developed severe P-deficiency symptoms. The symptoms of P-deficiency in the 5 and 0.5 μMP plants progressed to old leaf senescence, starting from the tip of the leaf blade and developing to complete senescence of the oldest leaves.

The Fe-deficiency symptoms were considerably reduced in the 50 μMP plants and alleviated in the 5 and 0.5 μMP plants (Photograph 4.1). This indicates that, in barley plants grown in a –Fe nutrient solution with low P level, Fe-deficiency symptoms could be alleviated, attenuated, or masked by the low P treatment. In other words, it was considered that the expression of Fe-deficiency symptoms of control plants might be

induced by the high P concentration in the growth medium. The roots of the low P and Fe-deficient plants (Photograph 4.1) showed similar characteristics to that of the sole low P (5 and 0.5 µMP) plants as shown previously in chapter 3 (Photograph 3.1).



Photograph 4.1. Barley plants as affected by Fe-deficient nutrient solutions with different levels of P (Phytotron condition).



Photograph 4.2. Barley plants as affected by Fe-deficient nutrient solutions with different levels of P (Green house condition in late spring 14 DAT).

4.3.2. Dry Matter Production

The plants with the –Fe and low P (50, 5, and 0.5 µMP) treatments displayed a higher dry matter yield in shoots and roots than control (500 µMP) plants (Fig. 4.1). Among the three low P levels, the highest value of the dry weight was obtained when the P concentration of the medium was 50 µM. In addition, the growth of the 5 and 0.5 µMP plants was more vigorous than that of control (500 µMP) plants, but was less vigorous than that of the 50 µMP plants. It was considered that the inferior growth of control (500 µMP) plants was due to the high P concentration of the medium, indicating that the retardation of growth in the control (500 µMP) plants might be due to the high P concentration in the growth medium. These results suggested that low P supply to barley plants grown under –Fe conditions alleviated Fe-deficiency symptoms and enhanced growth irrespective of the severity of the P-deficiency symptoms.

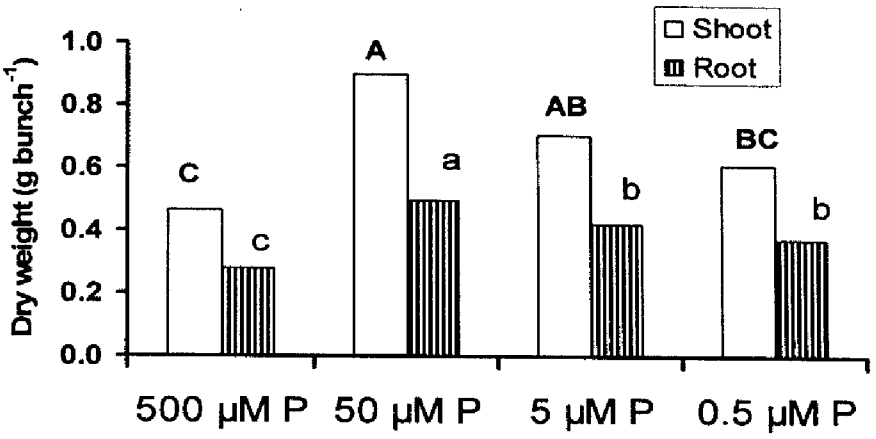


Figure 4.1. Dry matter weight of shoots and roots of barley plants grown in Fe-deficient nutrient solutions with different levels of P at 14 DAT. Different letters at the top of each bar indicate significant differences ($p<0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

4.3.3. Chlorophyll Index

The plants grown under the –Fe and low P conditions exhibited a higher chlorophyll concentration than control plants, although no significant differences were detected among the plants with the 3 low P treatments (50, 5 and 0.5 µMP) (Fig. 4.2). This result indicated that under –Fe and low P conditions, chlorophyll synthesis was enhanced. The chlorophyll indices of the young leaves were not significantly different among the low P plants (50, 5 and 0.5 µMP). The results of the chlorophyll index were consistent with the findings of Omar et al. (1971) who observed a relationship between increased phosphate (Pi) content and the degree of the severity of Fe chlorosis. They 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) of the leaves was markedly reduced by the increase in the P content, since the decrease in the P concentration of the –Fe medium led to

higher shoot Fe and chlorophyll contents. The close relationship between the contents of Fe and P in the appearance of chlorosis may be related to the fact that all the Pi in the plants with low P treatment was located in the cytoplasm and chloroplasm of the leaves (Foyer and Spencer, 1986), and that up to 90% of Fe accumulation in plant was located in the chloroplast (Price, 1968).

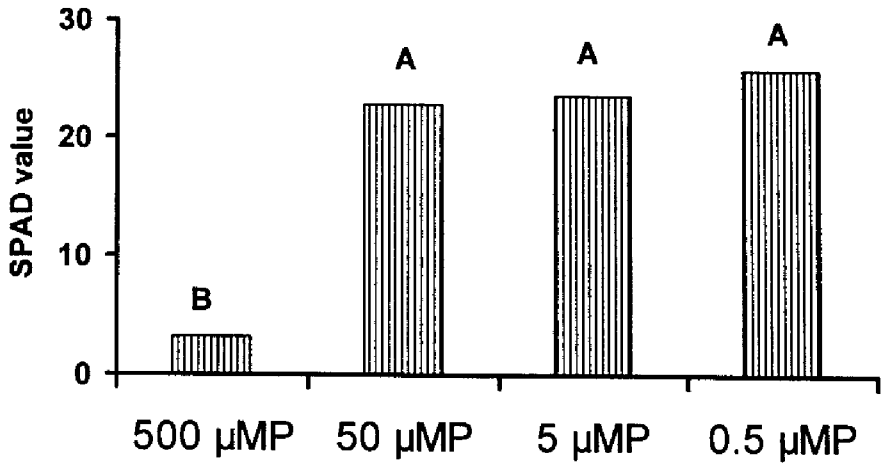


Figure 4.2. Chlorophyll concentration of new leaves of barley plants grown in Fe-deficient nutrient solutions at 14 DAT, as affected by different levels of P. Different letters at the top of each bar indicate significant differences ($p<0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

4.3.4. Phytosiderophore (PS) Release from Roots and Phytosiderophore Accumulation in Roots

The amount of PS released and its variation pattern were affected by the low P treatments (Fig. 4.3). The order of the amount of released PS was as follows: 7 DAT: 500 (control) >50 > 5> 0.5 µMP; 14 DAT: 500 = 50 >5 = 0.5 µMP. This release pattern indicated that PS release decreased with decreasing P concentration in the growth medium. It was observed that the amount of released PS decreased significantly under the lower P (5 and 0.5 µMP) conditions. The amount of released PS in 50 µMP plants

was similar to that of control plants. At 21 DAT, PS release of 500 and 50 μMP plants decreased because of severity of Fe deficiency while PS release increased in 5 μMP and 0.5 μMP plants which were still active.

In the plants grown under 50 μMP condition, the amount of PS released at 14 DAT was similar to that of control (500 μMP) 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 chlorosis (Fig. 4.2) was mild or not severe in the 50 μMP plants. These results showed that greening of leaves was not a reliable indicator for predicting the amount of PS release. It has been suggested that the mechanisms of PS release are genetically controlled and may be uncontrollable in special cases (Jolley and Brown, 1994). It is considered that the activity for formation of PS in roots may regulate the amount of PS release, which may be physiologically determined by the P and Fe status in shoots and roots in Gramineae.

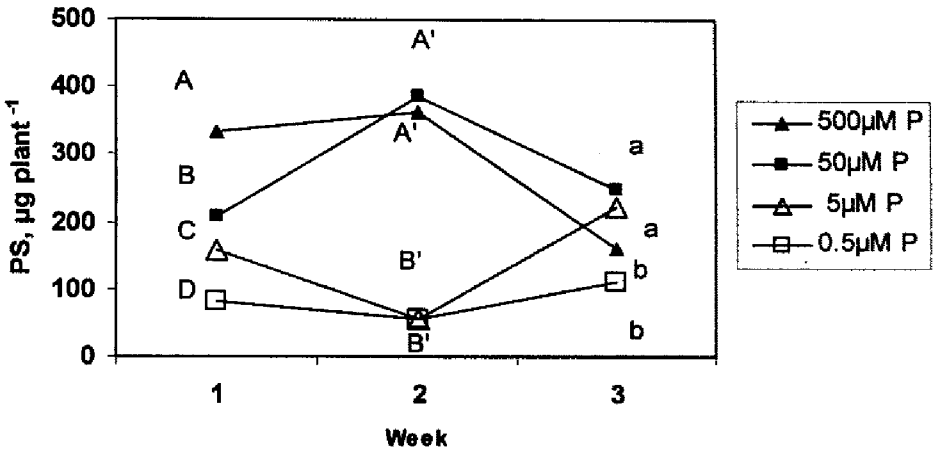


Figure 4.3. Phytosiderophore (PS) release from roots of barley plants grown in Fe-deficient nutrient solutions with different levels of P. Different letters at the top of each drawn line indicate significant differences ($p<0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

The pattern of PS accumulation in roots at 14 DAT was different from that of PS release from roots (Fig. 4.4). The amount of accumulated PS decreased with decreasing P level in the –Fe medium. These results indicated that the decrease in the P concentration in the –Fe medium decreased PS accumulation in roots. It was apparent that higher P conditions induced a higher PS accumulation in roots. However, it might be considered that low P conditions damaged the plasma membrane of the roots and enabled the leakage of PS accumulated in roots. This possibility should be examined in the future.

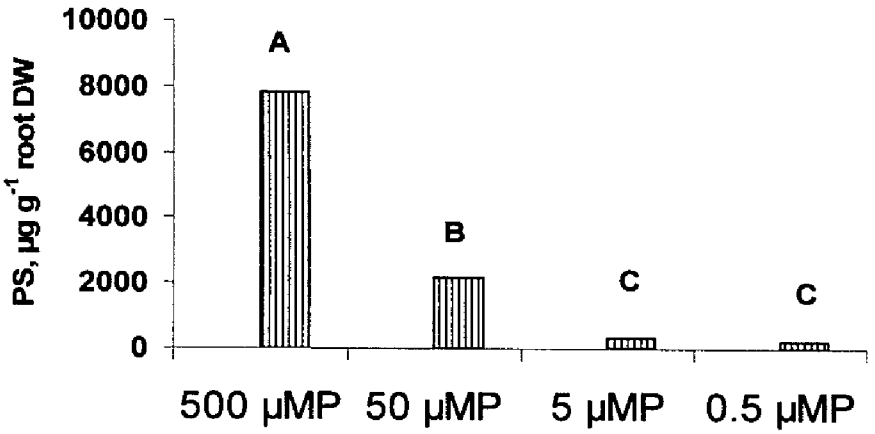


Figure 4.4. Phytosiderophore (PS) accumulation in roots of barley plants grown in Fe-deficient nutrient solutions at 14 DAT, as affected by different levels of P. Different letters at the top of each bar indicate significant differences ($p<0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

4.3.5 Relative Ratio of PS Release from Roots and PS Accumulation in Roots.

The relative ratio of PS release from roots and PS accumulation in roots at 14 DAT was higher in the plants with the –Fe and low P treatments, compared to that of the control plants (Fig. 4.5). The reason why a higher P concentration in the –Fe media reduced the relative ratio of PS release/ PS accumulation has not been known. 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, about 50% of the amount of PS accumulated in the roots was released in control plants (Fig. 4.5). In the plants with –Fe and low P treatments (50, 5, and 0.5 μMP), the relative ratio of PS exceeded 100%, indicating that the amount of released PS was larger than that of 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, it is considered that the origin of released PS needs to be elucidated. It is possible that potential PS which was not extracted with 80% ethanol might occur in the roots of the plants grown under –Fe 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.

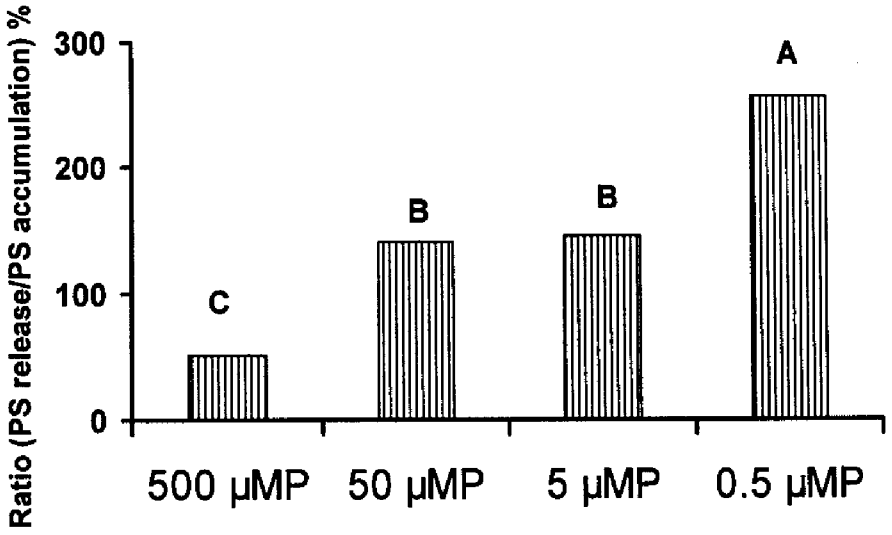


Figure 4.5. Relative ratio of PS release from roots and PS accumulation in roots of barley plants grown in Fe-deficient nutrient solutions at 14 DAT, as affected by different levels of P. Different letters at the top of each bar indicate significant differences ($p < 0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

4.3.6. Mineral Nutrition of Plants

Accumulation (mg plant^{-1}) and concentration (mg g^{-1} DW) of macro-nutrients in roots and shoots are shown in Table 4.1. and 4.2. The accumulation of N in shoots and roots in the plants with the 50 μMP treatment was higher compared to that of the

Table 4.1. Accumulation of nutrients in shoots and roots of barley plants grown in Fe-deficient nutrient solutions with different levels of P at 14 DAT

Treatment	mg plant^{-1}					μg plant^{-1}			
P(μM)	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

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test.

Table 4.2. Concentration of nutrients in shoots and roots of barley plants grown in Fe-deficient nutrient solutions with different levels of P at 14 DAT

Treatment	mg g $^{-1}$ DW					μg g $^{-1}$ DW			
P(μM)	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

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test.

control plants (Table 4.1). This higher accumulation of N in the plants with the 50 μMP treatment may be due to the higher Fe concentration in shoots and to the less severe P deficiency symptoms, since 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 along with the decrease of the P concentration in the medium.

The accumulation of K in shoots and roots was higher in 50 and 5 μMP plants than in control plants. However, K accumulation in the shoots and roots of the 0.5 μMP plants (lowest P treatment) was lower than that of control plants. The accumulation of K in shoots and roots was the highest in the 50 μMP plants. 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 to control plants. Therefore, K concentration may not be responsible for the greening of the leaves of the plants with the low P treatment.

The accumulation and concentration of Ca in shoots were not affected by the low P treatment. The accumulation of Ca in roots was higher in the 50 and 5 μMP plants. The low P treatment such as 50 μMP, enhanced Ca uptake by the plants, and the excess of Ca taken up may be accumulated in roots, which could account for the more vigorous root growth, since 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 treatment.

The accumulation of Mg, a component of chlorophyll, in shoots was not affected by the low P treatment (50, 5, and 0.5 μMP). The concentration of Mg in the shoots of the plants with the low P treatment was lower than that of the control plants. The increase of the chlorophyll index in the plants with the low P (50, 5, and 0.5 μMP) treatment (Fig.

4.2) 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 the plants with the low P treatment.

The accumulation (μg $plant^{-1}$) and the concentration (μg g^{-1} DW) of micro-nutrients in shoots and roots are shown in Tables 4.1 and 4.2. The accumulation of Fe in the shoots of the low P (50, 5, and 0.5 μMP) plants and the concentration of Fe in the shoots of the low P (5 and 0.5 μMP) plants were higher than those in control (500 μMP) plants. All of the Fe concentrations of the shoots 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 the plants with the low P treatment (50, 5, and 0.5 μMP), in spite of the low Fe concentration in shoots. It is considered that the appearance of 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 4.1 and 4.2 was higher in the plants with the low P treatment (50, 5, and 0.5 μMP) than that in control (500 μMP) plants. Total accumulation of Fe should be uniform among the treatments, because the –Fe media in all the treatments did not contain Fe. However, total accumulation of Fe was not uniform. The higher Fe accumulation in low P (5 and 0.5 μMP) plants may be derived from marginal contamination with Fe of the –Fe media which were prepared with deionized water.

It was considered that the plants grown under low P conditions were more efficient in the uptake of sparingly contaminated Fe. It was inferred that low P conditions might facilitate Fe uptake by roots.

The plants with the low P treatment (50, 5, and 0.5 μMP) showed a lower Fe concentration in roots and higher Fe concentration in shoots than the control (500 μMP) plants. The higher Fe concentration in the shoots of the plants may be due to the enhancement of internal Fe mobilization in the plant tissues. It appeared that the translocation of Fe from roots to shoots may be enhanced by the low concentration of P in shoots, although the accumulation of Fe in the roots of the low (50, 5, and 0.5 μMP) P plants was similar to that in control (500 μMP) 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 the plants increased with decreasing P concentration in the –Fe medium (Fig. 4.6). Our results were consistent with the findings previously reported. The higher concentration of P in control plants may lead to inactivation of Fe in the plant tissues, appearance of Fe-deficiency symptoms, and

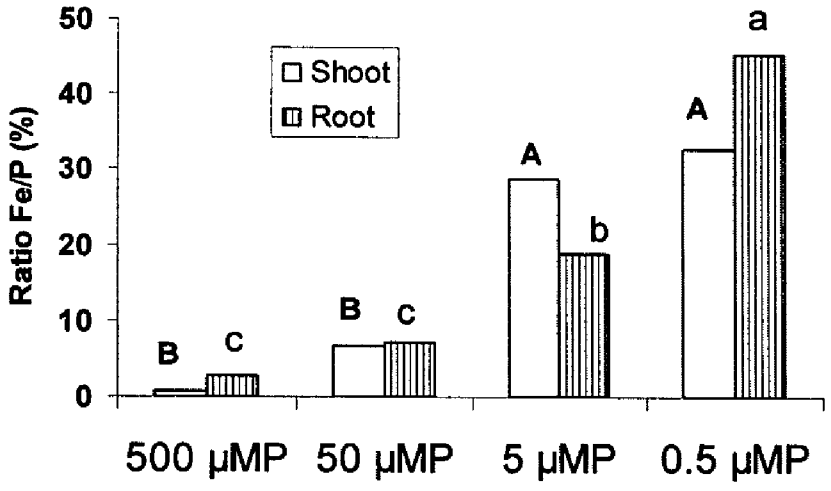


Figure 4.6. Ratio of Fe and P concentrations in shoots and roots of barley plants grown in Fe-deficient nutrient solutions with different P levels at 14 DAT. Different letters at the top of each bar indicate significant differences ($p<0.05$), according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

higher activity for PS formation. However, the mechanism of the inactivation of Fe by high P concentration in plant tissues still remained to be elucidated.

The accumulation of Cu in shoots was higher in the plants grown under low P conditions (5, 0.5 μMP) than that in control (500 μMP) plants, while the accumulation of Cu in roots was not significantly affected. This higher Cu accumulation in shoots is consistent with the report indicating that P deficiency resulted in a slightly higher Cu accumulation than that of the plants grown under adequate P conditions in bush bean (Wallace 1984). The concentration of Cu was the highest in the roots of the plants with the 5 μMP 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 the 5 μMP plants.

The accumulation of Zn in shoots was higher in the plants with the low P (5, 0.5 μMP) treatment, indicating the Zn-P antagonistic effect (Marschner and Cakmak, 1986) under –Fe conditions. However, Zn accumulation in roots of the plants with the low P treatment was similar to that of control plants. The concentration of Zn in the shoots was lower in the 50 μMP plants, but was not affected by the other lower P treatments, as compared to that of control (500 μMP) plants. The concentration of Zn in the roots of the 50, 5, and 0.5 μMP plants was much lower than that in control plants.

4.4. SUMMARY

Our results indicated that low P conditions ranging from 50 to 0.5 μMP alleviated Fe-deficiency symptoms, such as Fe chlorosis and higher release of PS by the barley roots, in spite of 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 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 conditions were not due to the low concentration of Mg or Fe but to the high P concentration which may repress the translocation of Fe from roots to shoots. It is considered that the lower ratio of Fe/P in the plants grown under 500 μMP (control) and –Fe conditions may be a major factor in the induction of Fe deficiency symptoms. Further studies focusing on the mechanism of inactivation of Fe by P in plants tissues should be carried out.

CHAPTER 5

EFFECT OF PHOSPHORUS-DEPLETION TREATMENT ON PHYTOSIDEROPHORE RELEASE AND CHLOROSIS IN BARLEY UNDER IRON-DEFICIENT CONDITION

5. Effect of phosphorus-depletion treatment on Phytosiderophores release and chlorosis in barley under Fe-deficient condition

5.1. INTRODUCTION

In practical agriculture, huge amount of phosphate (Pi) fertilizers are applied to crops in every region of the world to overcome the problem of P (phosphorus) deficiency in soils. This lack of P for plant growth in soils is mainly due to the fact that the Pi compounds found commonly in soils are mostly unavailable for plant uptake because they are fixed in insoluble forms (Brady and Weil, 2002). As responses of crops to tolerate P deficiency, a multiplication of roots and root hairs, a secretion or release of organic acids in order to uptake the sparingly soluble Pi of the growth medium were observed (Hoffland et al., 1989).

Interaction between P nutrition and cation uptake by plants was reported (von Wirén et al., 1996). Iron deficiency in soils with high pH may potentially be accompanied with P deficiency where both Fe and P might be unavailable for plant uptake. Phytosiderophores are released by the roots of strategy II plants (Takagi, 1993) in such condition. However, the effects of P deficiency on the synthesis or release of PS in strategy II plants remain to be clarified. It was previously found that PS release and accumulation in roots were enhanced by high P concentration (500 μMP) but depressed by low P (5 and 0.5 μMP) in Fe0 medium (Ladouceur et al., 2006). The plants treated with low P conditions showed lower expression of Fe chlorosis and enhanced growth. In order to clarify the role of P on the synthesis and release of PS in strategy II plants, an experiment was conducted with barley. In the experiment, the plants were grown in –Fe, +P medium for 7 days and for subsequent 14 days in –Fe, –P medium, comparing with

the plants grown continuously in –Fe, +P medium for three weeks. Plant growth, PS release from roots, and PS accumulation in roots were evaluated.

5.2. MATERIAL AND METHODS

5.2.1 Plant Growth

The seedlings of barley were grown hydroponically as previously described in Chapter 2. Ninety six bunches of plants (3 plants in bunch) were grown in seed box (10 L) filled with Hoagland-Arnon solution containing Fe for 15 days. Subsequently, the plants were transferred to the –Fe 1/2-strength modified Hoagland-Arnon solution (Takagi, 1993) containing 500 μMP supplied as NaH_2PO_4 (–Fe, +P medium) for 7 days to induce the Fe-deficiency symptoms as a "–Fe pretreatment". The plants pretreated for 7 days were referred to as "0 d plants", and then, 0 d plants were divided into two groups. After that, the roots of 0 d plants were washed with deionized water for 30 minutes. Half of 0 d plants was transferred to the nutrient solution where Fe and P were depleted (–Fe, –P medium) and designated as –P plants. The other half of 0 d plants was transferred to –Fe, +P medium, and designated as +P plants. The pH of the media was daily monitored and adjusted to 6.5. The media were renewed at 7 d intervals. Plants were harvested in triplicate at 0, 7, and 14 d after the treatments. Chlorophyll index (SPAD value), PS release from roots, PS accumulation in roots, plant growth, and the contents of Fe and P in plant materials were determined.

5.2.2. Measurement of Phytosiderophore Release from Roots

Roots of a bunch of plants in triplicate for each treatment were soaked in 500 mL beakers filled up with deionized water at the onset of light and were allowed to release PS for 4 hours at 0, 7, and 14 d. Concentration of PS in the root washings was measured as described in Chapter 2.

5.2.3. Measurement of Phytosiderophore Accumulated in Roots

Three bunches of plants were sampled in each treatment at the onset of light time, starting time of PS release, at the sampling days (0, 7, and 14 d) since PS concentration was the highest at that time (Kawai et al., 1993). The concentration of PS in the roots was measured as previously described in Chapter 2.

5.2.4 Measurement of Chlorophyll Index of Leaf

Chlorophyll index (SPAD value) of the youngest fully developed leaf of the plant was measured in 3 bunches of plants at 0, 7, and 14 d (This leaf corresponded to the 4^{th} leaf in the 0 d plants and to the 5^{th} leaf in the 7 d and the 14 d plants) by a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo, Japan).

5.2.5.Chemical Analysis of Plant Material

Three bunches of plants were collected at 0, 7, and 14 d, washed with deionized water, and divided into shoots and roots. The plant materials were dried in an oven at 55 °C for 24 hours continuously, weighed and digested in a mixture of nitric-perchloric acid (Piper and Piper 1950). The measurement of the mineral nutrients in the digested plant solutions was performed by the methods described in chapter 2.

5.2.6.Statistical Analysis

The experimental design was a completely randomized block with 3 replications. The data of the experiment were subjected to an analysis of variance (SAS Institute, 1988). The means were compared according to the Duncan Multiple Range Test ($p< 0.05$) using the computer "Origin 5" in Iwate University.

5. 3. RESULTS AND DISCUSSION

5.3.1. Visual Symptoms and Chlorophyll Index

Visual symptoms of Fe deficiency such as slight interveinal chlorosis were observed at 0 d (Photograph 5.1). In –P plants, the slight Fe chlorosis appeared at 0 d did not develop at 7 and 14 d. However, the P deficiency symptoms characterized by a slight purplish discoloration of the leaf edges started at 7 d and the senescence of the tips of the oldest leaves were observed at 14 d (Photograph 5.1). On the other hand, in the +P plants, the slight Fe chlorosis in the leaves at 0 d progressed towards severe chlorosis, whitish discoloration of the youngest leaves, for 14 d. The root system was larger and the roots were more dense in –P plants than in +P plants similarly to those of the low P (50, 5, and 0.5 μMP) plants and control (500 μMP) plants in photograph 4.1 of the previous experiment as shown in Photograph 5.2.

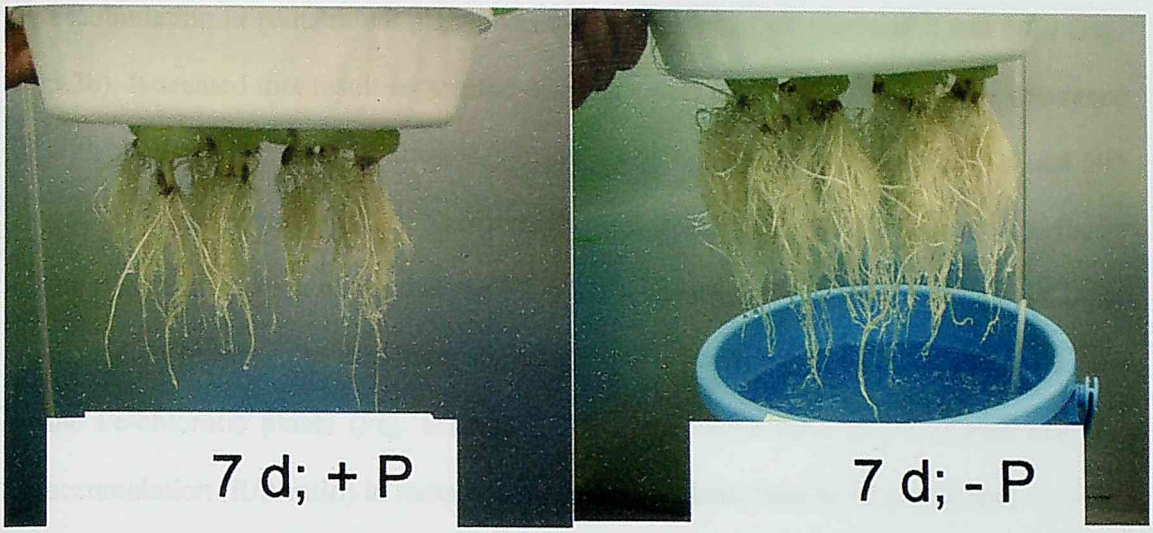
The chlorophyll index (SPAD value) of –P plants was higher than that of +P plants either at 7 or 14 d (Fig. 5.1). The chlorophyll index of +P plants, however, was decreased for 14 d. It was suggested that the P concentration of the medium played a major role in the depression of chlorophyll synthesis in barley grown in the –Fe medium. This result agreed with our previous result that chlorophyll index of barley grown with Fe0 and low P (0.5 μMP) was higher than that of the plants grown with Fe0 and high P (500 μMP) (Ladouceur et al. 2006). It was considered that plants could utilize their internal Fe absorbed in seed box before –Fe pretreatment for their metabolism and synthesize chlorophyll under –P condition.

It was indicated that the expression of Fe chlorosis in barley grown in –Fe medium was controlled by the P concentration of the medium. This finding was in accordance with the suggestion of Welch et al. (1991). The roots of +P plants were brownish and came to be shorter and less dense than those of –P plants, which were whitish, more dense, and longer.

5.3.2. Dry Matter Production

The dry weights in shoots (Fig. 5.2a) and in roots (Fig. 5.2b) were higher in –P plants than those in +P plants at 14 d. This result suggested that the –P treatment was beneficial to the growth of barley plants in the –Fe medium. It was inferred that the plants were grown under –P condition utilizing the small amount of internal Fe and P which were absorbed in seed box or in the –Fe pretreatment, which may result in higher growth than +P plants. Mimura et al. (1990) reported that the total P content in leaves might vary by a factor of 20 without affecting photosynthesis, and that the Pi concentration in the cytoplasm was regulated in a narrow range of available Pi.

Photogragh 5.1. Minus Fe barley plants as affected by +P or –P medium 7 DAT



Photogragh 5.2. Minus Fe barley plant roots as affected by +P or –P medium 7 DAT

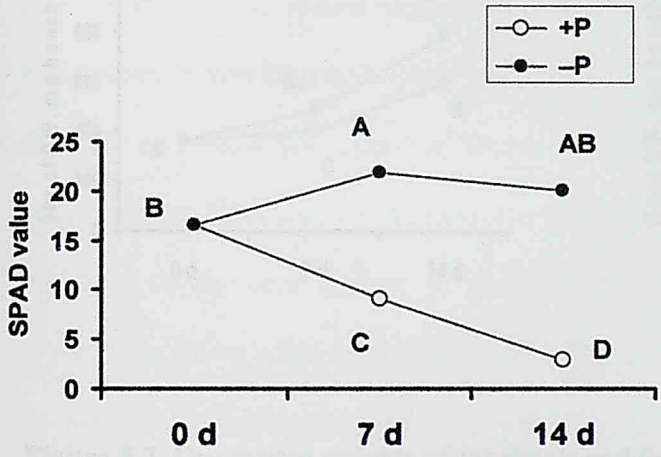


Figure 5.1. Chlorophyll index in the youngest leaves of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicates significant differences ($p< 0.05$) according to Duncan Multiple Range Test.

5.3.3. Phytosiderophore Release from Roots and Phytosiderophore Accumulation in Roots

The roots of –P plants released nearby 50% higher amount of PS at 7 d, and over 100% higher amount of PS at 14 d than those of +P plants (Fig. 5.3a). It seemed that result contradicted to our previous result that higher P concentration in the media induced higher PS release by roots in barley (Ladouceur et al., 2006). The amount of PS

accumulation in roots of +P plants was almost similar to –P plants at 7 and 14 d (Fig. 5.3b). It seemed that result contrasted with our result where PS accumulation decreased with decreasing P concentration in the Fe0 medium between the range of 0.5 and 500 μM (Ladouceur et al., 2006). These results have not been reported and are opposite to the common understanding that the PS release increases when Fe-deficiency symptom is severer, because our data showed that the green plants released much higher PS than the Fe-chlorotic plants (Fig. 5.1 and 5.3a). The relative ratio of PS release over PS accumulation (R/A ratio) in roots was higher in –P plants than in +P plants, and

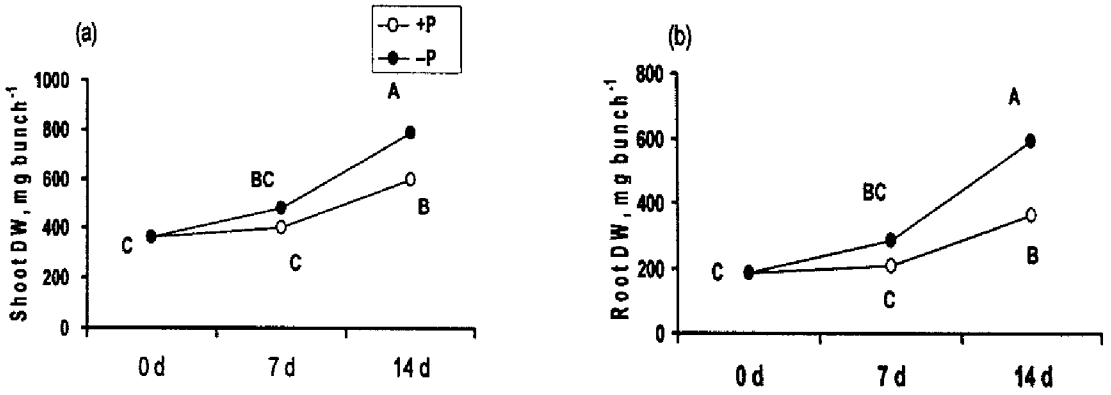


Figure 5.2. Dry matter weights of (a) shoots and (b) roots in barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p<0.05$) according to Duncan Multiple Range Test.

increased gradually from 0 d to 14 d in –P plants (Fig. 5.3c). In +P plants, the R/A ratio was almost stable between 0 and 14 d. The R/A ratio indicated that the released amount of PS from roots counted for about 50 % in +P plants but even more than 100 % in –P plants. It was noticeable that the amount of PS release by roots was higher than the

amount of PS accumulated in roots in –P plants. The reason why R/A could exceed 100 % is not known and needs to be investigated. This higher R/A ratio in –P plants than that in +P plants was consistent with the previous finding (Ladouceur et al., 2006). It seemed that the data contradicted to our previous results. However, both data are considered to be true because of the following reason. The results indicated that the activity of PS biosynthesis induced by the Fe-deficiency stress in 0 d plants was not reduced and kept being increased during the –P treatment, suggesting that the activity of the root cells for PS biosynthesis, once induced in –Fe pretreatment for 7 d, was maintained irrespective of the P status. On the other hand, PS release was much enhanced by –P treatment. It was speculated that –P treatment induced lower P concentration in membrane or putative transporter for release and reabsorption of free PS in roots. It may be possible that low P status in plant tissues changed the structure of the lipid or protein and enhanced largely PS release. Low P status might activate PS release and/or inactivate PS reabsorption by regulating the activities of the tools for PS transport on the root surface. It is known that released PS is reabsorbed without complexing Fe by roots even in the time of PS release (Kawai and Alam, 2006). Thus, the measured amount of released PS is the net amount of PS release minus the amount of reabsorbed PS by roots in the experiment. It is also possible in –P plants that only the activity for PS reabsorption might be lowered by low P treatment and higher amount of PS might be remained in root washing in the procedure of PS collection. The transporter for release and reabsorption of PS has not been characterized. The transporter for absorption of PS-Fe^{3+} complex was reported (Murata et al., 2006).

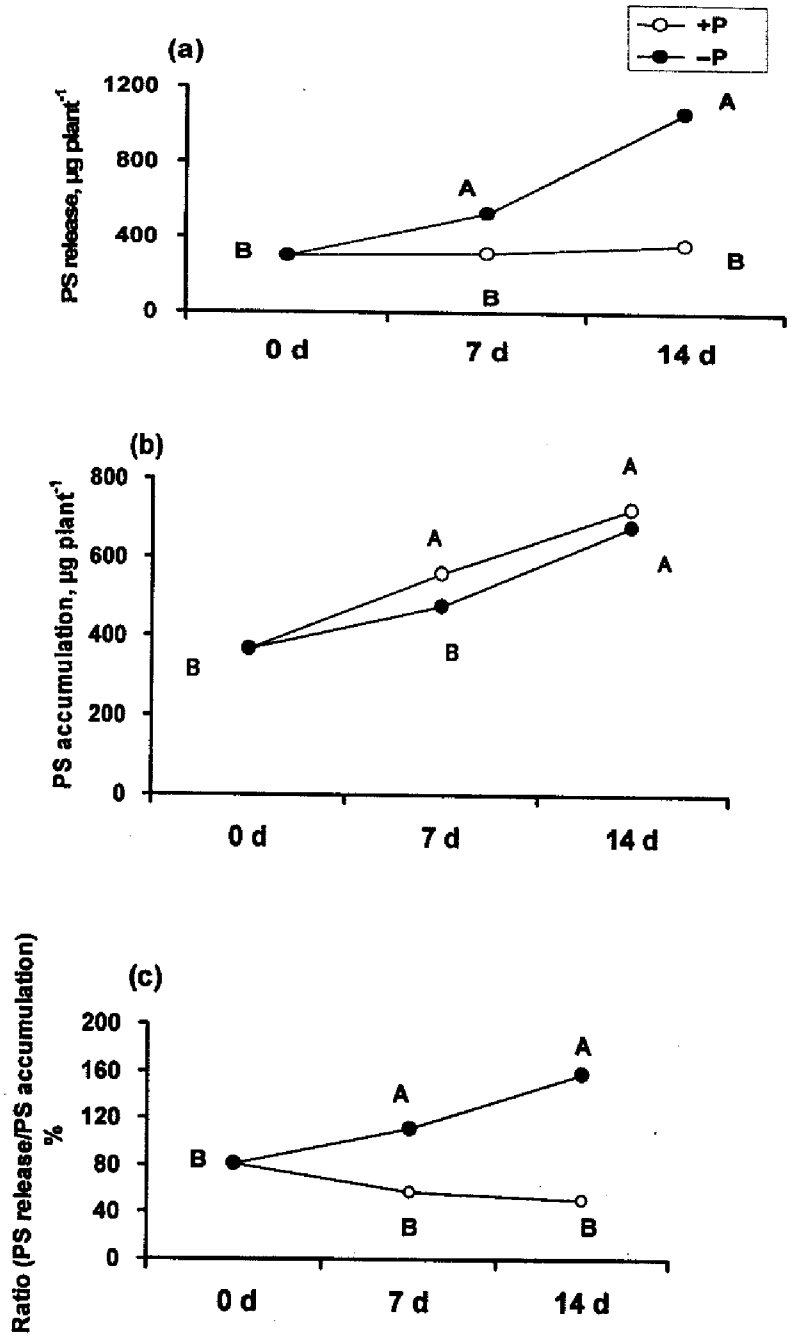


Figure 5.3. (a) Release amount of PS from roots, (b) accumulated amount of PS in roots, and (c) relative ratio (PS release/PS accumulation %) (R/A ratio) in roots in barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p<0.05$) according to Duncan Multiple Range Test.

The higher growth of –P plants (Fig. 5.2) may also be considered as one of the factors responsible for this higher PS release. However, the difference of the dry weights between –P and +P plants was small as compared to the difference of the amounts of their PS release. Shoot Fe content is known to regulate PS release (Gries et al., 1995). Our result, however, showed that Fe concentration of shoots was almost same at 14 d in both +P and –P plants (Fig. 5.5a).

The PS release by roots is known to be highly dependent on metabolic energy (Takagi, 1990). The amount of ATP for formation and release of PS in –P plants also needs to be measured. The necessity of ATP for PS re-absorption has not been reported. The mechanism of regulation of release and re-absorption of PS by low P condition remained to be clarified. It was also suggested that the tolerance of barley to Fe deficiency might be increased by decreased P concentration in rhizosphere because of the resulting increase of PS release.

5.3.4. Mineral Nutrition of the Plants

Phosphorus

Obviously, P concentration of shoots (Fig. 5.4a) and roots (Fig. 5.4b) and P accumulation in shoots (Table 5.1) and roots (Table 5.2) were lower in –P plants than those in +P plants. The P concentration of shoots and roots in –P plants decreased from 7 to 14 d due to the enhanced growth resulting in the dilution effect. Phosphorus concentration of shoots and roots in +P plants was almost stable between 7 and 14 d.

It was suggested that high P concentration of plant tissues might inactivate internal Fe, resulting in reduction of growth and development of the Fe chlorosis in leaves. It is considered that Fe is immobilized by the phosphate on the organic compounds. When P supply is abundant, the compounds with phosphate may increase and mobile Fe may be

decreased in cells. This result indicated that the Fe chlorosis and the low growth in +P plants were due to high P concentration in the plant tissues. This was consistent with our result (Ladouceur et al., 2006).

The remobilization of internal P in plants in P starved plants was reported (Smith et al., 1990). However, Michael (1939) reported that, in plants grown under –P condition, P in the membrane (phospholipids) and nucleic acid were not mobilized by P starvation.

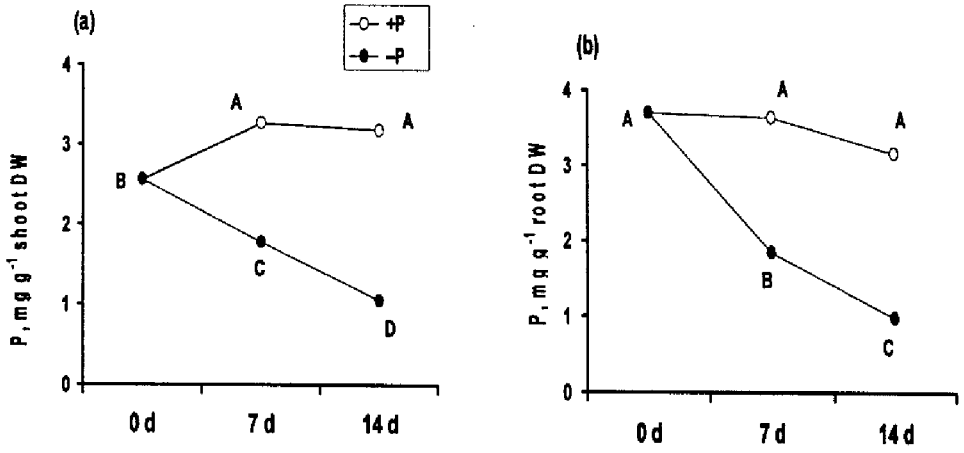


Figure 5.4. Concentration of P (a) in shoots and (b) in roots in barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p< 0.05$) according to Duncan Multiple Range Test.

Iron

Higher Fe concentration was obtained in shoots of –P plants than that of +P plants at 7 d, though it was similar to that of +P plants at 14 d (Fig. 5.5a). The Fe concentration of roots was slightly higher in –P plants than in +P plants at 7 and 14 d (Fig. 5.5b). This higher Fe amount in –P plants may be derived from higher uptake of the marginally contaminated Fe in deionized water used for –Fe media. The alleviation of Fe chlorosis in –P plants might be due to the higher Fe concentration of shoots in the –P plants at 7 d. However, the Fe concentration of shoots in –P plants was lowered to similar level of +P plants at 14 d. Therefore, Fe concentration of shoots may not be a main factor for the

alleviation of Fe chlorosis. The accumulation amounts of Fe in shoots and roots were higher in –P plants than in +P plants either at 7 or 14 d as shown in Table 5.1 and 5.2. Total accumulation (shoot + root) of Fe calculated from Table 1 and 2 was higher in –P plants at 7d as well as at 14 d than that in +P plants. The higher accumulation and concentration of Fe in roots in –P plants might be derived from greater ability of –P plants to uptake the marginally and sparingly contaminated Fe in the –Fe medium. This result is in agreement with our previous report that the total accumulation (shoot + root) of Fe was higher in the low P plants than in high P plants when grown in Fe0 media (Ladouceur et al., 2006).

The Fe concentration of shoots in +P and –P plants were always within the range of the critical deficiency level, 30-50 μg g^{-1} dry matter (Fig. 5a) (Römheld and Marschner, 1991), or lower than 65 μg g^{-1} dry matter (Tang et al., 1990). Therefore, it was certain that both –P and +P plants were in –Fe nutritional status, but large difference in the expression of Fe chlorosis was induced between –P and +P plants. The greener youngest leaves in –P plants could be due to the enhanced utilization of the internal Fe. This result indicated that the expression of Fe chlorosis can not be predicted based on Fe concentration in leaves only. It was suggested that the development of Fe chlorosis in plants grown in –Fe, +P medium was induced by not only the deficiency of Fe but also high concentration of P in plant tissues. Low P concentration of plant tissues must activate Fe in the apoplast or symplast and make it available for chlorophyll synthesis.

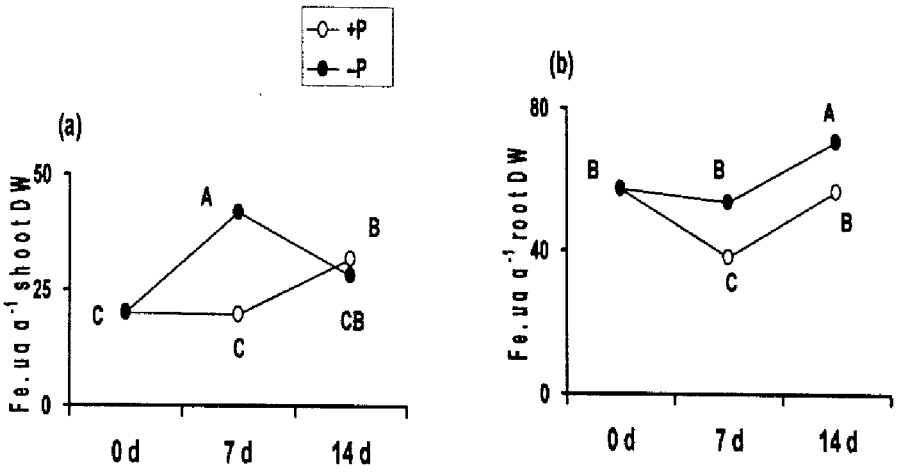


Figure 5.5. Concentrations of Fe (a) in shoots and (b) in roots in barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p < 0.05$) according to Duncan Multiple Range Test.

Ratio Fe/P

The ratio of the concentration of Fe over that of P (Fe/P) in shoots and in roots was continuously higher either at 7 d or at 14 d in –P plants than in +P (Fig. 5.6). This can be explained by the lower P concentration in shoots and roots, and higher Fe concentration in roots coupled with similar Fe concentration in shoots in the –P plants as compared to the +P plants. This result indicated that the –P treatment of the –Fe medium through the decrease in P concentration in plant tissues allowed plants to uptake the marginally and sparingly contaminated Fe from the –Fe media with higher efficiency than the +P treatment. As a result, the ratio Fe/P was higher and the greening of the youngest leaves occurred in –P plants. It was consistent with our previous report that barley showed higher Fe/P ratio in shoots and roots under low P (50, 5, and 0.5 μMP) and Fe0 condition than under high P (500 μMP) and Fe0 medium (Ladouceur et al., 2006). It is known that Fe deficiency in crop production can be caused by any factor that interferes with Fe absorption and translocation or impairs its utilization in metabolic processes

(Brown, 1961; Welch et al., 1991). This higher Fe/P ratio in the –P plants may be a causal factor of the greening of the youngest leaves of these plants, since it was reported that the severity of Fe chlorosis may be controlled by Fe/P ratio (DeKock and Alexander, 1955; Pushnik et al., 1984). The decrease in the P concentration in shoots of the plants by the –P treatment of the –Fe medium might enhance P remobilization in shoots which might result in the liberation of Fe in plant tissues and the greening of the leaves. The ratio Fe/P in the tissues of graminaceous plants grown in –Fe medium may counteract the expression of Fe chlorosis. However, the mechanism of the physiological competition between P and Fe in plant tissue needs to be investigated.

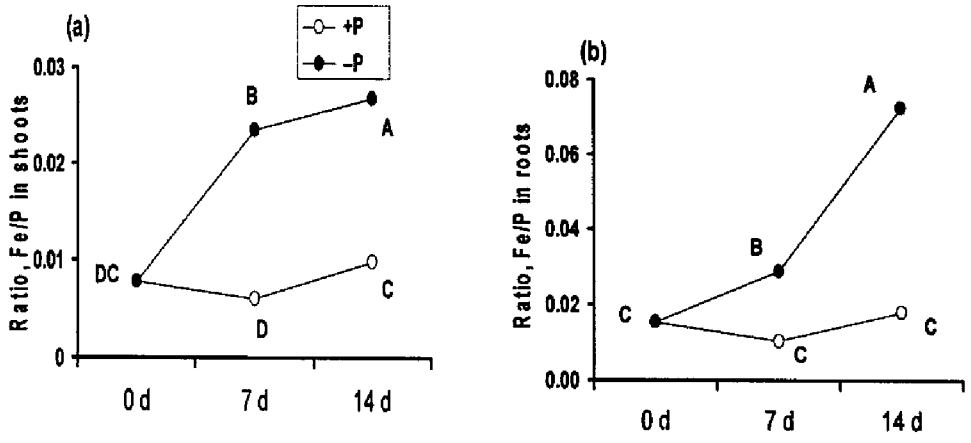


Figure 5.6. Ratio of Fe/P concentrations (a) in shoots and (b) in roots of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p < 0.05$) according to Duncan Multiple Range Test.

Potassium

The accumulations of K in shoots and roots were higher in –P plants than in +P plants as shown in Table 5.1 and 5.2 at either 7 d or 14 d. However, this increase in the K accumulation amounts in shoots of –P plants was not significant as compared to that of the +P plants at 14 d. The concentration of K in shoots in –P plants was higher at 7 d and similar at 14 d to that in +P plants, respectively (Fig. 5.7). The concentration of K in

Table 5.1. Accumulation of mineral nutrients in shoots of barley plants grown in –Fe media with P (+P) or without P (–P).

	mg plant $^{-1}$				μg plant $^{-1}$			
Treatment	K	P	Ca	Mg	Fe	Mn	Cu	Zn
				Accumulation in shoots at 0 d				
+P	10.4c	0.312c	1.15d	0.079c	2.43d	2.16c	1.79c	4.08d
–P	10.4c	0.312c	1.15d	0.079c	2.43d	2.16c	1.79c	4.08d
				Accumulation in shoots at 7 d				
+P	11.9c	0.443b	1.48cd	0.111b	2.59d	4.17b	1.82c	5.50c
–P	14.7b	0.281c	1.65c	0.113b	3.98c	4.55b	2.37b	6.48b
				Accumulation in shoots at 14 d				
+P	18.8a	0.710a	2.17b	0.164a	6.88b	5.41ab	2.70b	7.34ab
–P	20.4a	0.301c	2.65a	0.186a	8.05a	5.76a	3.54a	8.16a

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Duncan Multiple Range Test.

Table 5.2. Accumulation of mineral nutrients in shoots of barley plants grown in –Fe media with P (+P) or without P (–P).

	mg plant $^{-1}$				μg plant $^{-1}$			
Treatment	K	P	Ca	Mg	Fe	Mn	Cu	Zn
				Accumulation in roots at 0 d				
+P	5.41c	0.231b	0.516c	0.079c	3.54c	0.983b	1.42b	2.28b
–P	5.41c	0.231b	0.516c	0.079c	3.54c	0.983b	1.42b	2.28b
				Accumulation in roots at 7 d				
+P	5.54c	0.252ab	0.573bc	0.089bc	2.65d	1.25a	2.30a	3.31ab
–P	8.03b	0.181b	0.612bc	0.101b	5.08b	0.969c	1.93a	2.66b
				Accumulation in roots at 14 d				
+P	7.03bc	0.343a	0.631b	0.132a	6.06a	1.14ab	2.55a	3.92a
–P	12.7a	0.174b	0.804a	0.135a	6.72a	0.727c	2.23a	3.85a

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Duncan Multiple Range Test.

roots was not affected by the –P treatment at 7 d (Fig. 5.7). However, higher K concentration in roots was obtained in –P plants as compared to that in roots in +P plants at 14 d. Minus P treatment of the plants did not affect significantly the K concentration and accumulation in shoots, but increased the concentration and accumulation of K in roots. This result was similar to our previous result that the accumulation of K in roots in plants grown in low P (50, and 5 μM) and Fe0 medium was higher than that in roots in plants grown in high P (500 μM) and Fe0 medium (Ladouceur et al., 2006). This result indicated that the depletion of P in the –Fe medium might affect the K translocation from roots to shoots at 14 d in the plants. This higher K accumulation and concentration in roots of –P plants at 14 d may be one of the factor responsible for the higher root growth of the –P plants. It might also contribute to the alleviation of Fe chlorosis in these plants, since it was reported that K ameliorated Fe-deficiency in peanut grown in calcareous soil with corresponding increase in chlorophyll concentration (Barak and Chen, 1984). It was reported that additional K might enhance the tolerance of young rice to Mn toxicity by increasing Fe absorption without affecting Mn absorption (Alam et al., 2003). They reported that Mn-induced Fe-deficiency symptoms was ameliorated by additional K in the growth medium which increased as well chlorophyll index of the leaves.

Calcium

The accumulation of Ca in shoots (Table 5.1) and in roots (Table 5.2) of the –P plants was higher than those of the +P plants. The increase in Ca accumulation in shoots and roots was significant at 14 d. The concentration of Ca in shoots of –P plants was higher at 7 d and similar at 14 d to that in shoots of +P plants (Fig. 5.7). The pattern in roots was different where –P plants displayed lower Ca concentration at 7 d and higher Ca

concentration at 14 d than those in +P plants (Fig. 5.7). The –P treatment of the plants caused the concentration of Ca to decrease in roots and increase in shoots at 7 d, but it increased the concentration of Ca in roots without affecting that in shoots at 14 d, indicating that a decrease might occur in Ca translocation from roots to shoots at this period. This higher Ca concentration and accumulation in roots of the plants at 14 d may be one of the factors contributing to the significantly higher root growth shown in the –P plants, since Ca is a regulator of growth in length and involved in cell division. This result is consistent with that of our previous finding that low P and Fe0 medium did not affect Ca concentration in shoots of barley plants (Ladouceur et al., 2006).

Magnesium

The accumulation of Mg in shoots (Table 5.1) and in roots (Table 5.2), and the concentrations of Mg in shoots and in roots (Fig. 5.7) were not generally affected by the –P treatment of the plants grown in –Fe medium. However, an exceptionally high Mg concentration in shoots at 7 d was obtained in the –P plants. This result is in agreement with the previous finding that Mg accumulation in barley plants was not affected by low P and –Fe medium as compared to that of the plants grown in high P and –Fe medium (Ladouceur et al., 2006). Even if the –P plants showed higher growth and greener leaves than those of the +P plants, the latter displayed similar concentration and accumulation of Mg in shoots and roots as the former in spite of its chlorotic leaves. Though Mg has roles as chlorophyll constituent, regulator of cellular pH, and cation-anion balance, and turgor of cells (Marschner, 1995), it was not responsible for the greening and the higher growth of the –P plants grown in –Fe medium. It was reported that 6-25% of the total Mg is bound to chlorophyll depending on the magnesium nutritional status, and that the concentration of Mg, which is not firmly bound in organic structures but located in the

''metabolic pool'', has to be strictly regulated (Marschner, 1995). The concentration of Mg in the metabolic pool of leaf cells (in cytoplasm and chloroplasts) is assumed to be in the range of 2-10 mM (Leigh and Wyn Jones, 1986). It was reported that an increase in the Mg concentrations in the ''metabolic pool" of the sunflower leaf from 3-5 mM to 8-13 mM in the stroma of the chloroplasts inhibited photophosphorylation and photosynthesis (Rao et al., 1987). It might be possible that Mg concentration and accumulation in shoots and roots remained unaffected while imbalance between ''metabolic pool" and vacuoles concentration of Mg might have detrimental effect on chlorophyll synthesis, photophosphorylation and photosynthesis in +P plants. The –P treatment of the plants might have some positive effect on the balance between the Mg concentrations in these two groups of organelles and might enhance chlorophyll synthesis in –P plants. This phenomenon needs to be investigated.

Copper

The concentration (Fig.5.8) and the accumulation (Table 5.2) of Cu in roots were not generally affected by the –P treatment accordingly to our previous finding that Cu concentration in roots of barley grown in low P and Fe0 medium was not affected as compared to that of the plants grown in adequate P and Fe0 medium (Ladouceur et al., 2006). The concentrations of Cu in shoots and roots of the plants did not varied significantly with the –P treatment as compared to those in the +P treatment plants (Fig. 5.8). Higher accumulation amounts of Cu were obtained in shoots of the –P plants as compared to that of +P plants either at 7 d or at 14 d (Table 5.1). This higher Cu content in shoots was in agreement with that of our previous finding in Low P and Fe0 media with barley (Ladouceur et al., 2006). Wallace (1984) also reported that P deficiency resulted in higher Cu accumulation in plants than in the P-sufficient plants in bush bean.

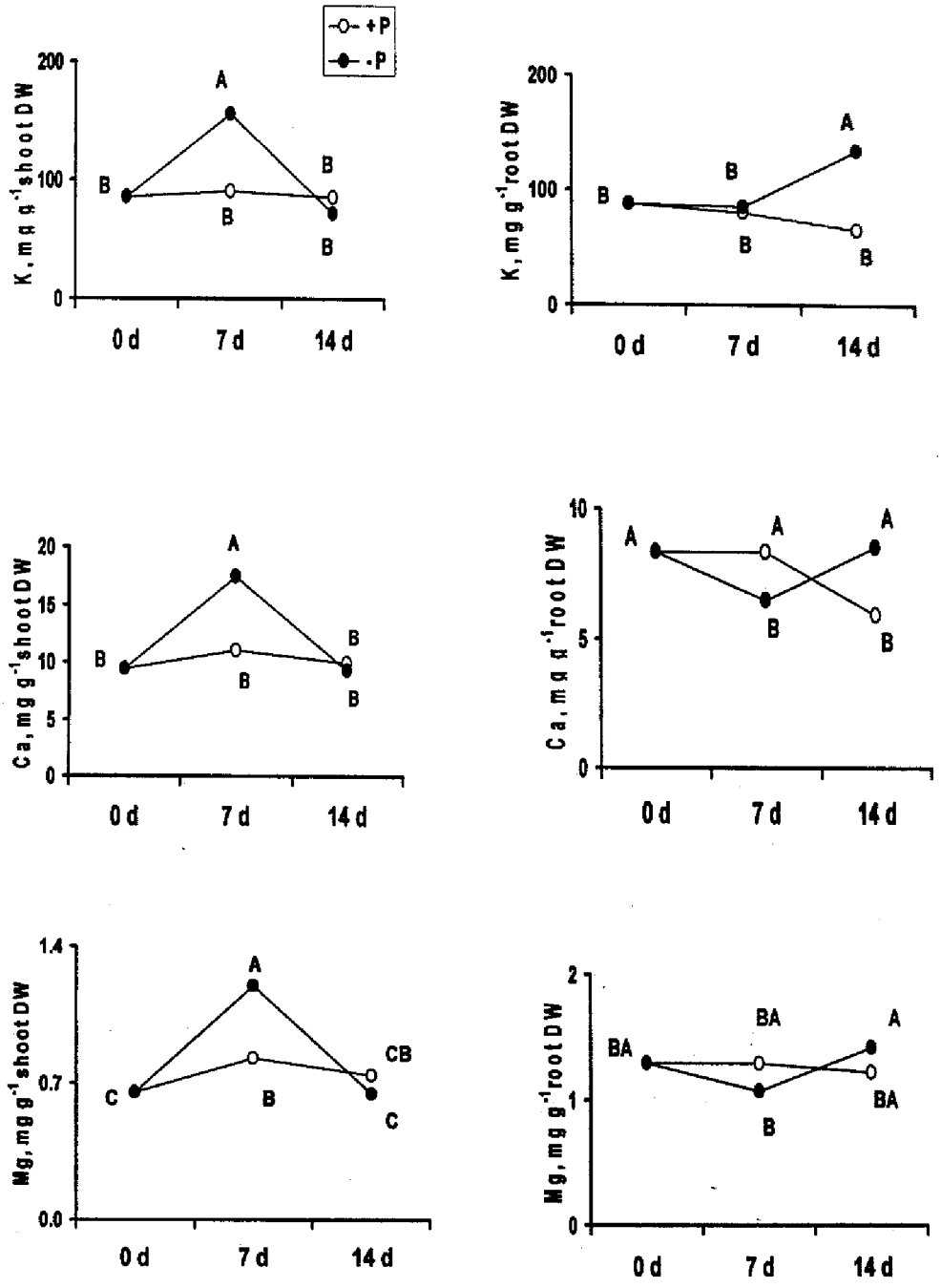


Figure 5.7. Concentrations of K, Ca, and Mg in shoot and roots of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p < 0.05$) according to Duncan Multiple Range Test.

This can be explained by the higher growth of the –P plant shoots and consequently to the concentration effect. However, higher concentrations of Cu in shoots and lower concentration of Cu in roots of the –P plants were obtained than those in the +P plants at 7 d, similarly to the other micronutrients. The Cu concentration in shoots and roots and the accumulation of Cu in roots were statistically similar for both plants at 14 d. This result indicated that the effect of the –P treatment on Cu concentration in shoots and roots in barley plants grown in –Fe medium was a very short-term increase at 7 d.

Zinc

The concentration (Fig. 5.8) and the accumulation (Table 5.1 and 5.2) of Zn in shoots of the plants were not affected by the –P treatment at 14 d similarly to the other micronutrients. The concentration and accumulation of Zn in shoots and roots of the plants were statistically similar for both treatments in +P plants and in –P plants at 14 d. This result indicated that the effect of the –P treatment in plants grown in –Fe medium on Zn concentration and accumulation in shoots and roots was not significant. The Zn-P antagonistic effect (Marschner and Cakmak, 1986) in –Fe conditions did not occurred, because P and Zn concentrations in both +P plants and –P plants were similar at 14 d. Loneragan et al. (1979) reported that high P contents in soils can decrease solubility of Zn in the soils. Decrease in Zn content in shoots due to high P known as P-induced Zn deficiency in plants was reported (Loneragan et al., 1979; Neilsen and Hogue, 1986). It was also reported that plants suffering from Zn phytotoxicity had lower shoot P levels (Boawn and Rasmussen, 1971). However, this result was consistent with the report of Pasricha et al. (1987) that the P-induced Zn deficiency may not always occur.

Manganese

The concentration (Fig. 5.8) of Mn in shoots was increased by the –P treatment of the plants at 7 d. However, Mn concentration (Fig. 5.8) and accumulation (Table 5.2) in roots were lower in –P plants than in +P plants at 14 d. In shoots the accumulation of Mn increased for 14 d and there was no difference between +P and –P plants. The accumulation of Mn in roots in –P plants decreased while that of +P plants increased for 14 d. Manganese concentration in shoots in –P plants was higher than that in +P plants at 7 d while the Mn concentration in roots was always lower in –P plants than that in +P plants, which suggested that Mn translocation was activated by –P treatment for a short period similarly to the other elements. The concentration of Mn in shoots was statistically similar in +P and –P plants at 14 d. The concentration and accumulation amounts of Mn in roots were significantly lower in –P plants than in +P plants, and Mn concentration in roots decreased with the –P treatment of the plants in –Fe medium. This decrease in the concentration of Mn in plant roots with the –P treatment of the medium, did not lead to higher Mn concentration and accumulation in shoots of –P plants. It is considered that the –P treatment reduced Mn accumulation and concentration in roots. This fact might be one of the factors involved in the activation of translocation of Fe, since the competition between Fe and Mn were reported by the past researchers. It is well known that Mn accumulates in barley roots in –Fe conditions (Alam et al., 2000). It is known that extreme Fe deficiency may increase the uptake of Mn to toxic levels and lead to complete crop failure (Chen and Barak, 1982). This result showed that the –P treatment of the –Fe barley allowed a significant decrease in Mn concentration and accumulation in roots. It was considered that low P concentration in roots may enhance Mn translocation to the shoots. Translocated Mn to the shoots may play a role in the recovery of the green color of the young leaves and the growth

enhancement by –P treatment. Manganese concentration and accumulation in shoots were not significantly affected by the –P treatment of the plants. In spite of the antagonistic effect between Mn and Fe, shoot Fe accumulation (Table 5.1) increased while Mn accumulation was not enhanced by –P treatment.

5.4. SUMMARY

After the –Fe pretreatment for 7 d, the plants grew better in –Fe, –P medium than in –Fe, +P medium. The –P treatment could keep the level of the chlorophyll index of the plants higher. The Fe concentration of shoots in both –P and +P plants, however, were in the range of critical deficiency level. It was suggested that the depression of chlorophyll synthesis in plants grown in –Fe medium was due to the combined effect of Fe-deficiency and high P status in plant tissues. Our results added evidence to the physiological competition between Fe and P in plant tissues. The Fe/P ratio may be the main factor for the induction and development of Fe chlorosis in plants under –Fe condition. The concentrations of macronutrients, such as K, Ca, and Mg, and those of the micronutrients, such as Fe, Mn, Zn, and Cu, in shoots were not significantly different from those of the +P plants at 14 d. Therefore, it was considered that the low Fe/P ratio of the +P plants was responsible for the progression of Fe chlorosis, and that the high Fe/P ratio of the –P plants induced the greening of their leaves and their higher growth.

The depletion of P in the –Fe medium increased drastically the amount of PS released by the roots of barley, though the accumulation of PS in the roots was similar between –P and +P plants. It was certain that short term depletion of P status in plant tissues enhanced PS release in –Fe plants after the induction of Fe chlorosis in leaves and the activation of PS synthesis and release in roots. The direct effect of P on the release sites

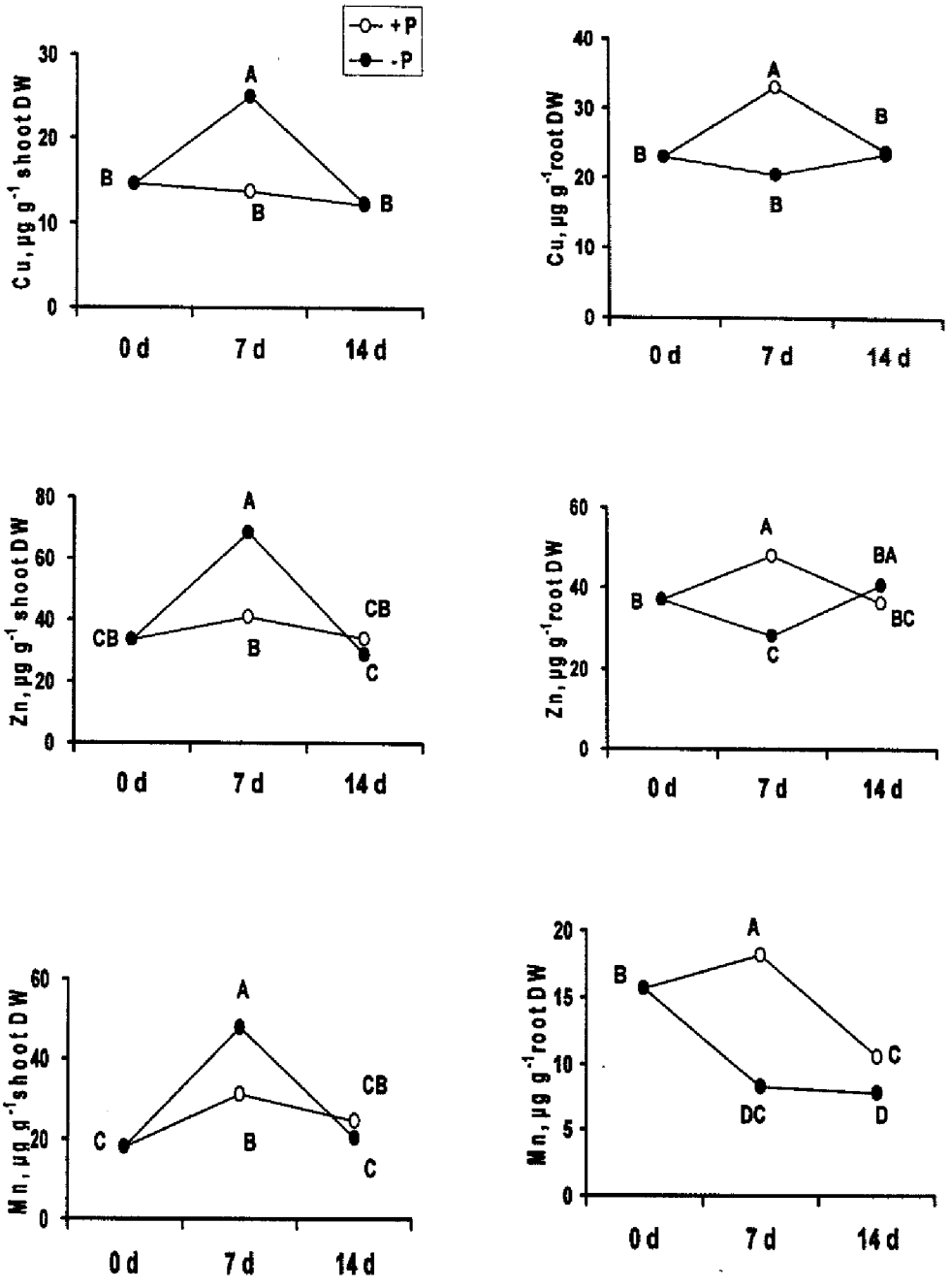


Figure 5.8. Concentrations of Cu, Zn, and Mn in shoot and roots of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p_< 0.05$) according to Duncan Multiple Range Test.

of PS in roots needs to be investigated. Further research is required focusing on the mechanism of the inactivation of Fe in plant tissues and the repression of PS release by high P in gramineae.

CHAPTER 6

PHOSPHORUS-DEPLETION TREATMENT AND MINERAL NUTRIENT DISTRIBUTION IN LEAVES OF BARLEY UNDER IRON-DEFICIENT CONDITION

6. Phosphorus-depletion treatment and mineral nutrient distribution in leaves of barley under Fe-deficient condition.

6.1. INTRODUCTION

The knowledge of the effect of decreasing nutrient supply on the continuation of plant development is important in crop production. The growth can be maintained to some extent by nutrient retranslocation within the plant after the decrease of the supply of mineral elements (Ascencio, 1988). The ability of plants to uptake P from soil is regulated by either internal or external utilization efficiency of P. The internal mechanisms enable plant to produce more dry matter. The external mechanisms enable plants to yield more because of an increased ability to extract P from the soil (Mackay et al., 1990). Our previous work showed that plant growth remained unchanged when P concentration of the medium was lowered to 10 times lower level (Chapter 3, Fig. 3.1). The livestock require very small amounts of P in the herbage (Sistani et al., 2003). However, the concentrations of P in the herbage of many pastures exceed livestock requirement (Reay and Grace, 1981; Smith and Cornforth, 1982). Plant growth and the distribution of N, P, or K in different organs have been measured for a large number of plants which were supplied with all nutrients up to a well defined stage of development and then transferred to deficient conditions (Cogliatti and Clarkson, 1983). The reports of the combined deficiencies of two nutrients are not much. In a previous attempt, we found that depletion of P in –Fe medium induced the greening of Fe chlorotic leaves and the growth of the plants. The decrease in P content of the shoots by the depletion of P did not affect the concentrations of most of the macronutrients and micronutrients.

Phosphorus is a readily mobile mineral in plants and can be translocated in upward or downward directions. For instance, in intact castor bean plants, young leaves of the

plant were supplied with Pi not only taken up by the roots but also from older leaves (Jeschke et al., 1997). Phosphorus remobilized from mature leaves is also transported via the phloem as retranslocated Pi formed by hydrolysis of organic P, but it is directed to the roots (Mengel and Kirkby, 2001). Phosphorus deficient tissues show an increase in phosphatase activity as a consequence of higher turnover rates of P and remobilization of Pi (Smith and Chevalier, 1984). The high turn over rate of P in the tissues of –P plant might contribute to the activation of Fe and the resulting the leaf greening (Chapter 5, Photograph 5.1). The mobilization of P might interact with other minerals within plant tissues. The shoot concentration of most minerals did not vary after P-depletion in barley under –Fe condition. The distribution of minerals among the leaves remains unknown. An experiment was designed in other to clarify the effect of P-depletion treatment on mineral nutrient distribution in barley leaves.

6.2. MATERIAL AND METHODS

The material and methods of this experiment were similar to those described in Chapter 2 and 5. The chlorophyll index of the new leaves, the content in roots and the release from roots of PS were measured at 0 d and 7 d. Plants were harvested at 0 d and 7 d, washed with deionized water, separated into roots, old leaves and new leaves. The 2 leaves with their leaf sheath from the top of the plant shoot were separated as new leaves while the remaining leaves with their sheath towards the bottom of the shoot were taken as old leaves. The plants materials were oven dried at 80 °C for 24 hours continuously, weighed, and digested. The content of mineral nutrients in roots, old leaves, and new leaves were measured in 7 d plants as described in chapter 5. The experimental design was a completely randomized block with six replicates. The data were analyzed as described in chapter 2.

6.3. RESULTS AND DISCUSSION

6.3.1. Visible symptoms

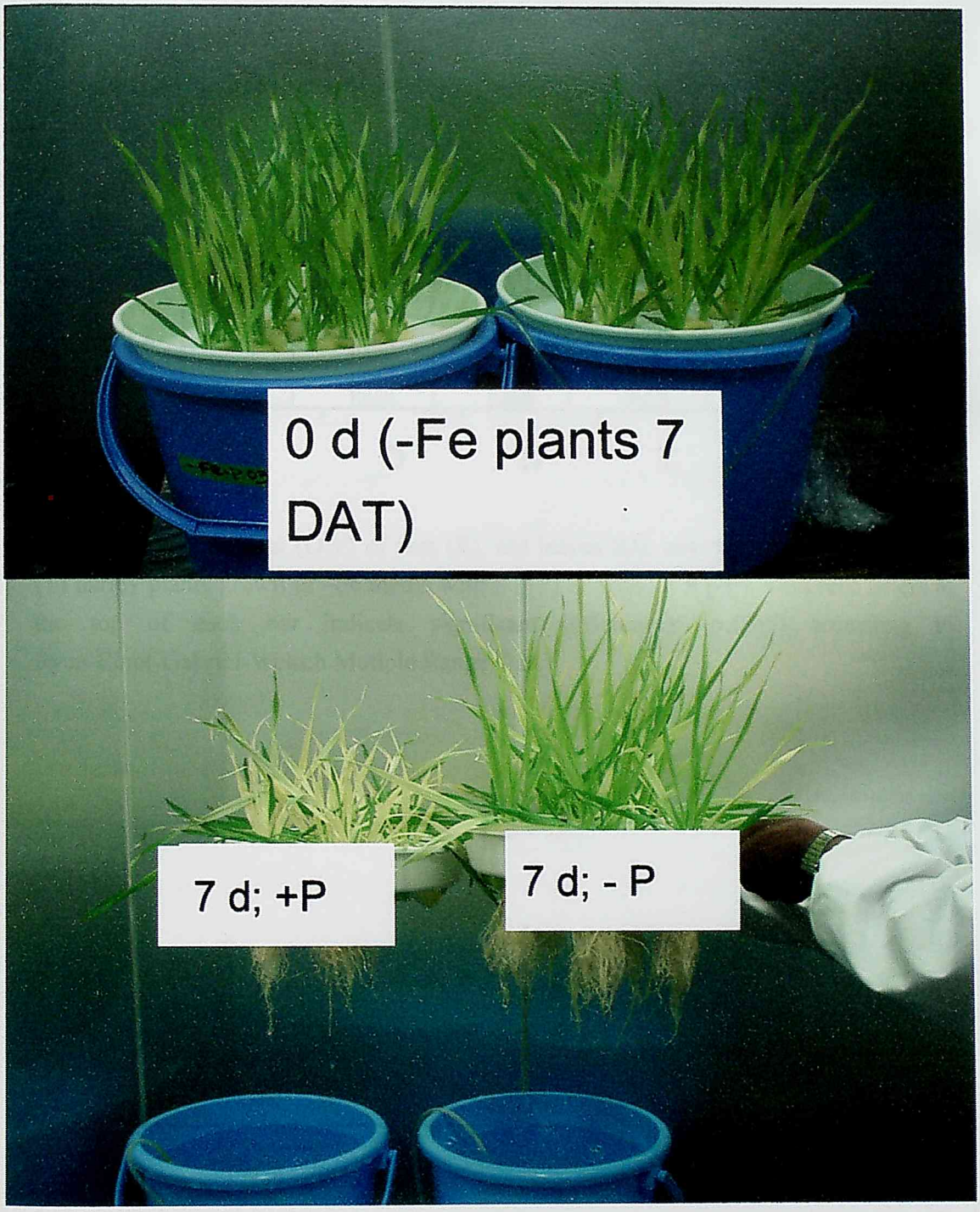
The visual symptoms of Fe deficiency in shoots and roots were obvious in 0 d plants and progressed in +P plants at 7 d as shown in Photograph 6.1 and described in chapter 5. The symptoms of P deficiency were not visible and the Fe chlorosis was alleviated in –P plants. The roots of the –P plants appeared as previously described in chapter 5.

6.3.2. Dry matter production

The total dry weight (DW) and the DW of roots, old leaves, and new leaves were higher in –P plants than in +P plants (Fig. 6.1). This result was consistent with that of previous experiment (Chapter 5) and our report of barley plants grown in low P and Fe0 medium (Ladouceur et al., 2006). The higher root growth of the –P plants was obvious, as it is known that the roots continue to grow not only by retaining most P but also by translocating P from shoots to roots in P-deficient condition (Smith et al., 1990).

6.3.3. Chlorophyll index

The chlorophyll index (SPAD value) of the new leaves (Fig. 6.2) was higher in –P plants than in +P plants similarly to the result of chapter 5.



Photograph 6.1. Barley plants grown in –Fe media with P (+P) or without P (–P) (7 DAT).

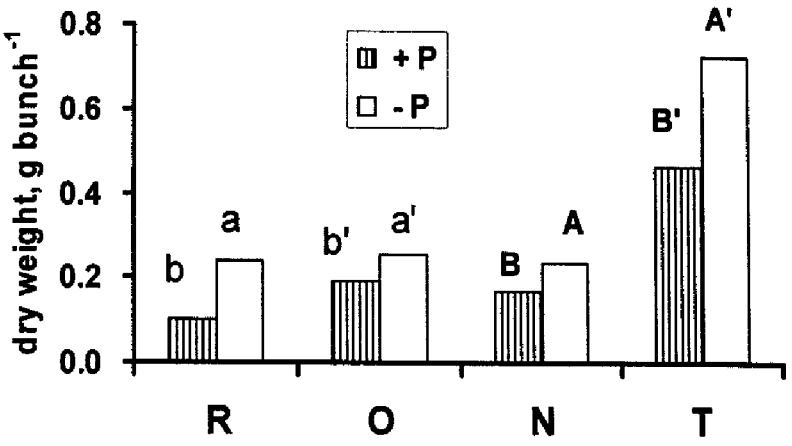


Figure 6.1. Dry weight (DW) of root (R), old leaves (O), new leaves (N) and of total (T) barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each bar indicate significant differences ($p<0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

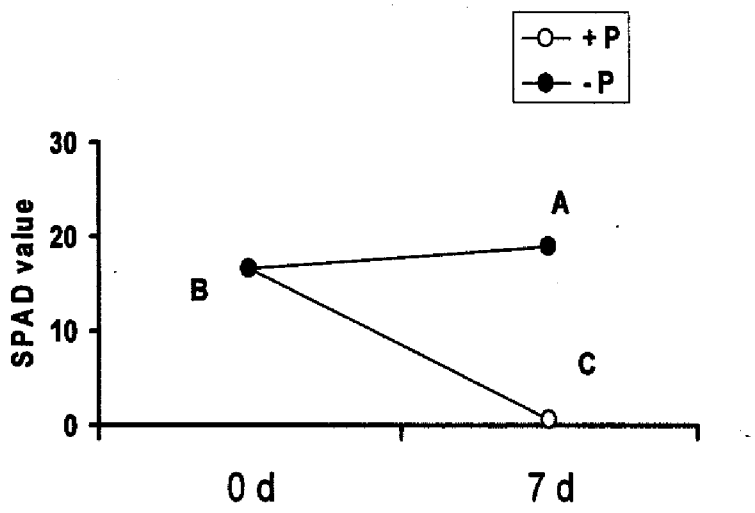


Figure 6.2. Chlorophyll index of the new leaves of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each bar indicate significant differences ($p< 0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

6.3.4. Phytosiderophores release from roots and Phytosiderophores accumulation in roots

The amount of PS release (R) from roots (Fig. 6.3a), the PS accumulation (A) (Fig. 6.3b) in roots as well as the ratio (R/A) (Fig. 6.3c) was higher in –P plants than in +P plants. The results of the pattern of PS release and the ratio were consistent with the previous experiment (Chapter 5). The accumulation amount of PS in roots was increased with the –P treatment in plants and was higher than the released amount of PS. The ratio (R/A) did not exceed 1 or 100%, and this ratio was higher in –P plants than +P plants similarly to the experiment of chapter 5. It is again suggested that PS synthesis and PS release may be enhanced by low P treatment.

6.3.5. Distribution of mineral element in plant organs

Phosphorus

The accumulation (Table 6.1) and the concentration (Table 6.2) of P of roots, old leaves, and new leaves were obviously lower in –P plants than in +P plants. The –P treatment decreased the concentration and accumulation of P in roots more than in leaves. It is known that P is remobilized in plants under –P condition. Jeschke et al. (1997) reported the mobilization of P towards the roots from mature leaves via the phloem. The concentration of Pi in the phloem from shoot to root is a feedback signal to regulate P uptake of plant (Drew and Saker, 1984). Our result suggested that in –P and –Fe conditions the mobilization of P may be directed much towards the leaves. This mobibilization of P towards the leaves under –Fe condition is different from the discussion of Smith et al. (1990) that the roots retained most of the plant P by importing P from shoot under P-starved conditions. The drastic decrease in P concentration in roots may be one of the cause of the higher PS release from roots of the –P plants.

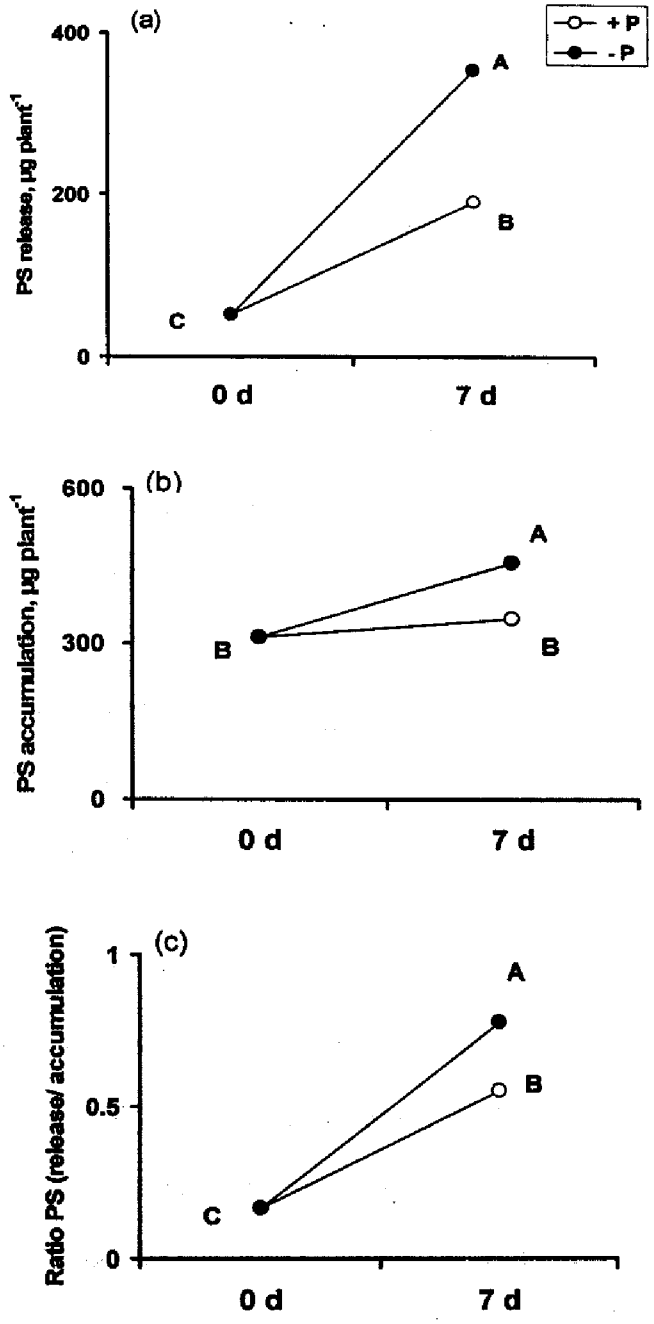


Figure 6.3. (a) Release amount of PS from roots, (b) accumulated amount of PS in roots, and (c) relative ratio (PS release/PS accumulation %) (R/A ratio) in roots of barley plants grown in –Fe media with P (+P) or without P (–P). Different letters at the top of each line indicate significant differences ($p< 0.05$) according to Duncan Multiple Range Test.

Iron

The accumulation of Fe in roots and old leaves were higher in –P plants than in +P plants (Table 6.1). The concentration of Fe in roots of –P plants was lower than that of +P plants. This may be due to dilution effect, because the dry weight of the –P plants was higher than that of the +P plants. The Fe concentration was not affected in the old and new leaves by –P treatment (Table 6.2). It can be considered that the greening was not due to higher Fe concentration, but to Fe mobilization within the plants induced by low P status in –P plants. This finding is consistent with the report that Fe deficiency may not always be characterized by chlorotic leaves but simply by a reduction of leaf growth (Kosegarten et al., 1998). Mengel et al. (2001) also reported that a leaf of plant showing Fe deficiency symptoms might have same Fe concentration as a green leaf, and that was due to more internal precipitation of Fe than to external Fe concentration of the growth medium.

Ratio Fe/P

The Fe/P ratios of roots, old leaves, and new leaves were higher in –P plants than in +P plants (Fig. 6.4). It was reported that Fe-deficient plants with low Fe/P showed Fe chlorosis while plants with high Fe/P displayed alleviated or no Fe chlorosis (Ladouceur et al., 2006). The high ratio in roots and in leaves may be the responsible factor of the greening in leaves of the –P plants. The precipitation of Fe in old leaves as insoluble oxides or phosphates or as complexes with phytoferritin was reported. Phytoferritin can form complex with Fe not only in leaves but in other plant parts (Oh et al., 1996). The precipitation of Fe diminishes subsequent mobilization of the metal into the phloem for long-distance translocation (Taiz and Zieger, 2002). The low P status of the tissues of

–Fe plants might reduce immobilization or precipitation of Fe by P compounds and increase Fe availability for chlorophyll synthesis.

Table 6.1. Accumulation of nutrient in roots and old and new leaves of barley plants grown in Fe-deficient and P depleted nutrient solutions (7 DAT)

	mg plant $^{-1}$			μg plant $^{-1}$			
Treatment	P	Ca	Mg	Fe	Mn	Cu	Zn
				New leaves			
+P	0.568a	0.103b	0.085b	2.56a	1.72a	0.628b	3.92a
–P	0.460b	0.276a	0.141a	3.54a	1.98a	0.998a	4.29a
				Old leaves			
+P	0.538a	0.798b	0.181b	3.87b	3.46b	0.739a	2.33b
–P	0.425b	1.75a	0.233a	5.27a	6.44a	0.771a	4.85a
				Roots			
+P	0.266a	0.088b	0.060b	4.36b	1.01a	1.71a	1.07b
–P	0.097b	1.19a	0.128a	5.76a	1.21a	1.62a	1.77a

Note: Means followed by different letters in each column are significantly different ($p < 0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test.

Table 6.2. Nutrient concentration in roots and old and new leaves of barley plants grown in Fe-deficient and P depleted nutrient solutions (7 DAT)

	mg g^{-1}			μg g^{-1}			
Treatment	P	Ca	Mg	Fe	Mn	Cu	Zn
			New leaves				
+P	9.52a	1.94b	1.62a	48.2a	32.5a	11.7a	73.9a
–P	5.53b	3.68a	1.86a	46.1a	26.1a	13.2a	57.3a
			Old leaves				
+P	8.45a	12.5b	2.81a	60.6a	53.7b	11.5a	36.1b
–P	4.89b	20.5a	2.75a	62.9a	75.7a	9.12b	57.1a
			Root				
+P	6.98a	2.58a	1.76a	128.3a	29.4a	50.2a	31.4a
–P	1.18b	2.39a	1.65a	73.6b	15.2b	20.6b	22.5a

Note: Means followed by different letters in each column are significantly different ($p<0.05$) according to Ryan- Einot- Gabriel -Welsch Multiple Range Test.

Magnesium

The Mg accumulation in roots, old and new leaves were higher in –P plants than +P plants (Table 6.1). However, the Mg concentration in these organs was not affected by the –P treatment (Table 6.2). These results confirmed that the chlorosis in –Fe conditions was not due to the Mg concentration of the leaves. This similar Mg content in the old and new leaves of –P plant in addition to the fact that their leaf Fe

concentration was not affected by –P treatment indicated that Mg and Fe content did not control chlorophyll formation in leaves.

Calcium

Calcium accumulation (Table 6.1) and Ca concentration (Table 6.2) were higher in the leaves of –P plants than +P plants. The root Ca accumulation was also higher in –P plants but the root Ca concentration of these plants was similar to that of +P plants. This result suggested that the treatment of –P under –Fe conditions enhanced Ca uptake of the plants and confirmed that there may be antagonistic effect of Ca and P uptake by plants. This higher Ca concentration in the leaves of –P plants may be responsible for the higher growth of these plants, as it is well known that Ca is involved in cell division.

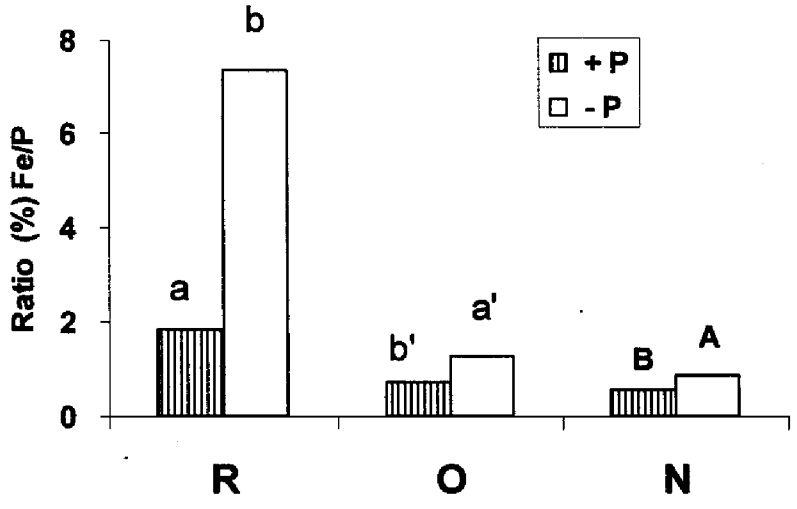


Figure 6.4. Ratio (Fe/ P) of concentrations in roots, old and new leaves of barley plants grown in –Fe and P-depleted nutrient solutions 7 DAT. Different letters at the top of each bar indicate significant differences ($p<0.05$) according to Ryan-Einot-Gabriel-Welsch Mutiple Range Test.

Copper

Copper accumulation in roots and old leaves were not significantly affected by the –P treatment (Table 6.1), but the concentration of Cu in these plant organs was lower in –P plants than +P plants (Table 6.2). However, Cu accumulation in the new leaves was significantly higher in –P plants than in + P plants. Copper accumulation in roots was similar and Cu concentration in roots was lowered. It is considered to be dilution effect induced by increase of root growth by –P treatment. This result indicated that the –P treatment did not interfere Cu translocation toward the new leaves.

Manganese

With the –P treatment, the accumulation (Table 6.1) and the concentration (Table 6.2) of Mn in the old leaves were higher in –P plants than in +P plants. The Mn accumulation in roots was not significantly affected, but the root Mn concentration was significantly lower in –Plants than in +P plants which is considered to be dilution effect. However, neither the Mn accumulation nor the Mn concentration in new leaves was significantly affected by –P treatment. This result suggested that there might be no interference of Mn translocation from roots to shoots by the –P treatment. However, the reason why accumulation of Mn in the old leaves of –P plants was higher is not known. This unchangeable content of Mn in the new leaves may contribute to the maintenance of photosynthesis of –P plants.

Zinc

The concentration and the accumulation amount of Zn in old leaves were significantly higher in –P plants than +P plants, which was similar to those of Mn. However, the –P treatment did not significantly affect the accumulation (Table 6.1) and the concentration (Table 6.2) of Zn in the new leaves. It is considered that concentrations of metal

micronutrients were regulated stable even under low P status in plants. In the roots, the Zn concentration was not significantly affected, but the accumulation amount was higher in –P plants. It was considered that more Zn was absorbed under –P condition, which might be Zn and P antagonistic effect. It was reported that Zn deficiency can also lead to P toxicity through restriction of Pi transport from shoot to root and thus impair transmission of the signal which controls Pi uptake (Marschner and Cakmak, 1986).

6.4. SUMMARY

The results of this experiment showed that the greening and growth enhancement occurring with the depletion of P from the growth medium could enhance mineral uptake in the plants. The –P treatment of the plants enhanced P remobilization within the plants and decreased the concentration of P in roots and leaves (old and new). The P concentration of roots was much decreased than that of the leaves in –P plants. Phosphorus movement towards the shoots might be active under –Fe and P-deficient conditions. Even though the Fe concentration of both +P and –P plants remained in the deficiency range, the –P treatment allowed a mobilization of Fe within the plants resulting in increased Fe accumulation in roots and old leaves. The higher PS release of –P plants may increase the Fe uptake activity of the sparingly contaminated Fe of the –Fe medium.

Manganese concentration in roots was also reduced through dilution effect and translocation of Mn towards shoots was resulted in increased Mn concentration in the old leaves. That reduction of Mn concentration in roots by –P treatment may play a role in the mobilization of Fe within the plants. The high Fe/P ratio in plant organs may be responsible for the greening and high Ca and Mg concentration of the leaves might contribute to the increased growth of the –P plants in –Fe medium.

CHAPTER 7

EFFECT OF LOW PHOSPHORUS TREATMENT ON CONCENTRATIONS OF PHYTOSIDEROPHORE AND MINERAL NUTRIENT IN THE XYLEM SAP OF IRON-DEFICIENT BARLEY

7. Effect of low phosphorus treatment on concentrations of phytosiderophore and mineral nutrient in the xylem sap of Fe-deficient barley

7. 1. INTRODUCTION

The xylem sap composition of plant may reflect its nutritional status under a specific environmental condition. There are many reports of the studies of mineral nutrient composition of the xylem sap and its relationship with metal complexation and translocation from roots to shoots (Graham, 1979; white et al., 1981). The form in which nutrients are translocated through the xylem tube may vary among plant species. Iron was detected in xylem sap of tomato (Strategy I) plants as Fe-citrate complex (Tiffin, 1967). In rice (Strategy II) plants grown in –Fe condition, about 12% of the Fe content in the xylem sap was in the form of PS-Fe^{3+} complex (Mori et al.,1991). The identification of PS in the xylem sap of –Fe barley plants was reported by Kawai et al. (2001).

In natural conditions, the deficiencies of Fe and P in some soils may potentially occur simultaneously. There is no report on the role of P on mineral and organic solutes concentration of the xylem sap of –Fe plants. The objective of this study was to analyze the effects of low P and –Fe conditions on the concentrations of mineral elements, phytosiderophore, and other organic solutes in the xylem sap of barley plants.

7.2. MATERIAL AND METHODS

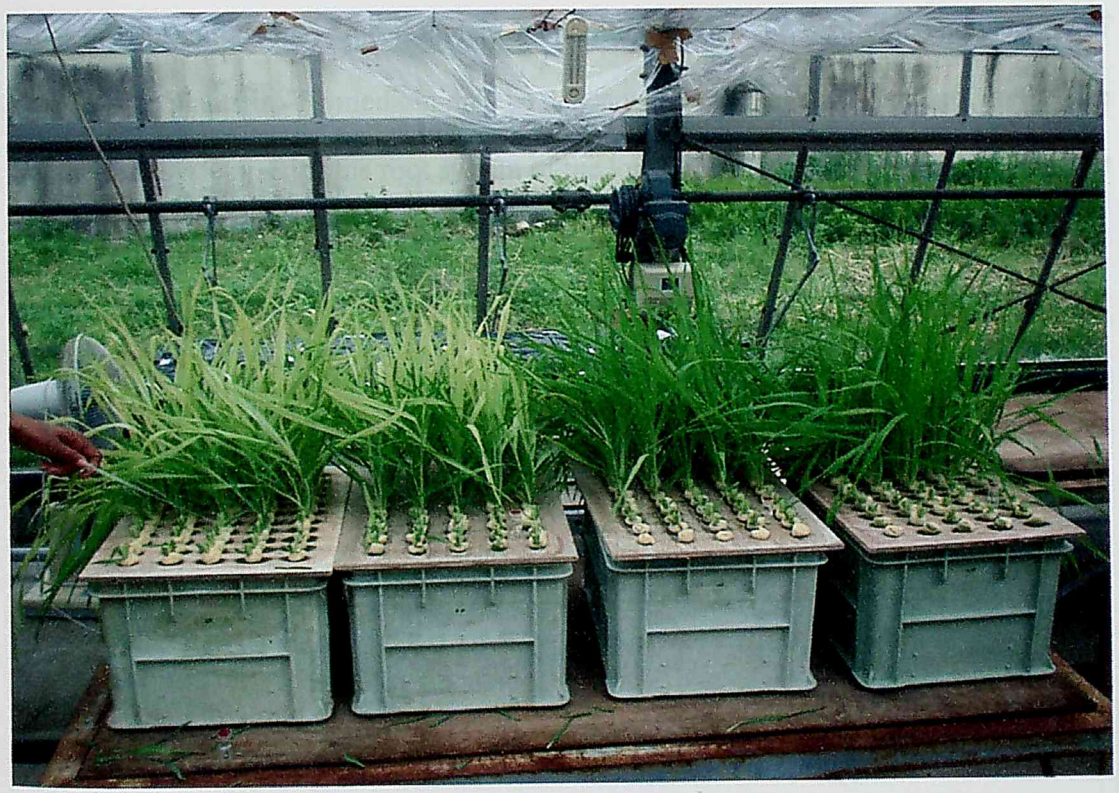
7.2.1. Plant Growth

The procedure for the preculture of barley seeds and the transplantation of seedlings in 35-L buckets were the same as those described previously in chapter 2. Plants were grown hydroponically in –Fe medium with 4 P levels (500, 50, 5, and 0.5 μMP) similarly to the methods described in chapter 4, in a greenhouse at the Faculty of

Agriculture of Iwate University in the spring season for 14 days. The nutrient solution pH was daily monitored and adjusted to 6.5. Nutrient solution was continuously aerated, weekly renewed, and the solution level of the bucket was maintained by the addition of deionized water during the experiment.

7.2.2. Collection of Xylem Sap

The material and methods used for the collection of the xylem sap are previously described in chapter 2. Plants were decapitated at about 2 cm above the roots with stainless-steel razor blade at at 13: 00 where no PS release was observed from roots. Subsequently, the xylem sap was collected continuously for 3 h from 16 bunches of plants per treatment in duplicate by attaching a capillary glass equipped with rubber tube to the remaining part of the plant stems as shown in Photograph 7.1. Collection of xylem sap were conducted with 16 bunches in duplicate.



Photograph 7.1. Decapitated Fe-deficient with high or low P barley plants for xylem sap collection.

7.2.3. The Parameters Measured

The measured parameters were the following:

(a) The amount of xylem sap

(b) The concentration of minerals in the xylem sap (concentration)

(c) The amount of minerals translocated per hour (translocation)

(d) The concentration of PS in the xylem sap

(e) The amount of PS translocated per hour (translocation)

(f) The concentration of organic acids (citrate, malate, and succinate) in the xylem sap

7.3. RESULTS AND DISCUSSION

7.3.1. Amount of Xylem Sap

The amount of collected xylem sap was lowered according to decrease of P concentration in the media (Fig. 7.1). It was suggested that water flow driven by the root pressure in plants reduced under –P condition of the –Fe medium. The velocity of sap flow in plants in –Fe medium may be dependent on the level of P and not on the size of the root system, since the root system of the low P (50, 5, 0.5 μM P) plants was larger than high P (500 μMP) plants in –Fe media (Ladouceur et al., 2006).

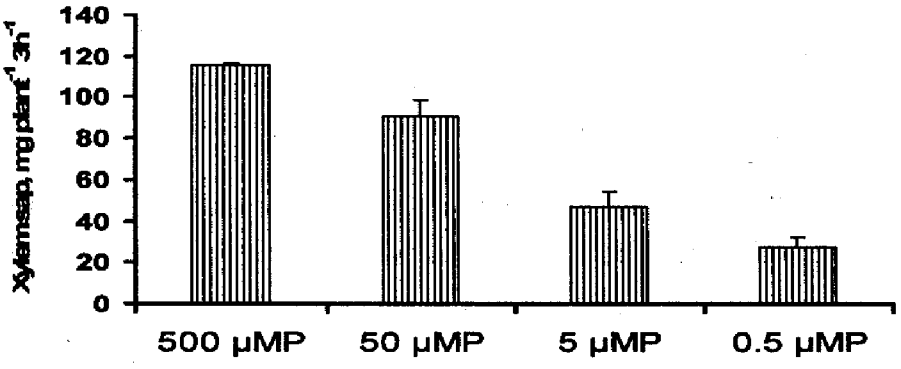


Figure 7.1. Flow rate of xylem sap of barley plants as affected by different P levels in Fe-deficient condition. Values represent the mean ± SE of 2 replications.

7.3.2. Phytosiderophore in Xylem Sap

The PS concentration in the xylem sap was higher in the plants grown in medium with the lowest P concentration (0.5 μMP) than in control (500 μMP) plants (Fig. 7.2).

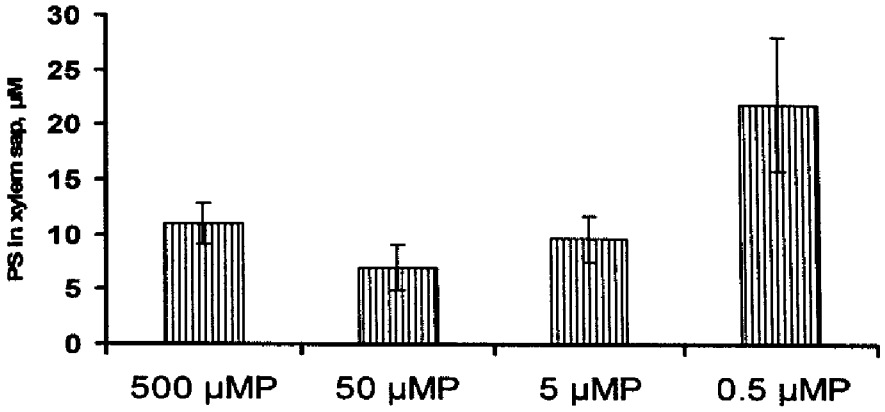


Figure 7.2. Concentration of Phytosiderophore of the xylem sap of barley plants as affected by different P levels in Fe-deficient condition. Values represent the mean ± SE of 2 replications.

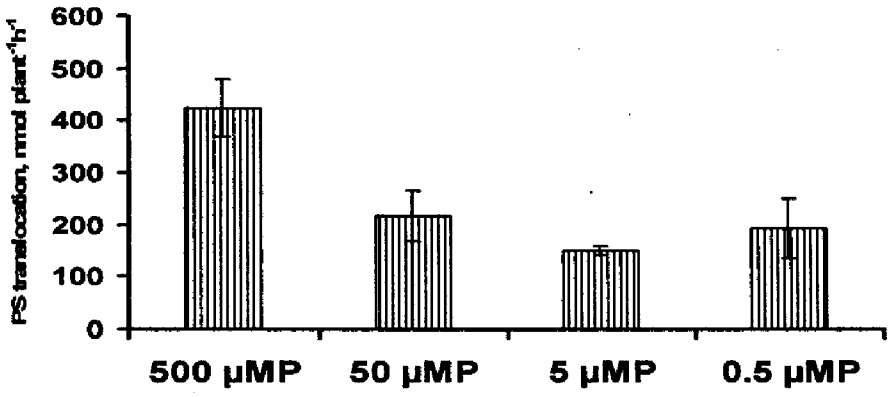


Figure 7.3. Phytosiderophore in the xylem sap of barley plants as affected by different P levels in Fe-deficient condition. Values represent the mean ± SE of 2 replications.

The concentration of PS in the xylem sap of the 50 or 5 μMP plants was similar to that of control plants. The translocation amounts of PS through the xylem sap were lower in low P (50, 5, 0.5 μMP) plants than in the control (500 μMP) plants (Fig. 7.3). This may

be explained by the lower amounts of xylem sap of the low P plants. It was suggested that PS may enhance Fe translocation in plants under severe –P condition, since PS-Fe^{3+} complex was found in the xylem sap of Strategy II plants (Mori et al., 1991). The PS of the xylem sap of barley plants grown under –Fe condition was identified as mugineic acid (Fig. 7.4). The PS of the xylem sap of barley plants grown with 0.5 μMP under –Fe condition is shown in Figure 7.5.

7.3.3. Mineral Element in Xylem Sap

The translocation of P (Table 7.1) and the concentration of P (Table 7.3) in the xylem fluid exudate were lower in low P (50, 5, and 0.5 μMP) plants than control (500 μMP) plants. The translocation amounts of other macronutrients such as K, Ca, Mg (Table 7.1) was lower in the low P plants (5 and 0.5 μMP) than in control (500 μMP) plants. The translocation amount of Mg and K was even higher in the xylem sap of 50 μMP than in control (500 μMP) plants. The concentration of K and Mg were higher in low P (50, 5, and 0.5 μMP) plants (Table 7.3) than in control (500 μMP) plants. This was due to concentration effect since the xylem flow of the low P plants (Fig. 7.1) was lower than that of the control (500 μMP) plants. The translocation (Table 7.2) and the concentration (Table 7.4) of micronutrients such as Fe, Mn, and Zn were higher in the xylem sap of the low P plants (5 and 0.5 μMP) than in those of the sap of the control (500 μMP) plants. It is suggested that –P treatment activated translocation of Fe, Mn, and Zn.

The translocation of Cu through the xylem was lower in low P (5 and 0.5 μMP) plants than in control (500 μMP) plants, but the concentration of Cu in the xylem sap of low P plants was higher than that of the high P plants which was concentration effect. The high micronutrient translocation and low P translocation in the xylem fluid of low P (5 and 0.5 μMP) plants under –Fe conditions may have effect on growth and chlorophyll

index of low P plants which were higher (Ladouceur et al., 2006) than those of high P plants. It is well known that Fe is involved in chlorophyll synthesis, Mn and Cu are involved in photosynthetic activity, and Zn is involved as a component of a number of enzymes in plants. Phytosiderophores might be involved in the enhancement of micronutrients transport via the xylem. It was reported that PS solubilized effectively Cu, Fe, Mn, and Zn from calcareous soils (Treeby et al., 1989).

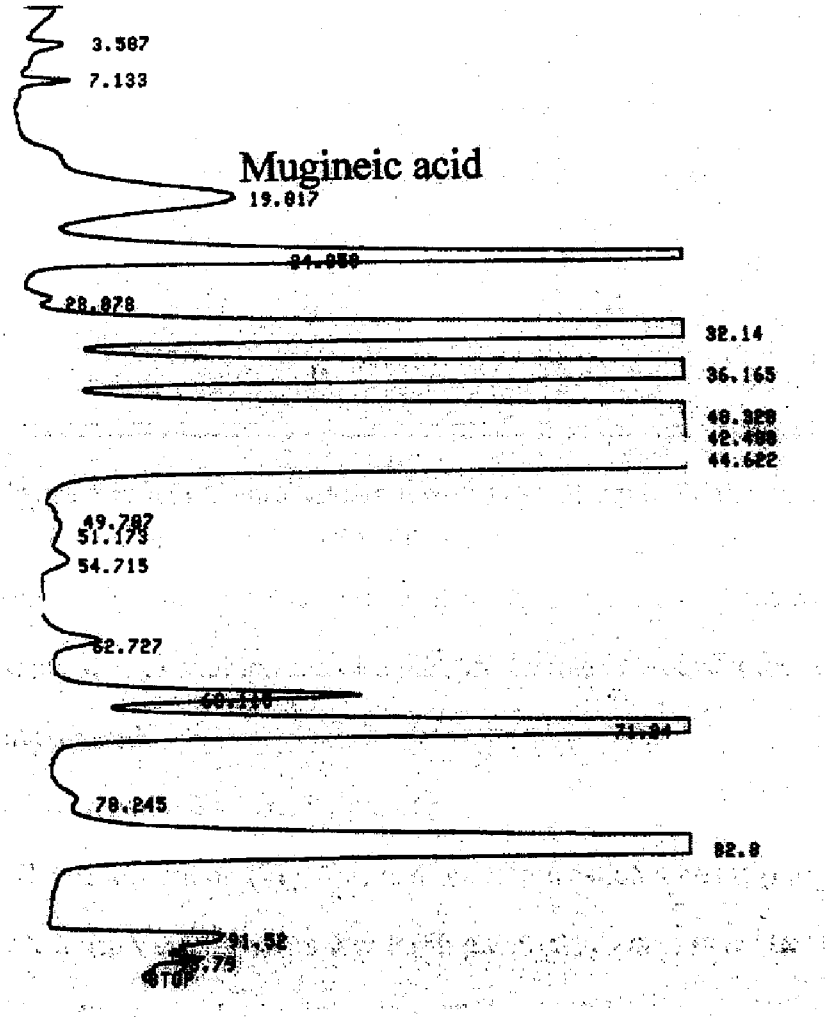


Figure 7.4. Standard HPLC chromatogram of the xylem sap of Fe-deficient barley plants grown in nutrient solutions with 500 μMP. Value in each peak represents the retention time

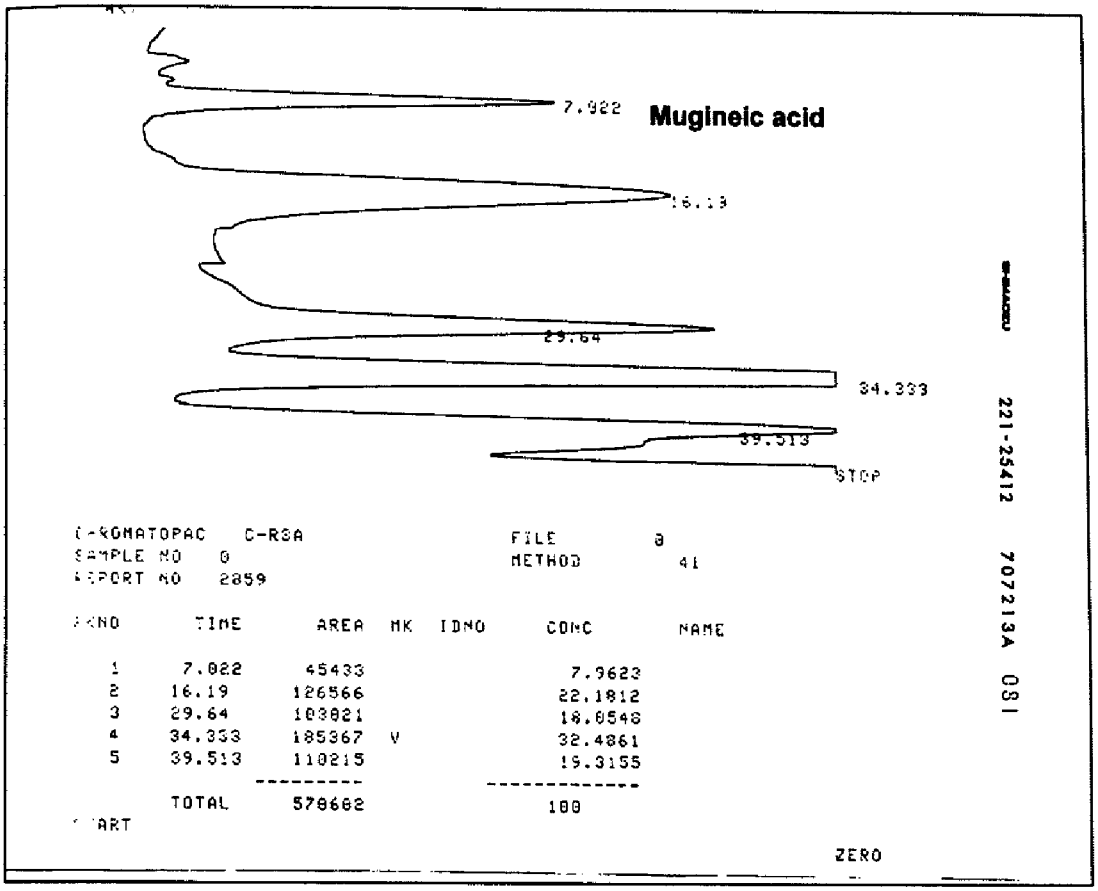


Figure 7.5. HPLC chromatogram of the xylem sap of Fe-deficient barley plants grown in nutrient solutions with 0.5 μMP. Value in each peak represents the retention time.

The mechanism by which low P plants translocate higher micronutrients than high P plants in –Fe condition and also the specific role of PS under low P condition need to be investigated.

7.3.4. Organic Acids in Xylem Sap

The concentration (Fig. 7.6) and the translocation amount (Fig. 7.7) of citrate in the xylem sap were higher in low P (50 μMP) plants than in control (500 μMP) plants. In 0.5 μMP plants, the concentration of citrate of the xylem sap was not affected, but its translocation amount was even reduced because of their lower amount of xylem sap as compared to that of the control plants. It was suggested that the concentration of citrate

of the xylem sap may be increased in plants under low P condition. However, in low P (0.5 μM) and –Fe conditions, the translocation amount of citrate through the xylem sap may be even decreased. The concentrations of malate were higher in the xylem sap of the low P (5 and 0.5 μMP) plants than in the control (500 μMP) plants (Fig. 7.6), but the translocation amounts were not significantly affected as compared to the control plants (Fig. 7.7). The concentrations (Fig. 7.6) and the translocation amounts of succinate in the xylem sap (Fig. 7.7) were lower in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants. It was indicated that succinate concentration and translocation in xylem sap decreased in plants under low P and –Fe conditions.

The release of organic acids under low P condition is well known as plant reactions to uptake sparingly soluble P of the medium (Hoffland et al., 1989; Ohwaki and Hirata, 1992). Our result showed that succinate concentration in xylem sap of plants may be depressed under low P and –Fe conditions. It is well documented that Fe can be transported in xylem sap as Fe-citrate complex in Strategy I plants (Tiffin, 1967). It would be interesting to investigate whether the citrate of the xylem sap of Strategy II plants (barley) under –P and –Fe conditions is carrying Fe or not.

Table 7.1. Translocation (nmol plant^{-1} h^{-1}) of macronutrients in xylem sap of barley plants as affected by different P levels in Fe-deficient medium.

Treatment P (μM)	P	K	Ca	Mg
500	3880±65.5	28430±3027.0	6521±819.5	1284±186.88
50	226±35.3	36318±3369.2	6260±204.5	2027±167.76
5	34±2.14	25427±1627.4	2190±44.5	1027±108.72
0.5	3±1.15	15716±2920.8	1883±411.1	558±94.61

Values represent the mean ± SE of 2 replications

Table 7.2. Translocation (nmol plant^{-1} h^{-1}) of micronutrients in xylem sap of barley plants as affected by different P levels in Fe-deficient medium.

Treatment P (μM)	Fe	Mn	Zn	Cu
500	5.52±0.09	23.67±4.27	11.01±2.27	3.506±0.538
50	6.09±1.62	15.52±3.81	10.53±0.51	2.427±0.119
5	12.95±1.68	39.01±2.07	23.22±0.15	2.399±0.301
0.5	13.24±4.17	33.69±8.06	22.96±3.88	2.016±0.426

Values represent the mean ± SE of 2 replications.

Table 7.3. Concentration (μM) of nutrients in xylem sap of barley plants as affected by different P levels in Fe-deficient medium.

Treatment P (μM)	P	K	Ca	Mg
500	100.45±3.08	737.8±22.3	169.3±7.8	33.16±1.16
50	7.53±0.50	1200.1±36.2	207.8±10.5	67.01±2.24
5	2.32±0.38	1627.3±49.0	141.1±6.9	66.68±2.19
0.5	0.33±0.01	1711.4±51.5	205.0±10.3	60.64±2.03

Values represent the mean ± SE of 2 replications.

Table 7.4. Concentration (μM) of micronutrients in xylem sap of barley plants as affected by different P levels in Fe-deficient medium.

Treatment P (μM)	Fe	Mn	Zn	Cu
500	0.14±0.01	0.62±0.03	0.29±0.015	0.091±0.008
50	0.20±0.01	0.51±0.03	0.35±0.020	0.081±0.009
5	0.82±0.04	2.52±0.12	1.49±0.075	0.153±0.012
0.5	1.44±0.07	3.67±0.19	2.50±0.127	0.219±0.016

Values represent the mean ± SE of 2 replications.

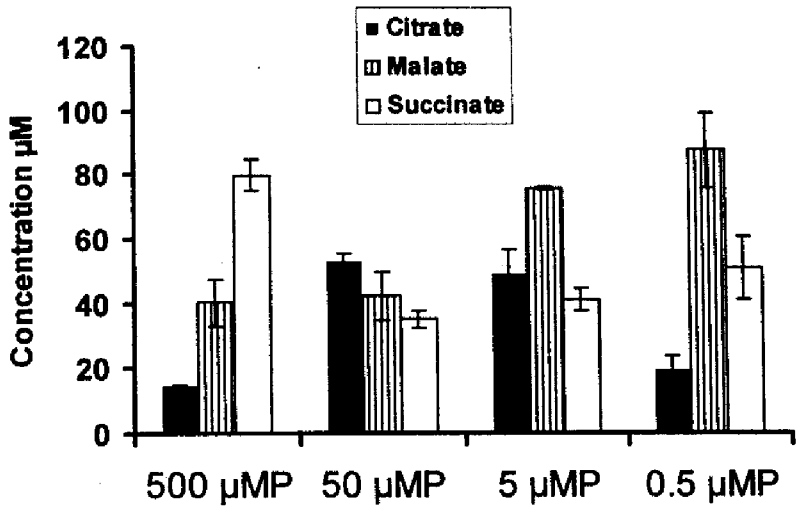


Figure 7.6. Organic acid (citrate, malate, succinate) concentrations in xylem sap of barley plants as affected by different P levels in Fe-deficient media. Values represent the mean ± SE of 2 replications.

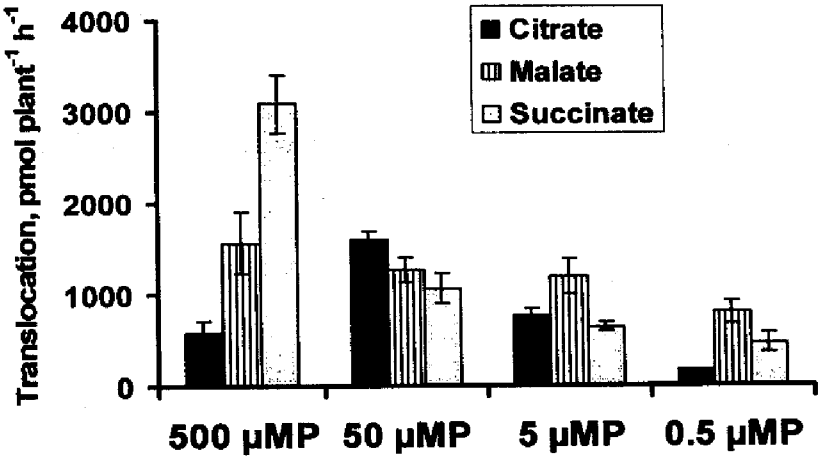


Figure 7.7. Translocation amounts of organic acids (citrate, malate, succinate) through xylem sap of barley plants as affected by different P levels in Fe-deficient media. Values represent the mean ± SE of 2 replications.

7.4. SUMMARY

The low P treatment with the –Fe medium of the plants decreased the flow rate of the xylem sap from roots to shoots. The translocation of macroelements were decreased, however, the translocation of microelements were increased according to the decrease of P concentration of the media with the exception of Cu. It was indicated that P represses translocation of microelements. The concentrations of macronutrient such as K, Ca, and Mg were higher in the xylem sap of low P (50, 5, and 0.5 μMP) plants than in that of high P (500 μMP) plants in –Fe condition. The concentration of the micronutrients such as Fe, Mn, Zn, and Cu increased in the xylem sap of the plants with decreasing P concentration of the –Fe medium. The concentration of PS of the xylem sap of the plants increased in lowest P (0.5 μMP) and –Fe medium. The concentration of the xylem sap of the plants in organic acids such as citrate and malate was increased while that of succinate was decreased by the deficiency of P in the –Fe medium. It is important to examine the mechanisms of the absorption and translocation of the micronutrients in relation with PS and citrate in plants under low P conditions.

CHAPTER 8

EFFECT OF SUPPLIED PHYTOSIDEROPHORE ON ^{59}IRON ABSORPTION AND TRANSLOCATION IN IRON-DEFICIENT BARLEY GROWN HYDROPONICALLY IN LOW PHOSPHORUS MEDIA

8. Effect of supplied phytosiderophore on ^{59}iron absorption and translocation in Fe-deficient barley grown hydroponically in low phosphorus media

8.1. INTRODUCTION

Iron (Fe) and phosphorus (P) are two essential mineral elements for plant growth. The deficiency in these two elements are limiting crop yield world wide. In calcareous soils, Fe forms water-insoluble oxides and oxy-hydroxides and is poorly available to plants, even though total Fe concentration can be relatively high (Lindsay and Schwab, 1982; Hartwig and Loeppert, 1993). Iron in soil solution usually ranges from 0.1 to 10% of the total needed by plants, especially in calcareous soils (Lindsay, 1984). The chemical forms of Fe found in most soils are sparingly soluble in soil solution at biological pH (Lindsay, 1984). Iron chlorosis induced by high pH or carbonate is probably due to the lack of available Fe for chlorophyll biosynthesis and chlorophyll binding proteins (Römheld and Marschner, 1991).

Phosphorus is the second most limited nutrients after nitrogen for vegetative growth (Vance et al., 2003). The deficiency of P in soils is mainly due to the fixation of P by Fe and Al in acidic conditions, and by calcium in alkaline or calcareous condition into insoluble complex forms rendering this nutrient unavailable for plant uptake (Mengel and Kirkby, 2001). The combined deficiencies of P and other micronutrients can potentially be occurred in calcareous and alkaline soils. For instance, most of the chickpea-growing regions of India are deficient in P as well as micronutrients (Srinivasarao et al., 2006).

The role of phytosiderophores (PS) release by graminaceous plants (Strategy II plants) for their Fe acquisition in Fe-stressed condition is well known (Takagi et al., 1984; Römheld and Marschner, 1986). Takagi et al. (1984) reported that the addition of PS to

the medium caused marked increase in Fe uptake by rice seedlings, resulting in greening of the chlorotic leaves. High P concentration in Fe0 medium increased PS release and PS accumulation in roots (Ladouceur et al., 2006). Alam et al. (2005) reported an increase in ^{59}Fe absorption and translocation from roots to shoots in barley with the addition of PS to the media. The effect of PS and/ or P on Fe absorption and translocation in strategy II plants under –Fe stress has not been reported. In an attempt to specify the role of PS for absorption and translocation of Fe in –Fe plants grown under low P levels of the medium, a feeding experiment with radioactive ^{59}Fe to the barley plants grown under varied P levels in –Fe condition was conducted with or without added PS, and the absorption and the translocation of ^{59}Fe in the plants were measured.

8.2. MATERIAL AND METHODS

8.2.1. Plant Culture and Growth

Seedlings of barley plants were cultivated hydroponically by the method described in the previous report (Ladouceur et al., 2006). Plants were transplanted in bunch of 3 plants wrapped with sponge rubber and transferred to 10-L plastic buckets (16 bunches $bucket^{-1}$) filled up with 1/2–strength Hoagland-Arnon solution for 2 days. Subsequently, the plants were transferred to the –Fe 1/2–strength modified Hoagland-Arnon solution (Takagi, 1993) with 4 P levels, 500 (control), 50, 5, and 0.5 μMP. Phosphorus was supplied as NaH_2PO_4. The plants were grown in a phytotron as described in chapter 2. The pH of the nutrient solutions was daily monitored and adjusted to 6.5. The nutrient solutions were weekly renewed. The plants were allowed to grow for 12 days after treatment (DAT) prior to ^{59}Fe feeding experiment.

8.2.2. Chlorophyll Index of Leaves

The chlorophyll content in the 4th leaves was measured in three bunches of plants at the harvest day using a SPAD-502 chlorophyll meter as described in chapter 2.

8.2.3. Source of Used PS and ^{59}Fe

Mugineic acid, one of the major PS released by the barley cultivar, was collected from the root washings of –Fe barley by the method previously described (Takagi, 1993). The ^{59}Fe-radionuclide was purchased from Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA, USA.

8.2.4. Feeding with ^{59}Fe

At 12 DAT, plant roots were washed with deionized water. Subsequently, plants were transferred to feeding solution with the 4 P levels where ^{59}Fe as $^{59}FeCl_3$ (10 μM) was added with or without PS (10 μM). Plants were fed with ^{59}Fe in triplicate for 4 hours in the time, when PS were not released by roots, in beakers wrapped with aluminum foil containing 100 mL of feeding solution as shown in photograph 8.1. The starting time of ^{59}Fe feeding was 8 hours after the onset of light in the phytotron. The radioactivity of ^{59}Fe in each beaker was 37 KBq. The apoplastic ^{59}Fe in roots was solubilized and removed after the feeding time by the method of Bienfait et al. (1985). After the removal of apoplastic ^{59}Fe, the plants were throughout washed with tap water, divided into shoots and roots, oven dried at 70°C for 1 d, and weighed.

8.2.5. Measurement of ^{59}Fe

Dried shoots and roots of the plants were digested in concentrated nitrate as described by Zarcinas et al. (1987). The radioactivity of ^{59}Fe of the digested plant solutions or the root washing containing apoplastic ^{59}Fe was determined by using a gamma scintillation counter (Auto Well Gamma System, AccuFLEX ARC-7000, Aloka, Tokyo, Japan). The amount of the extracellular ^{59}Fe in the root apoplast was not included in the ^{59}Fe content

in roots. The total absorption represents the sum of the amount of ^{59}Fe in shoots and roots, and the absorption activity per root dry weight (DW) represents the total amount of ^{59}Fe in plant divided by root dry weight. The translocation per plant represents the shoot content of ^{59}Fe, and the translocation per shoot DW was also calculated. The relative translocation rate represents the percentage of the ^{59}Fe translocated from roots to shoot to the total absorption of ^{59}Fe per plant.

8.2.6. Statistical Analysis

The experiment was arranged in completely randomized block design with 3 replicates. Data were subjected to an ANOVA (SAS Institute, 1988). The means of treatment were compared according to Duncan's Multiple Range Test ($P<0.05$) using the computer "Origin 5" in Iwate University.

Photograph 8.1. Low P and –Fe barley plants fed with ^{59}Fe or ^{59}Fe-PS contained in nutrient solutions.

8.3. RESULTS AND DISCUSSION

8.3.1. Dry weight and Chlorophyll Index of the Plant

The dry weights of shoots and roots of –Fe plants grown under low P (50, 5, and 0.5 μMP) media were higher than control (500 μMP) plants (Table 8.1). The chlorophyll index was higher in the low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants. The leaves of low P plants were green, while those of control plants showed Fe chlorosis as previously (Ladouceur et al., 2006).

8.3.2. Total Absorption and Root Absorption Activity of ^{59}Fe in Plant

The total absorption of ^{59}Fe was higher in control (500 μMP) plants than that in low P (50, 5, and 0.5 μMP) plants both in the case with or without fed PS (Fig. 8.1a). In the absence of fed PS, the total absorption was similar among these low P (50, 5, and 0.5 μMP) plants. The addition of PS to the feeding solution increased the total absorption of ^{59}Fe in plants by 1.3 to 3.7 fold without being related with the P level of the medium. The absorption activity of ^{59}Fe of plant per gram root DW (Fig. 8.1b) showed similar pattern as that of the total absorption of ^{59}Fe in plant. It was clear that low P plants had lower activity for Fe uptake than control plants, in spite of larger root system and greener leaves of the former (Table 8.1). It was indicated that low P depressed the absorption activity of ^{59}Fe in plants either with or without fed PS. It was shown that Fe chlorotic plants with control concentration in shoot and roots had higher absorption activity of ^{59}Fe than non-chlorotic plants with low P concentration in shoot and roots. This higher absorption activity of ^{59}Fe in control plants may be due to intensified response to Fe deficiency in plants. The plants grown in –Fe and high P (control) medium may express chlorosis and show the higher absorption activity of Fe. The

activity of the transporter for Fe in the root plasma membrane of plants might be enhanced by –Fe and high P conditions.

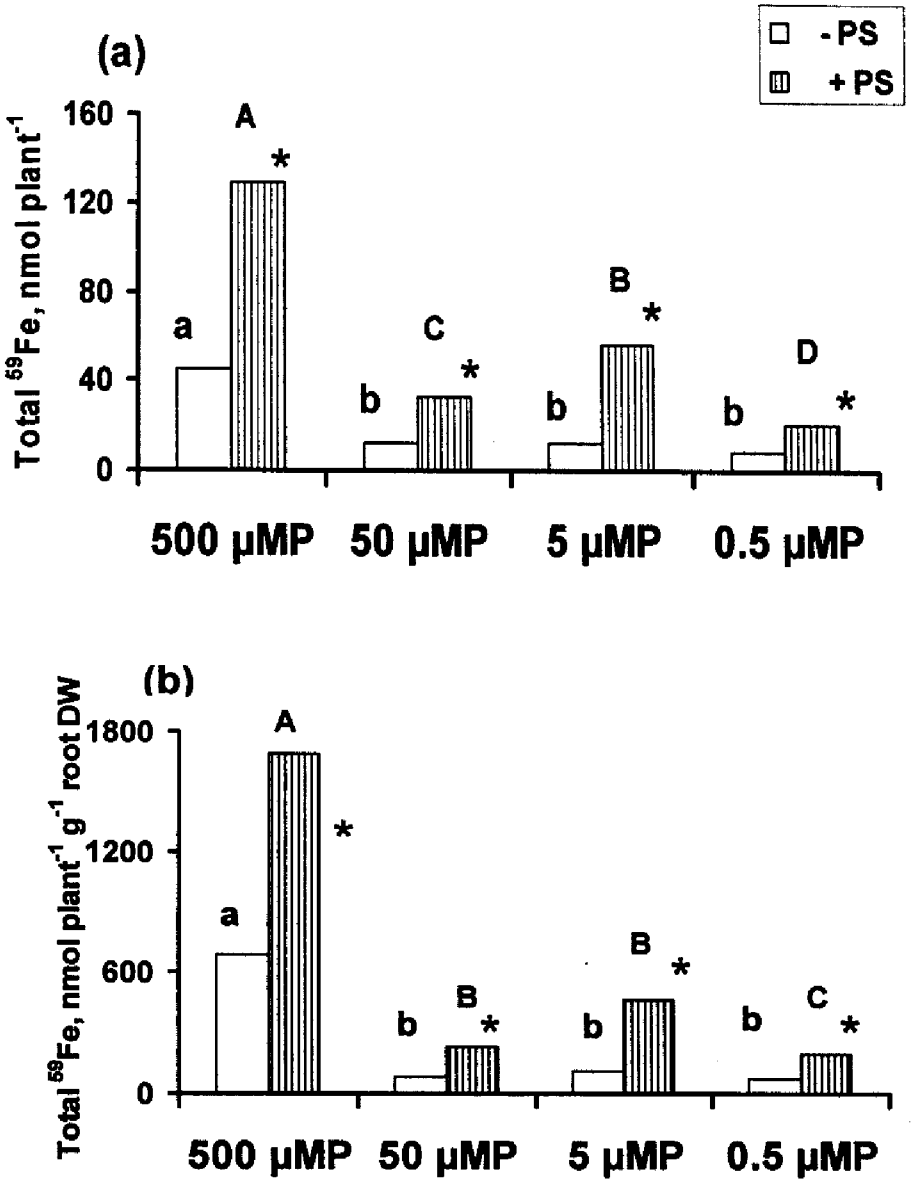


Figure 8.1. (a) Total absorption and (b) root absorption activity of ^{59}Fe in barley plants grown in –Fe medium as affected by different P levels (small letter for plants without PS and capital letter for plants with PS) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

Table 8.1. Dry weight (DW) and chlorophyll index (SPAD value) in barley plants grown in Fe-deficient medium with different P levels at 12 DAT

Treatment (μM) P	500	50	5	0.5
Shoot DW (mg bunch^{-1})	467 ± 45.9 c	812 ± 44.5 a	669 ± 32.4 ab	576 ± 12.8b
Root DW (mg bunch^{-1})	213 ± 9.0 b	418 ± 8.3 a	348 ± 21.4 a	329 ± 20.4 a
SPAD value	3 ± 0.58b	23 ± 1.9 a	24 ± 1.6 a	26 ± 1.8 a

Note: Means with standard deviation followed by different letters in each column are significantly different ($p<0.05$) according to Duncan Multiple Range Test.

It was indicated that PS could enhance total ^{59}Fe absorption per plant (Fig.1a) and ^{59}Fe absorption activity per root DW (Fig. 8.1b) in all P levels. Release of PS is dependent on ATP (Takagi, 1990). The ATP concentration in plant roots might be lowered by low P level (50, 5, and 0.5 μMP) of the media. However, it was noticeable that enhancement of the absorption of ^{59}Fe by PS was shown even under lowest P (0.5 μMP) condition. In this experiment, PS was equally fed to the roots of all the plants grown under varied P levels, and ATP consumption for PS release may not be critical. However, the amounts of ^{59}Fe absorption by low P plants were still lower than that by control plants. The ATP concentration in roots might affect the absorption of PS-^{59}Fe complex. Relationship with PS-Fe absorption and ATP concentration in roots needs to be studied. The enhancement of Fe absorption by adding PS to the medium was first described in Takagi et al. (1984), where the addition of PS (1-15 μM) caused marked increase in Fe uptake by rice seedlings from the nutrient solution.

8.3.3. Translocation and Relative Translocation rate of ^{59}Fe to Shoots

The translocation of ^{59}Fe to shoots in plants without PS feeding (Fig. 8.2a) was not clearly affected by low P level. Generally, addition of PS significantly enhanced

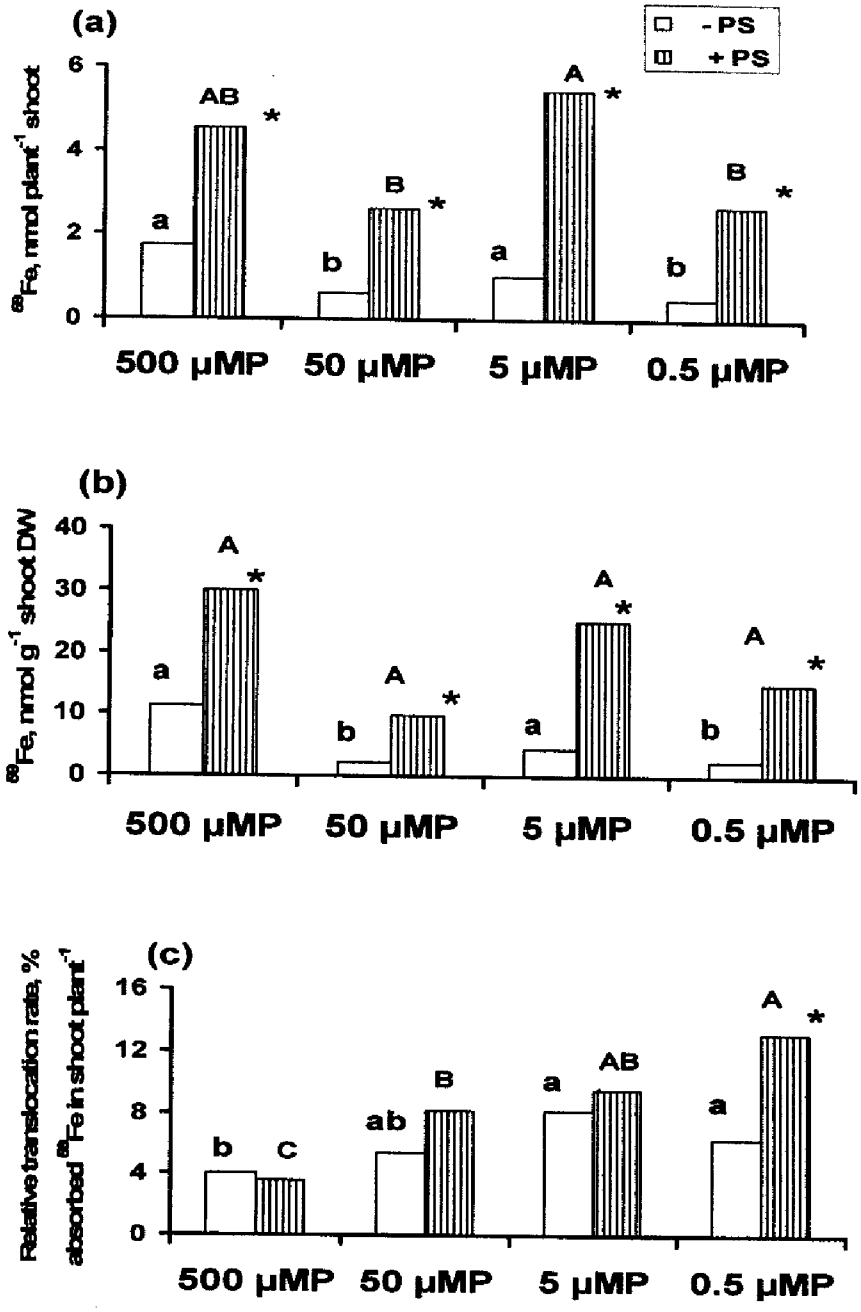


Figure 8.2. (a) Translocation, (b) concentration in shoots and (c) relative translocation rate of ^{59}Fe to shoots in barley plants grown in –Fe medium as affected by different P levels (small letter for plants without PS and capital letter for plants with PS) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

translocation of ^{59}Fe in each P level. The translocation of ^{59}Fe was enhanced 1.5 fold in control (500 μMP) plants and 3.2 to 4.3 fold in low P (50, 5, and 0.5 μMP) plants, respectively. The concentration of ^{59}Fe in shoots (Fig. 8.2b) followed the same pattern as the translocation of ^{59}Fe to shoot (Fig. 8.2a).

Phytosiderophore always enhanced ^{59}Fe translocation in all P levels. $^{59}Iron$ translocation in low P (50, 5, and 0.5 μMP) plants was enhanced by PS with higher ratios than that in control (500 μMP) plants. It is known that PS-Fe^{3+} is absorbed via a specific transporter in roots (Murata et al., 2006) and then translocated to the other parts of the plant (Römheld and Marschner, 1986; Takagi et al., 1984). Kawai and Alam (2006) reported that addition of PS to the medium enhanced largely Fe translocation into xylem tubes in –Fe barley. The protein of root membrane that mediated the absorption of PS-Fe^{3+} complex in maize (Zea mays L.) and the levels of ys1 mRNA increased in both shoots and roots under Fe-deficient condition (Curie et al., 2001). The activity for Fe translocation at the loading site to xylem may not be affected much in low P (50, 5, and 0.5 μMP) plants.

The relative translocation rate of ^{59}Fe was higher in the low P (50, 5, and 0.5 μMP) plants than that in control (500 μMP) plants with or without PS (Fig. 8.2c). It was indicated that high P condition depressed relative translocation rate of ^{59}Fe. This lower relative translocation rate of ^{59}Fe in control (500 μMP) plants might be due to a higher immobilization of Fe in the form of Pi-Fe complexes in root cells. The immobilization of Fe was reported in the older leaves and other plant parts as insoluble oxides, phosphates or formation of complexes with phytoferritin as a Fe-binding protein (Oh et al., 1996). The immobilization of Fe may diminish subsequent movement of Fe into the phloem for long-distance translocation (Taiz and Zieger, 2002). It was suggested that

the low relative translocation rate of ^{59}Fe induced by high P in –Fe plants may be a major factor responsible for the induction of Fe chlorosis. The low mobilization of Fe might result in low availability of Fe in the sites of chlorophyll synthesis in leaves. By contrast, the higher relative translocation rate of the low P plants may enhance the supply of Fe for the sites of chlorophyll synthesis in leaves.

There was no difference in the relative translocation rate between plants with and without PS in the plants under 500 (control), 50, and 5 μMP. However, at the lowest P level (0.5 μMP), this parameter was significantly enhanced by PS. It was considered that PS could enhance effectively the relative translocation rate of ^{59}Fe under lowest P (0.5 μMP) condition. The physiological mechanism about it needs to be investigated.

8.3.4. Apoplastic ^{59}Fe in Roots

In plants without fed PS, the root apoplastic ^{59}Fe per plant was not significantly affected by the P level of the media (Fig. 8.3a). When fed with PS, the apoplastic ^{59}Fe per plant was higher in the low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants (Fig. 8.3a). It was indicated that PS depressed formation of apoplastic ^{59}Fe in high P plants (control) probably through solubilizing Fe in the media, preventing adsorption of Fe to cell wall or membrane. The Fe in the root apoplast, where entry port for the transporter of PS-Fe complex is located, may be insoluble Fe-phosphate complex, bound Fe with protein, or adsorbed Fe to phospho-lipid of membrane of the root. The formation of such insoluble complexes of phosphate with Fe in plant tissues was reported (Oh et al., 1996). The supply of PS might depress the formation of such iron-phosphate complex in high P condition.

The apoplastic ^{59}Fe per root DW of the plants without fed PS was lower in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants (Fig. 8.3b). In plants fed with

PS, the concentration of apoplastic ^{59}Fe was not significantly affected by the P level of the medium. It was shown that PS did not affect this parameter in low P (50, 5, and 0.5 μMP) plants, but PS decreased it in control (500 μMP) plants.

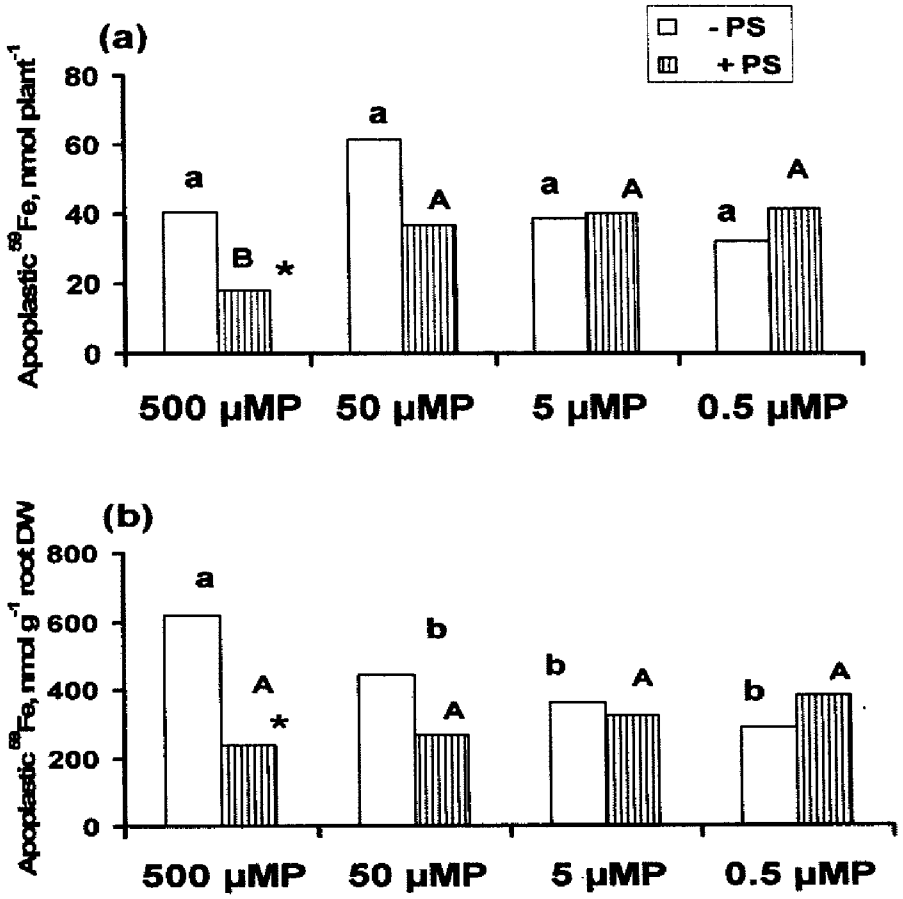


Figure 8.3. Apoplastic ^{59}Fe in roots in barley plants grown in –Fe medium as affected by different P levels (small letter for plants without PS and capital letter for plants with PS) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

Without PS feeding, the ratio of the ^{59}Fe content in root apoplast (Fig. 8.3a) to the total absorption of ^{59}Fe per plant (Fig. 8.1a) was 0.9 in control (500 μMP) plants but was 5.2, 3.3, and 3.9 in low P (50, 5, and 0.5 μMP) plants, respectively. With PS feeding, the

ratio was 0.14 at control (500 μMP) plants but 1.1, 0.7, and 2.0 at low P (50, 5, and 0.5 μMP) plants, respectively. Generally, the ratio was higher in –PS condition suggesting that PS enhanced Fe absorption and prevented formation of apoplastic Fe in this 4 hours experiment. In addition, the ratio was much lower in control (500 μMP) plants than in the low P (50, 5, and 0.5 μMP) plants. It was considered that there was a balance between absorption and formation of apoplastic Fe. It seemed that the plants with higher Fe demand, such as control (500 μMP) plants induced the lower ratio indicating that Fe was preferentially absorbed rather than being accumulated in apoplast. The mechanism of the formation of apoplastic Fe has not been clearly elucidated, and the function of apoplastic Fe has not been discussed sufficiently other than the role for reservoir of Fe in roots of wheat (Zhang et al., 1991). It was also suggested in soybean that the accumulation of short term reserve of Fe in the root apoplast and the translocation of Fe to the shoot may be important characteristics for the resistance of some cultivars to Fe chlorosis (Longnecker and Welch, 1990). In addition to the ratio of apoplastic Fe and total absorption of Fe, low relative translocation rate in control (500 μMP) plants was shown (Fig. 8.2c). It indicated that high P condition immobilized more Fe in roots. The spatial and chemical characteristics of the repression of Fe translocation in high P plant roots remains to be investigated. Further investigation about the mechanism of the immobilization of Fe by P is necessary.

8.4. SUMMARY

The results showed that the plants grown in control (500 μMP) medium had higher absorption activity of ^{59}Fe than the plants in low P (50, 5, and 0.5 μMP) media either with or without added PS. This higher absorption activity of ^{59}Fe may be induced by plant response to Fe deficiency.

The relative translocation rate of ^{59}Fe increased according to the decrease of the P level of the medium. Phytosiderophores enhanced the absorption of ^{59}Fe and its translocation from roots to shoots regardless of the P level of the media. The relative translocation rate of ^{59}Fe was significantly enhanced by PS in the lowest P (0.5 μMP) plants. The lower relative translocation rate of Fe in control plants may be induced by the physiological immobilization of Fe in the roots of plants.

CHAPTER 9

UPTAKE AND TRANSLOCATION OF ^{59}IRON IN LOW PHOSPHORUS BARLEY PLANT AS AFFECTED BY PHYTOSIDEROPHORE

9. Uptake and Translocation of Iron in Low Phosphorus barley as affected by Phytosiderophore

9.1. INTRODUCTION

Phosphorus follows N as the second most limited nutrients for plant growth in soils (Vance et al., 2003). It was reported that less than 15% of a Pi fertilizer applied to a soil is usually recovered by a crop grown immediately after application (Greenwood, 1981). The deficiency of P in high pH soils may potentially be accompanied with micronutrient deficiencies. For instance, in India, most of the soils in the chickpea-growing regions are simultaneously P- and micronutrient-deficient (Srinivasarao et al., 2006). The effect of P on the uptake and translocation of micronutrients such as Fe in plants is fairly understood. An equilibrium balance between P and Fe concentrations was reported as a regulating factor for chlorophyll synthesis in leaves of macadamia (Jones et al., 1972). There is no report on the mechanism for Fe absorption and translocation in plants under low P condition. The release of PS by Fe-stressed gramineae (Strategy II plants) to complex and convey Fe to the roots is well known (Takagi et al., 1984). Phytosiderophores are effective chelators that involve for not only the uptake but also the translocation of Fe in plants (Murata et al., 2006). The release of PS and the uptake of PS-Fe^{3+} by plants in calcareous or alkaline soils might potentially occur under the deficiencies of both P and Fe in field condition. However, it remains unknown whether P status affect the activity of PS for Fe uptake and translocation in plants or not. In order to examine the effect of PS in varied P status in graminaceous plants on the absorption and translocation of Fe, an experiment was conducted with barley plants grown in low P media for 14 days after treatment (DAT). The plants were fed with ^{59}Fe solution with or without added PS.

9.2. MATERIAL AND METHODS

9.2.1. Plant Culture and Growth

Seedlings of barley plants were cultivated hydroponically by the method described in the previous report (Ladouceur et al., 2006). Plants were transplanted as a bunch of 3 plants wrapped with sponge rubber to 10-L plastic buckets (16 bunches bucket^{-1}) filled up with 1/2–strength Hoagland-Arnon solution at pH 5.5 and for 2 days. After that, the plants were transferred to the 1/2–strength modified Hoagland-Arnon solution (Takagi, 1993) with 4 P levels, 500 (control), 50, 5, and 0.5 μMP supplied as ammonium dihydrogen phosphate ($NH_4H_2PO_4$). The plants were grown in a phytotron as described in Chapter 2. The pH of the nutrient solutions was daily monitored and adjusted to 5.5. The nutrient solutions were weekly renewed. The plants were allowed to grow until 14 days after treatment (DAT) prior to the feeding experiment.

9.2.2. Chlorophyll Index of Leaves

The chlorophyll index of the new leaves was measured as previously described in Chapter 3.

9.2.3. Origin of used PS and ^{59}Fe

The origin of the used ^{59}Fe and that of PS were the same as those described earlier in chapter 8.

9.2.4. Feeding with ^{59}Fe and PS

After washing the roots with deionized water, the plants were transferred to feeding solution with the 4 P levels added ^{59}Fe as $^{59}FeCl_3$ (10 μM ^{59}Fe) with or without PS (10 μM). Bunches of 3 plants were fed with ^{59}Fe (37 KBq) for 4 hours in beaker wrapped with aluminum foil containing 100 mL feeding solution as shown in Photograph 9.1. The apoplastic ^{59}Fe in roots was solubilized and removed by the method of Bienfait et al.

(1985) after feeding of ^{59}Fe. Subsequently, the plants were washed with tap water, divided into shoots and roots, oven dried at 70°C for 1 d, and weighed.

9.2.5. Measurement of ^{59}Fe

The dried plant materials were digested in concentrated nitric acid (Zarcinas et al. 1987). The radioactivity of ^{59}Fe of the digested plant solutions and of the root washing containing apoplastic ^{59}Fe were measured by the gamma scintillation counter as described in chapter 8. The extra-cellular ^{59}Fe in the root apoplast was not included in the ^{59}Fe content of roots. The calculation procedures and the definition of each measured parameter including the total plant absorption of ^{59}Fe, the translocation to shoots of ^{59}Fe, and the relative translocation rate of ^{59}Fe were same as those described in chapter 8.

9.2.6. Statistical Analysis

The experiment was a completely randomized block design with three replicates. Data were subjected to an ANOVA (SAS Institute, 1988). The means of treatment were compared according to Duncan's Multiple Range Test ($P<0.05$) using the computer "Origin 5" in Iwate University.

9.3. RESULTS AND DISCUSSION

9.3.1. Dry Biomass and Leaf Chlorophyll Index

The dry weight of the shoots was lower in the 5 and 0.5 μM P plants than in 50 or 500 (control) μM P plants (Table 9.1). There was no difference between the shoot dry matter of the control plants and that of 50 μM P plants. It was indicated that plant could maintain its growth under 50 and 500 μM P conditions. Phosphorus deficiency symptom occurred in plants grown at the lowest P level (5 μMP). It is known that the Pi concentration in the cytosol of plant tissue was maintained at fairly constant

concentrations in the range of 5 to 8 mol/m^3 (Lauer et al., 1989) regardless of the external P concentration in the medium, except under severe P deficiency (Lee and Ratcliff, 1993). The root dry weight of the low P (50, 5, and 0.5 μMP) plants was higher than that of control (500 μMP) plants (Table 9.1).

The chlorophyll index of the leaves was slightly lower in the low P plants (5 and 0.5 μMP) than in control (500 μMP) plants (Table 9.1). Iron is involved in chlorophyll synthesis. The accumulation of anthocyanin in shoots was reported as typical response of plants to P deficiency (Jain et al., 2007), being accompanied with low leaf photosynthetic activity (Rao et al., 1989). However, the accumulation of anthocyanin was not clearly observed in this experiment, though purple color on the basal part of stem was slightly observed.

Table 9.1. Dry weight (DW) and chlorophyll index (SPAD value) in barley plants grown with different P levels at 14 days after treatment

Treatment P (μM)	500	50	5	0.5
Shoot DW (mg bunch^{-1})	1327 a	1285 a	973 b	940 b
Root DW (mg bunch^{-1})	383b	531 a	457a	521 a
SPAD value	38 a	37 a	30 b	31 b

Note: Means followed by different letters in each row are significantly different ($p<0.05$) according to Duncan Multiple Range Test.

9.3.2. Absorption and Root Absorption activity of ^{59}Fe in Plant

The total absorption of ^{59}Fe was higher in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants with and without added PS in the feeding media (Fig. 9.1a).

The pattern of the root absorption activity of ^{59}Fe (Fig. 9.1b) was similar to that of the total absorption of ^{59}Fe in plants. The supply of PS to the plants increased 2.9 to 6.5 fold the total absorption and the absorption of ^{59}Fe per root DW in all of the P levels. The efficiency of PS in increasing ^{59}Fe uptake was the highest in 50 μMP plants (6.5 fold). It was indicated that low P treatment enhanced Fe absorption in plants regardless of the

Photograph 9.1. Sufficient (500 μMP) and low P (50 μMP) barley plants in ^{59}Fe or ^{59}Fe-PS containing feeding solutions.

P concentration. The low chlorophyll index (Table 9.1) of the plants grown under P-deficient (5 and 0.5 μMP) condition may be related to higher Fe absorption activity. Our result suggested also that enhancement activity of PS for Fe absorption in plant varied depending on plant P status. The chelating and transporting activity for Fe of PS

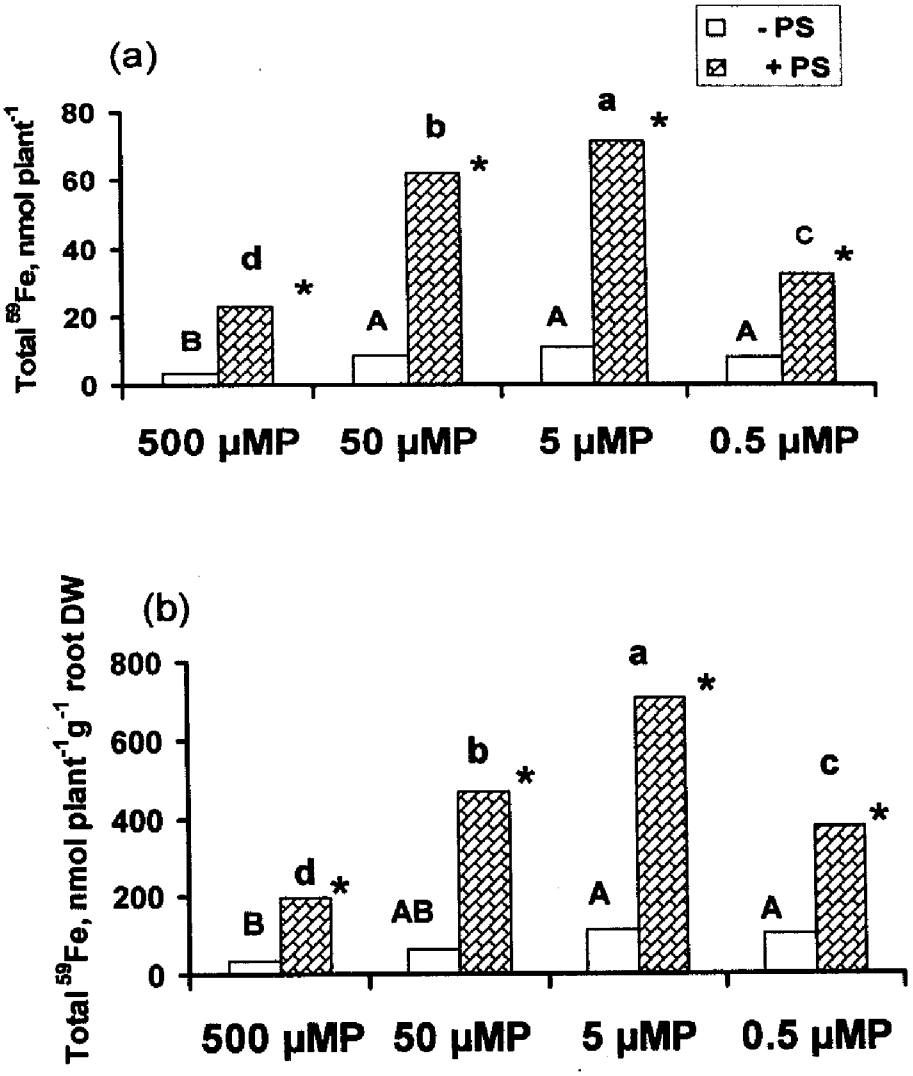


Figure 9.1. (a) Total absorption and (b) root absorption activity of ^{59}Fe in barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

occurred in plants not only in –Fe medium (Römheld and Marschner, 1986) but also in +Fe medium. The lower chlorophyll index of the low P plants might activate the transporter of Fe to the roots. The release of PS is known to be dependent on ATP

(Takagi, 1990), the absorption of PS-Fe^{3+} may also be dependent on ATP. However, the plants fed with PS in low P (50, 5, and 0.5 μM) media absorbed higher amount of ^{59}Fe than the control plants, suggesting that ATP for PS-Fe^{3+} uptake was still available even in the lowest P (0.5 μM) plants.

9.3.3. Translocation and Relative Translocation rate of ^{59}Fe to Shoots

In the absence of added PS, the translocation of ^{59}Fe from roots to shoots in plants fed with ^{59}Fe was not affected by low P (50, 5, and 0.5 μMP) as compared to that of the control (500 μMP) plants (Fig. 9.2a). The low P (50, 5, and 0.5 μMP) plants showed higher translocation of ^{59}Fe than control (500 μMP) plants when both plants were fed with ^{59}Fe with added PS. Plants fed with PS showed higher translocation of ^{59}Fe than plants without fed PS in each P level. It was considered that the shoot Fe demand for chlorophyll synthesis in P–deficient plants might be greater than that in P-sufficient plants. Otherwise, the translocation of PS-^{59}Fe might be more prompt in plants in low P status than in high P status. The pattern of the translocation of ^{59}Fe per shoot DW (Fig. 9.2b) was similar to that of the translocation of ^{59}Fe per plant. The efficiency of PS for the increase of the translocation of ^{59}Fe was much higher in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants, suggesting that PS activity for Fe translocation may be more enhanced under low P condition. It meant that much higher amount of ^{59}Fe was absorbed in the form of PS-^{59}Fe and translocated to the shoots in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants. It is known that PS-Fe^{3+} complex absorbed at the root surface via a specific transporter (Murata et al., 2006) and then translocated to different parts of the plant (Römheld and Marschner, 1986). Kawai and Alam (2006) reported the detection of PS-Fe^{3+} complex in xylem tubes of –Fe barley. It

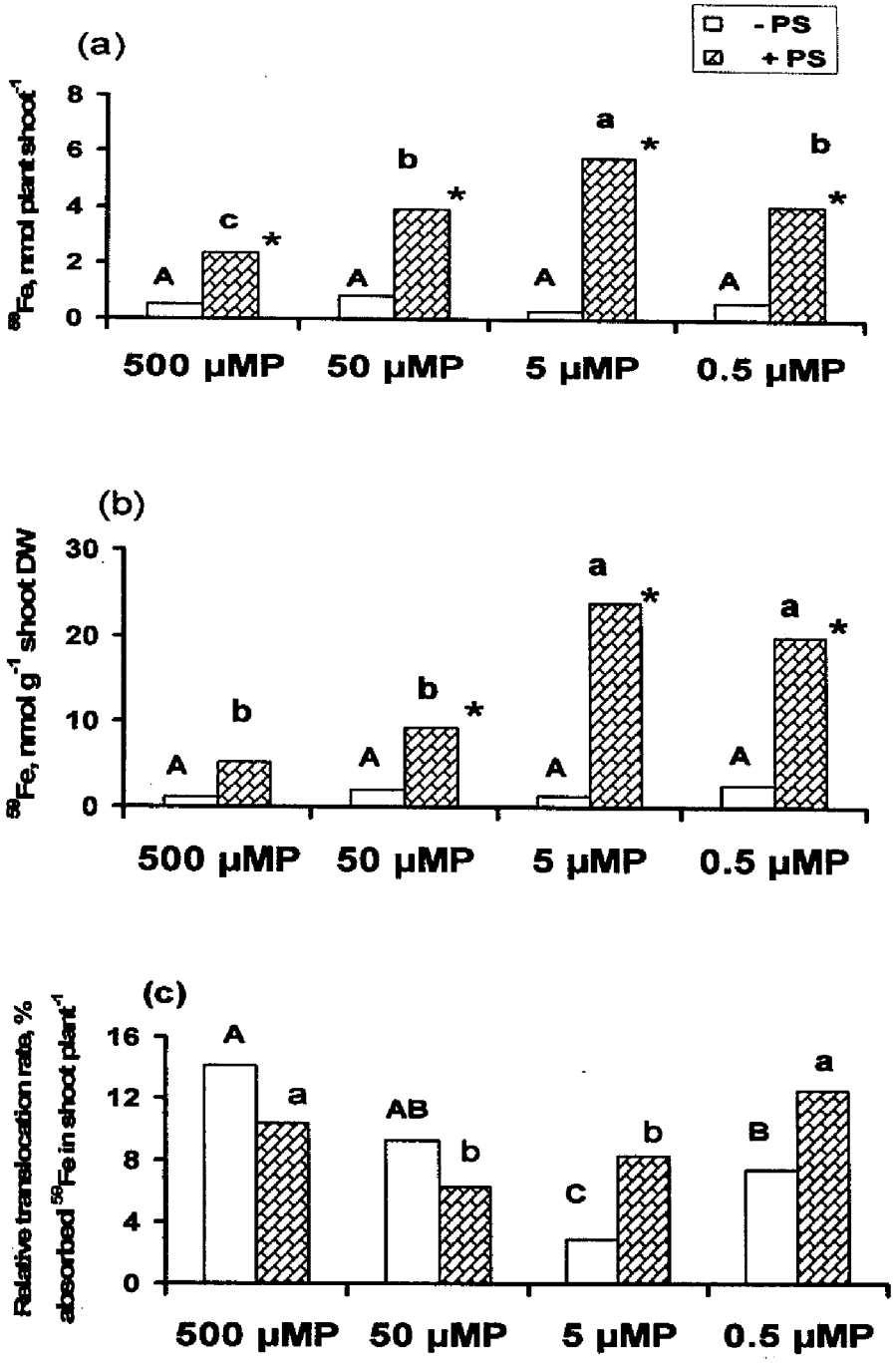


Figure 9.2. (a) Translocation, (b) concentration in shoots and (c) relative translocation rate of ^{59}Fe to shoots in barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

is considered that absorbed PS-Fe^{3+} might be actively loaded into xylem tubes. The protein mediator at the membrane for the absorption of PS-Fe^{3+} complex in maize (Zea mays L.) and the levels of ys1 mRNA increased in both shoots and roots in –Fe condition (Curie et al., 2001). The activity for Fe translocation at the loading site to xylem may be affected by low P status in plants. In our data obtained without PS, the result was not consistent with the past researcher's discussion that Fe translocation from roots to leaves may be hampered by high P condition in plants (Hue and Nakamura, 1988). However, the data in the PS added condition showed that Fe translocation was much decreased in plants grown under P-sufficient condition. It was suggested that Fe translocation was activated in plants not only by Fe deficiency (Bauer and Hell, 2006) but also by P deficiency. It is considered that PS is involved in this activation.

The relative translocation rate of ^{59}Fe was lower in the plants grown under 5 and 0.5 μMP condition than in control (500 μMP) plants when both plants were fed with ^{59}Fe without added PS (Fig. 9.2c). The relative translocation rate of ^{59}Fe in plants fed with added PS was lower in low P plants (50 and 5 μMP) than in control (500 μMP) plants (Fig. 9.2c). Addition of PS to the media did not affect significantly the relative translocation rate in all the P levels. The precipitation of Fe was reported in the older leaves and other plant parts as insoluble oxides, phosphates or formation of complexes with phytoferritin an iron-binding protein (Oh et al., 1996). The precipitation of Fe may diminish subsequent mobilization of the metal into the phloem for long-distance translocation (Taiz and Zieger, 2002). The low mobilization of Fe for long distance translocation might result in low availability of Fe in the sites of the synthesis of chlorophyll in leaves.

9.3.4. Apoplastic ^{59}Fe in Roots

The apoplastic ^{59}Fe per plant in roots was higher in the the low P (50, 5, and 5 μMP) plants than in control (500 μMP) plants when fed with ^{59}Fe without PS (Fig. 9.3a), suggesting that low P conditions enhanced ^{59}Fe accumulation in root apoplast. In the

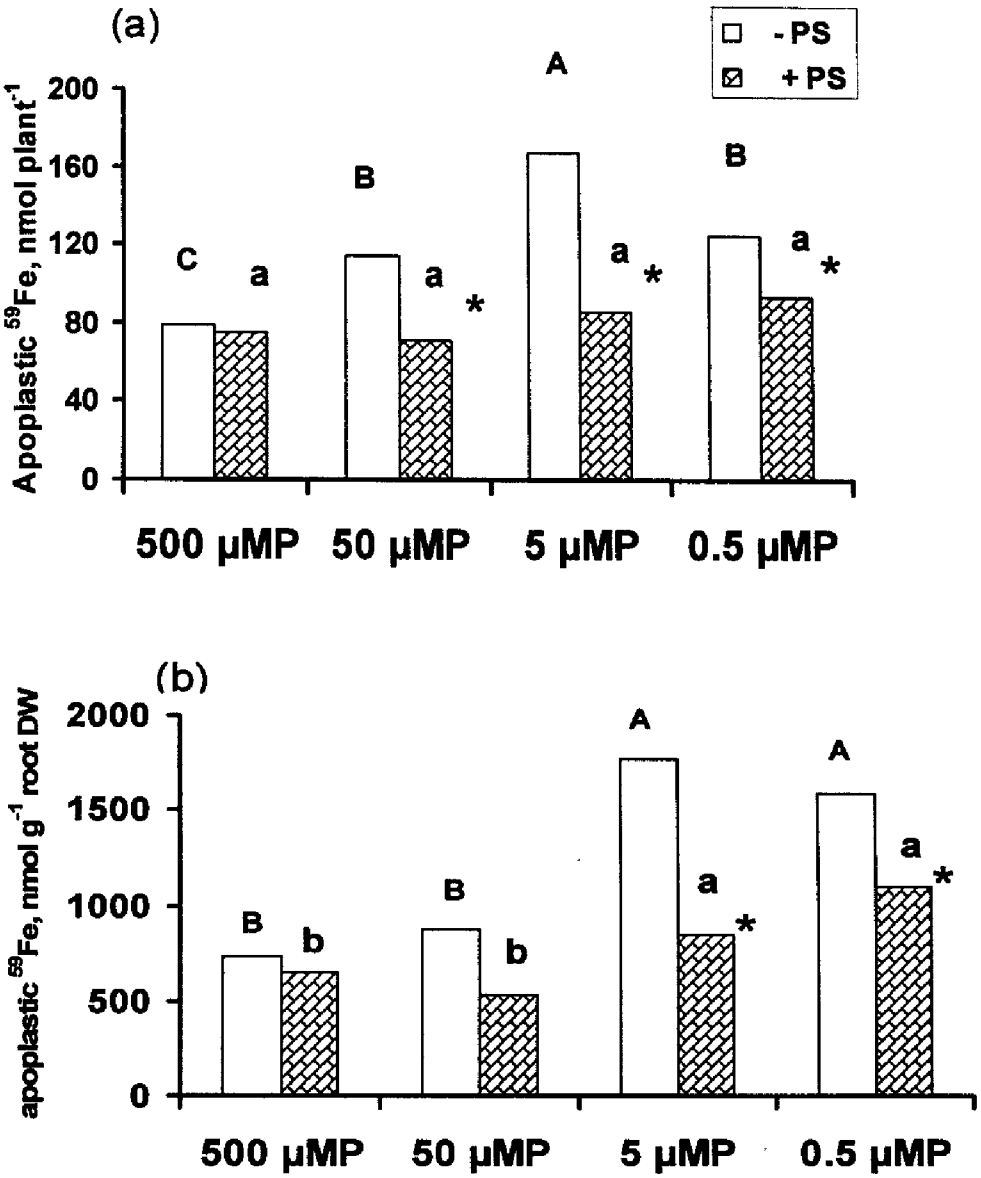


Figure 9.3. Apoplastic ^{59}Fe in roots in barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

presence of PS, the root apoplastic ^{59}Fe was similar in all P levels, suggesting that PS reduced the formation of ^{59}Fe in the apoplast of the roots of the low P (50, 5, and 5 μMP) plants to similar level as that of control (500 μMP) plants. Zhang et al. (1991) reported the mobilization of Fe from root apoplast by PS in Fe-deficient wheat. The supply of PS to the media reduced the root apoplastic ^{59}Fe in all of the low P (50, 5, and 0.5 μM) plants while not affecting it in control (500 μMP) plants. The apoplastic ^{59}Fe per plant root DW showed similar tendency (Fig. 9.3b) either with or without fed PS.

9.4. SUMMARY

Our results showed that low P concentration of the medium enhanced the total absorption of ^{59}Fe without affecting its relative translocation rate in plants. The addition of PS to the medium enhanced the total absorption of ^{59}Fe and its translocation in plants at all of the P levels of the media. The supply of PS to the medium reduced the apoplastic ^{59}Fe in roots of plants in the low P (50, 5, and 0.5 μM) media. The mechanism of the enhancement of Fe absorption and translocation by PS in low P condition needs further investigation.

CHAPTER 10

ABSORPTION AND TRANSLOCATION OF ^{59}IRON IN LOW PHOSPHORUS AND IRON-DEFICIENT BARLEY PLANTS AS AFFECTED BY DIFFERENT IRON CHELATORS

10. Absorption and translocation of ^{59}iron in low phosphorus and iron-deficient barley plants as affected by different iron chelators

10.1. INTRODUCTION

From the experiment results described in the previous chapter, low P condition enhanced growth and chlorophyll index of plants grown under –Fe condition. We also found that short-term depletion of P from –Fe medium increased the release amount of PS. The concentrations of PS, citrate, and Fe in the xylem sap of –Fe barley plants were higher in low P medium. It was also shown that PS increased ^{59}Fe absorption and translocation to shoots either in high or low P plants, but with greater effectiveness in the low P plants grown under –Fe condition. Our results showed that the relative translocation rate of Fe was higher in low P plants than in control plants under –Fe condition. The release of organic acids, particularly citrate, is well known as a reaction of P-starved plants (Hoffland et al., 1989) and the release of PS is well documented in Strategy II plants such as barley in –Fe condition (Römheld and Marschner, 1986; Takagi et al., 1976). The increase of citrate in roots is also known as a reaction of Strategy I plants to acquire Fe from Fe-deficient soils (Brown and Chaney, 1971; Landsberg, 1986). Iron was found as Fe-citrate in the xylem sap of tomato (Strategy I) plants. In some soils P- and Fe-deficiency may occur simultaneously. It is not known whether citrate and PS together, or PS alone are involved in Fe translocation in barley (Strategy II) plants under low P and –Fe condition. This experiment was conducted in order to examine the absorption and translocation of Fe of barley plants in –Fe and low P medium when added with Fe chelator such as PS, citrate, or EDTA.

10.2. MATERIAL AND METHODS

10.2.1. Plant Culture and Growth

Seedlings of barley plants were cultivated hydroponically by the method described previously in chapter 8. Plants were transplanted in bunch of 3 plants wrapped with sponge rubber and transferred to 10-L plastic buckets (16 bunches bucket^{-1}) filled up with 1/2–strength Hoagland-Arnon solution for 2 days. Subsequently, the plants were transferred to the –Fe 1/2–strength modified Hoagland-Arnon solution (Takagi, 1993) with 2 different levels of P, 500 (control), 50 μMP. Phosphorus was supplied as NaH_2PO_4. The plants were grown in a phytotron as described in chapter 2. The pH of the nutrient solutions was daily monitored and adjusted to 6.5. The nutrient solutions were weekly renewed. The plants were allowed to grow for 13 days after treatment (DAT) prior to ^{59}Fe feeding experiment in medium added PS, citrate or ethylene diamine tetraacetic acid (EDTA). Plants are shown in Photograph 10.1 with the typical Fe chlorosis in control (500 μMP) plants which was partially alleviated in 50 μMP plants.

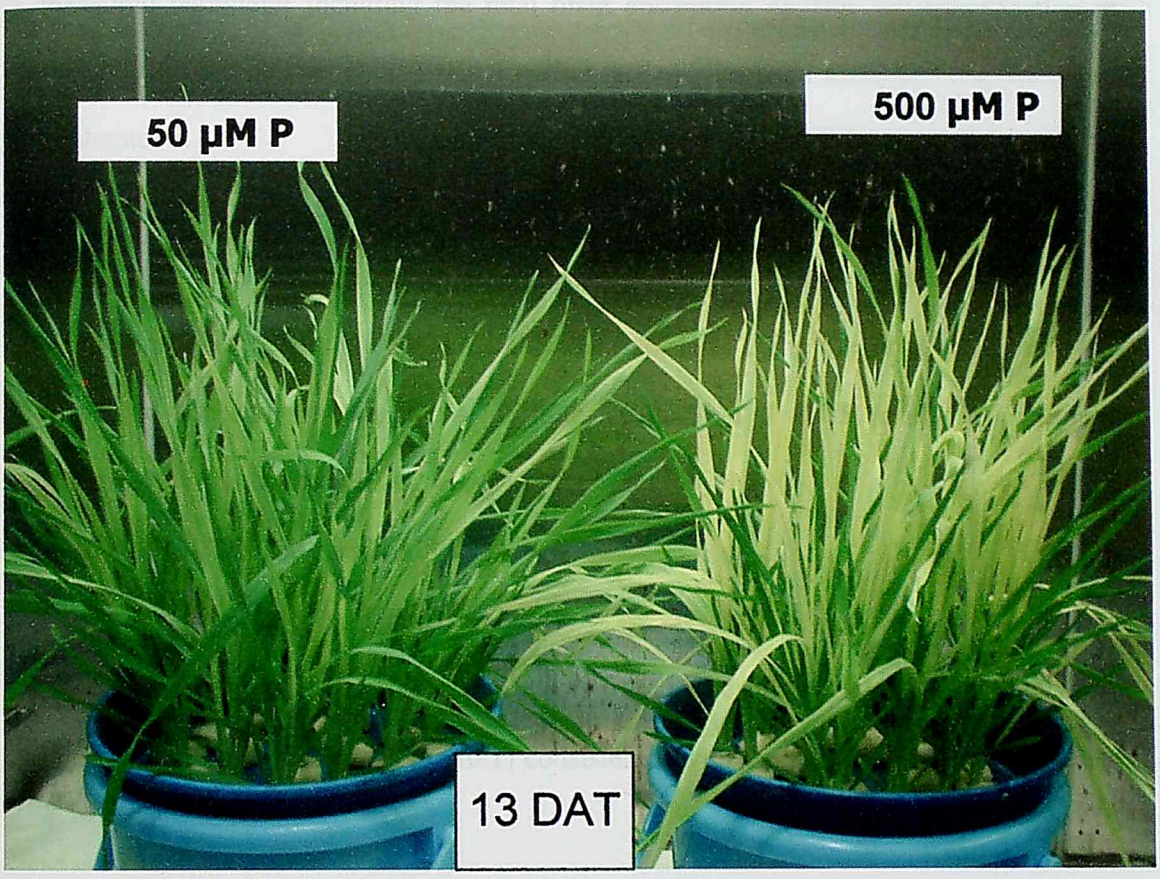
10.2.2. Source of used PS, Citrate, EDTA, and ^{59}Fe

Mugineic acid, one of the major PS released by the barley cultivar, was collected from the root washings of –Fe barley by the method previously described (Takagi, 1993). Citrate and EDTA were reagents purchased from Kanto Chemical Co., Inc., Tokyo, Japan. The ^{59}Fe-radionuclide was purchased from Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA, USA.

10.2.3. Feeding with ^{59}Fe

At 13 DAT, plant roots were washed with deionized water. Subsequently, plants were transferred to feeding solution with the 2 P levels where ^{59}Fe as $^{59}FeCl_3$ (10 μM) was added with or without added either PS (10 μM), citrate (10 μM) or EDTA (10 μM),

respectively. Plants were fed in triplicate for 4 hours in time, when PS were not released by roots, in beakers wrapped with aluminum foil containing 100 mL of feeding solution.



Photograph 10.1. Barley plants as affected by two different P levels in Fe-deficient medium.

The starting time of the feeding of ^{59}Fe of plants was 8 hours after the onset of light in the phytotron. The radioactivity of ^{59}Fe in each beaker was 37 KBq. The apoplastic ^{59}Fe in roots was solubilized and removed after the feeding time by the method of Bienfait et al. (1985). After that, the plants were throughout washed with tap water, divided into shoots and roots, oven dried at 70˚C for 1 d, and weighed.

10.2.4. Measurement of ^{59}Fe

Dried shoots and roots of the plants were digested in concentrated nitrate as described by Zarcinas et al. (1987). The radioactivity of ^{59}Fe of the digested plant solutions or the

root washing containing apoplastic ^{59}Fe was determined by using the methods described in chapter 8. The amount of the extracellular ^{59}Fe in the root apoplast was not included in the ^{59}Fe content in roots. The calculation procedures and the definition of each measured parameter including the total plant absorption of ^{59}Fe, the translocation to shoots of ^{59}Fe, and the relative translocation rate of ^{59}Fe were same as those described in chapter 8.

10.2.5. Statistical Analysis

The experiment was arranged in completely randomized block design with 3 replicates. Data were subjected to an ANOVA (SAS Institute, 1988). The means of treatment were compared according to Duncan's Multiple Range Test ($P<0.05$) using the computer "Origin 5" in Iwate University.

10.3. RESULTS AND DISCUSSION

10.3.1. Dry Weight of the Plants

The dry weights of the shoots and the roots were higher in low P (50 μMP) plants than in control (500 μM) plants (Fig. 10.1) consistently with the results described in chapter 5.

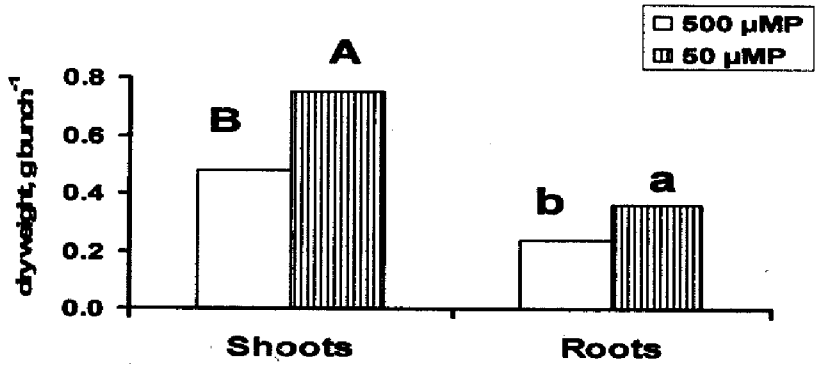


Figure 10.1. Dry weight of barley plants as affected by two different P levels in –Fe media. Different letter at the top of each bar indicates significant differences ($P<0.05$) according to Duncan Multiple Range Test.

10.3.2. Total Absorption of ^{59}Fe in Plant

The total absorption of ^{59}Fe was higher in low P (50 μMP) plants than in control (500 μMP) plants fed with ^{59}Fe both with and without Fe chelator (Fig. 10.2a). The supply of

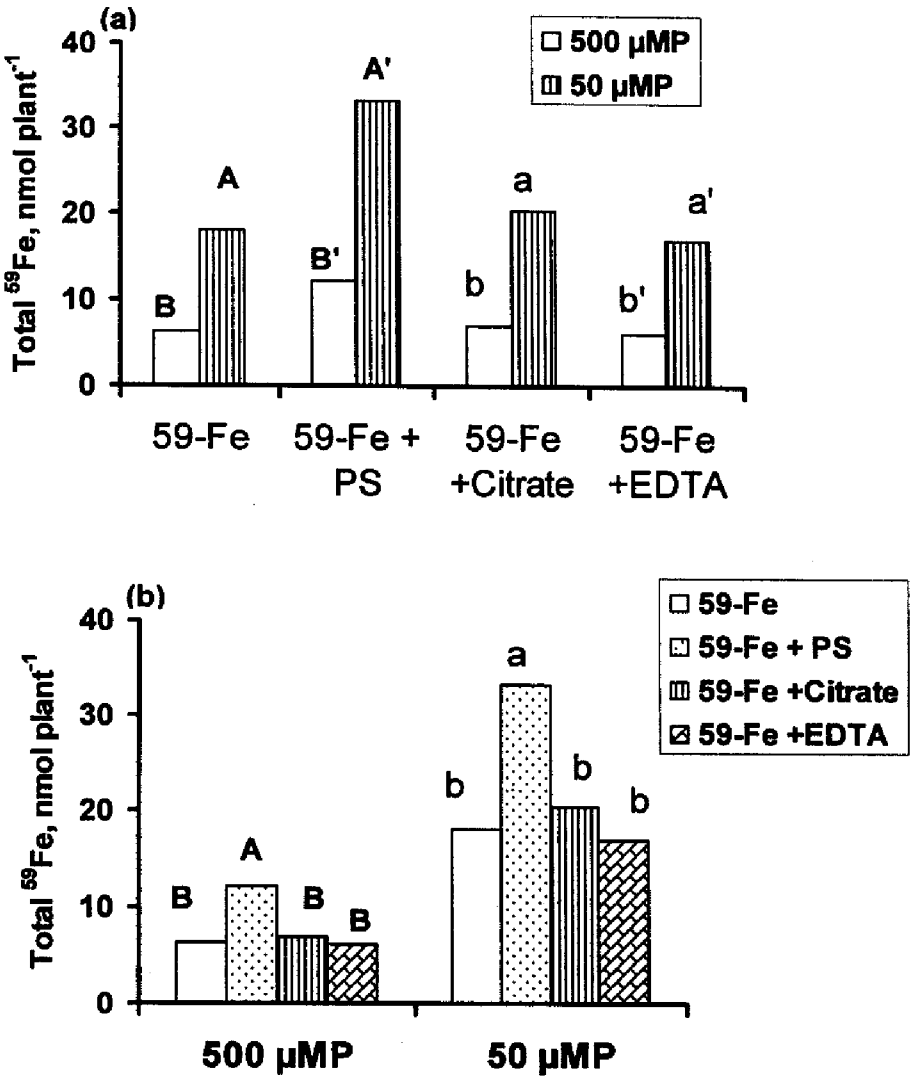


Figure 10.2. Total absorption of ^{59}Fe in barley plants grown with two different P levels in –Fe medium as affected by three different Fe chelators (PS, citrate, EDTA). Different letter at the top of each bar indicates significant differences ($P<0.05$) between P levels (a) or among chelators (b) in each P level, respectively, according to Duncan Multiple Range Test.

PS enhanced the absorption of ^{59}Fe of plants under control (500 μMP) and low P (50 μMP) condition (Fig. 10.2b). The addition of citrate and EDTA did not affect the absorption of ^{59}Fe of plants at both P levels of the medium. It was suggested that low P condition enhanced ^{59}Fe absorption in plants regardless of the Fe. This result confirmed the specific efficiency of PS for the enhancement of Fe absorption. Takagi et al. (1984) reported that addition of PS increased Fe uptake by rice seedlings in nutrient solution. Citrate and EDTA were ineffective for the enhancement of Fe absorption by plants either under low or high P conditions.

10.3.3. Translocation of ^{59}Fe to Shoot

The translocation of ^{59}Fe was higher in low P (50 μMP) plants than in control (500 μMP) plants fed with ^{59}Fe both with and without Fe chelator (Fig. 10.3a). Phytosiderophore enhanced the translocation of ^{59}Fe to shoots at both of P levels, but its effect was greater in low P (50 μMP) plants than in control (500 μMP) plants. Citrate and EDTA did not affect significantly ^{59}Fe translocation in plants both in control and low P plants (Fig. 10.3b). It was indicated that low P treatment enhanced Fe translocation and PS efficiency on Fe translocation to shoots in –Fe plants.

10.3.4. Relative Translocation rate of ^{59}Fe to Shoots

The relative translocation rate of ^{59}Fe to shoots was not significantly affected by the P level or by the addition of PS or EDTA to the medium (Fig. 10.4a) However, the supply of citrate to the plants enhanced the translocation rate of ^{59}Fe in low P (50 μMP) medium as compared to that in control (500 μMP) medium. It was suggested that citrate may enhance Fe relative translocation rate in low P and –Fe plants. The Fe chelators (PS, citrate, and EDTA) used in the experiment did not affect the relative translocation rate of plants both in control (500 μMP) and low P (50 μMP) medium (Fig. 10.4b).

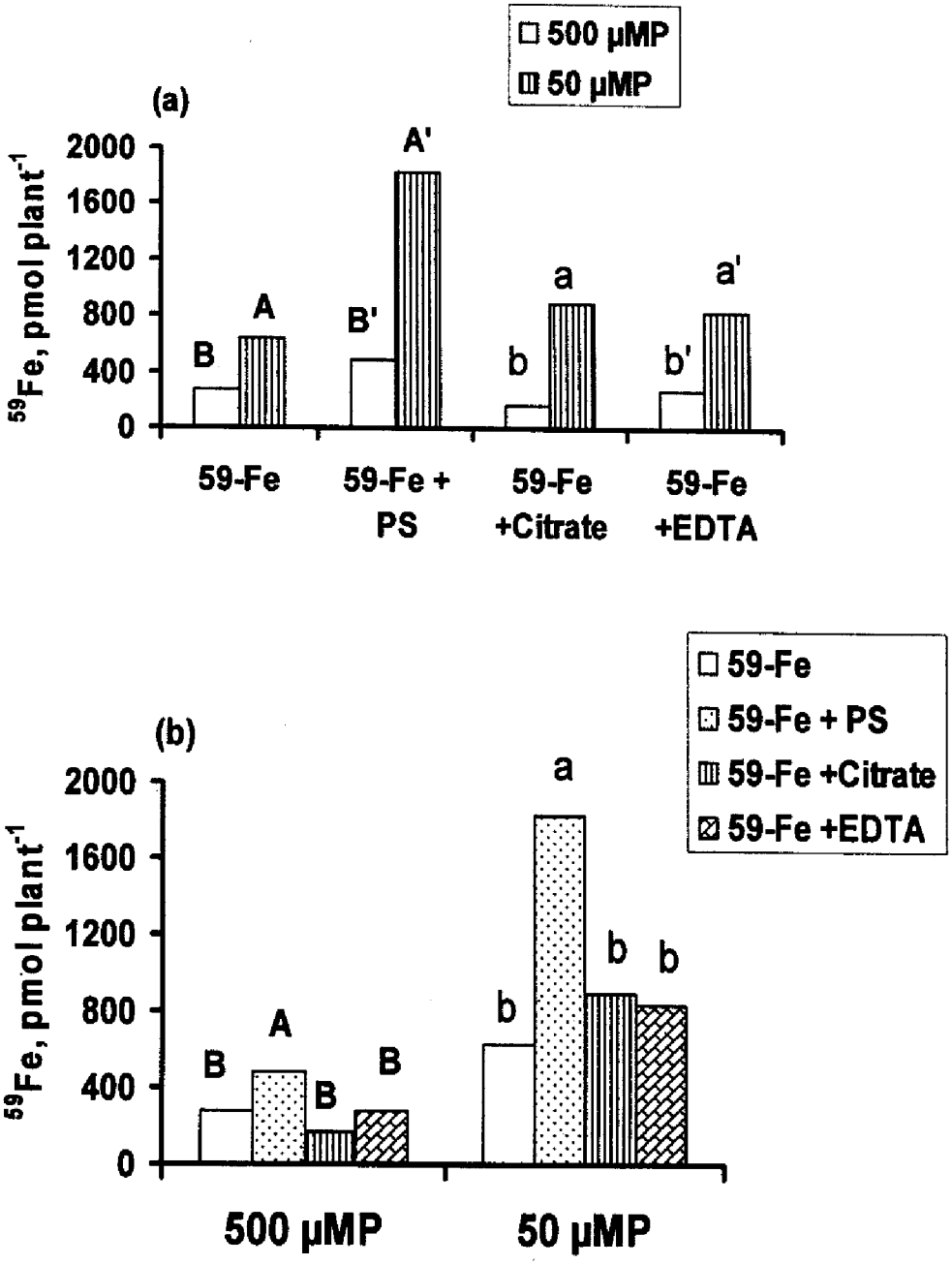


Figure 10.3. Translocation of ^{59}Fe from roots to shoots in barley plants grown with two different P levels in –Fe medium as affected by three different Fe chelators (PS, citrate, EDTA). Different letter at the top of each bar indicates significant differences ($P<0.05$) between P levels (a) or among chelators (b) in each P level, respectively, according to Duncan Multiple Range Test.

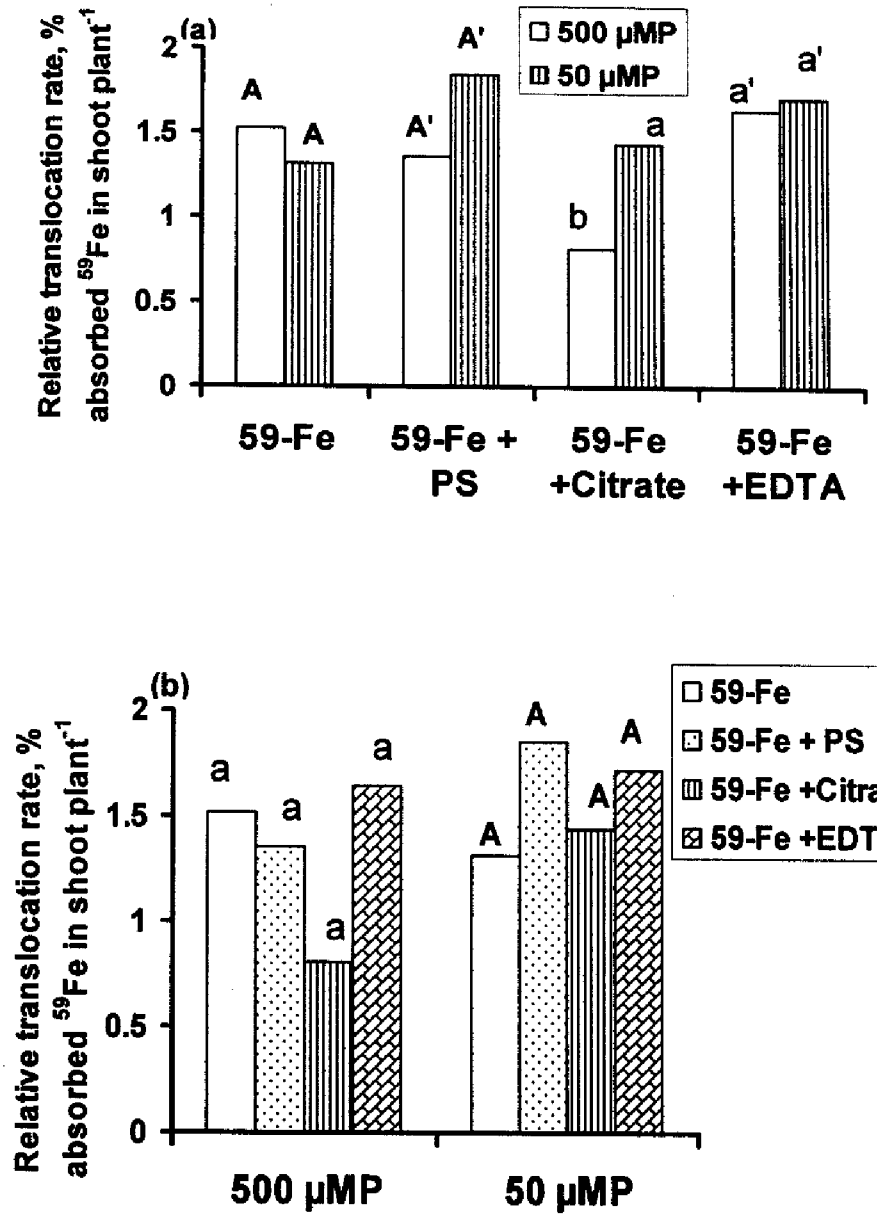


Figure 10.4. Relative translocation rate of ^{59}Fe from roots to shoots in barley plants grown with two different P levels in –Fe medium as affected by three different Fe chelators (PS, citrate, EDTA). Different letter at the top of each bar indicates significant differences ($P<0.05$) between P levels (a) or among chelators (b) in each P level, respectively, according to Duncan Multiple Range Test.

10.3.5. Apoplastic ^{59}Fe in Roots

The content of apoplastic ^{59}Fe in roots was similar for both control (500 μMP) plants and low P (50 μMP) plants (Fig. 10.5a). The supply of the Fe chelators (PS, citrate, EDTA) in low P (50 μMP) plants did not affect significantly the accumulation of ^{59}Fe in

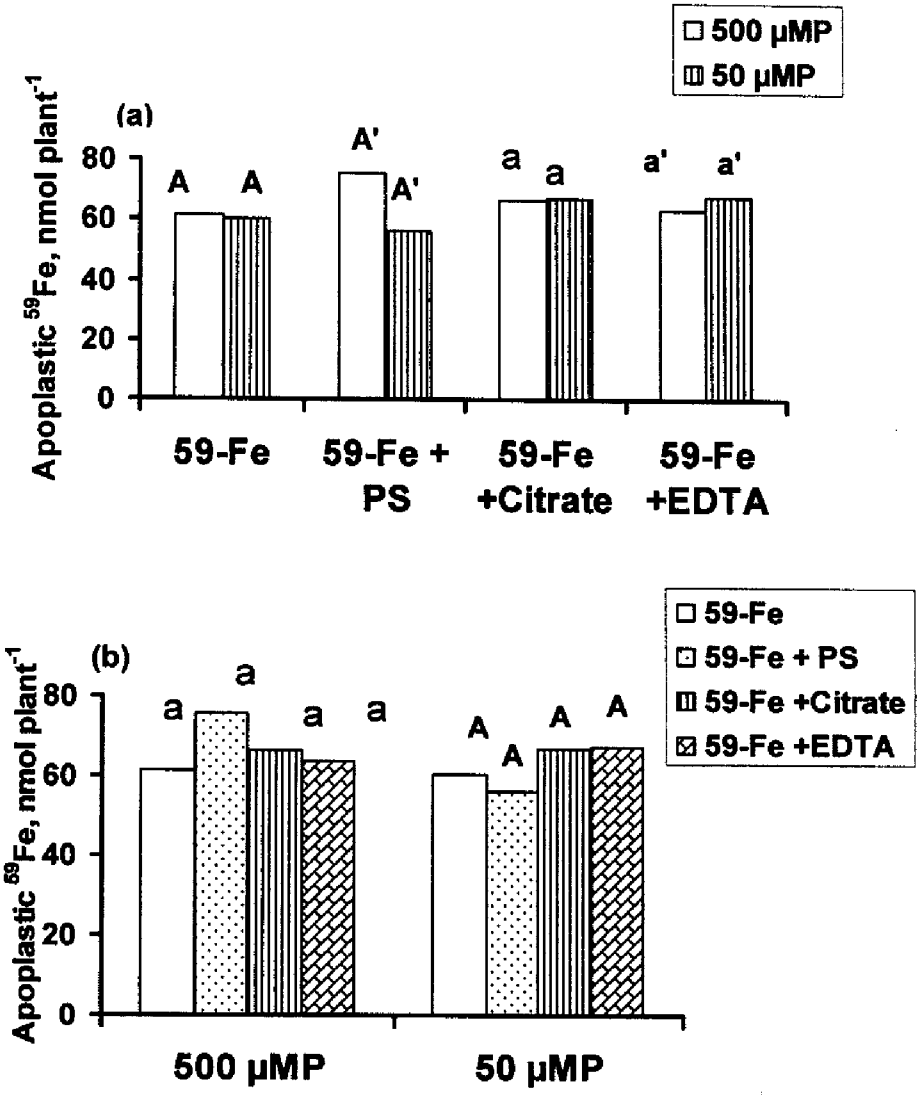


Figure 10.5. Apoplastic ^{59}Fe in roots of barley plants grown with two different P levels in –Fe medium as affected by three different Fe chelators (PS, citrate, EDTA). Different letter at the top of each bar indicates significant differences ($P<0.05$) between P levels (a) and among chelators (b) in each P level, respectively, according to Duncan Multiple Range Test.

in roots as compared to those of the control (500 μMP) plants. Apoplastic ^{59}Fe in plant roots was not affected by the addition of Fe chelators such as PS, citrate or EDTA to the feeding solution in each P concentration of the medium (Fig. 10.5b).

10.4. SUMMARY

The results of this experiment showed that low P (50 μMP) condition enhanced ^{59}Fe absorption and translocation to shoots. The supply of PS enhanced ^{59}Fe absorption and translocation of the plants in low P (50 μMP) and control (500 μMP) medium with greater efficiency under low P (50 μMP) condition. The other Fe chelators, citrate and EDTA did not affect the absorption and translocation of ^{59}Fe in plants both in control (500 μMP) and low P (50 μMP) conditions of the medium. It was suggested that citrate and EDTA are ineffective on Fe absorption and translocation in –Fe barley plants. The relative translocation rate of absorbed ^{59}Fe to shoots in plants was not significantly affected, neither by the P level of the medium nor by the presence of Fe chelator in control (500 μMP) or low P (50 μMP) medium. However, the supply of citrate enhanced the relative translocation rate of the low P (50 μMP) plants as compared to that of the control (500 μMP) plants. It was suggested that citrate might also involve in Fe translocation in –Fe and low P conditions of the medium.

The increased growth and partial alleviation of Fe chlorosis in plants under low P (50 μMP) and –Fe conditions may be due to the higher Fe absorption and translocation capacity and the greater efficiency of PS for enhancing Fe absorption and translocation to shoots under the condition. Additionally, citrate may also contribute to enhance the relative translocation rate of the absorbed Fe to shoots in low P and –Fe plants. There is no report that xylem sap of Strategy II plants contains Fe in the form of Fe-citrate. The

form of Fe in xylem sap of low P plants and the mechanism for the enhancement of relative translocation rate of absorbed Fe under low P condition need to be clarified.

CHAPTER 11

ABSORPTION AND TRANSLOCATION OF ^{65}ZINC IN LOW PHOSPHORUS BARLEY PLANTS AS AFFECTED BY PHYTOSIDEROPHORES UNDER pH 5.5

11. Absorption and translocation of ^{65}Zinc in low phosphorus barley plants as affected by phytosiderophores under pH 5.5

11.1. INTRODUCTION

The zinc (Zn)-phosphorus (P) antagonistic interaction in soils and in plant tissues was reported by several researchers (Loneragan et al., 1979; Neilsen and Hogue, 1986; Boawn and Rasmussen, 1971). According to the report of Pasricha et al. (1987), this interaction may not always occur. The Zn content of the plant dry matter was not decreased while the physiological availability of Zn (solubility and mobility of Zn both within the cells and in long-distance transport to the shoot apex) was decreased by high P content of the shoot (Cakmak and Marschner, 1986; Cakmak and Marschner, 1987). The ability of PS of to chelate and convey Fe to plant roots in the rhizosphere was also reported for other metal micronutrients, such as Zn, Cu, and Mn, in calcareous soil conditions (Treeby et al., 1989; Singh et al., 1992). Our preliminary work described in chapter 7 showed that the concentrations of several micronutrients including Zn increased in the xylem sap of the plants under low P and –Fe conditions. This experiment was conducted with barley plants grown hydroponically with 4 different P levels and fed with ^{65}Zn with or without added PS. The objective was to examine the effect of PS and low P on the absorption and translocation of Zn of barley plants.

11.2. MATERIALS AND METHODS

11.2.1. Plant Culture and Growth

Seedlings of barley plants were cultivated hydroponically by the method described previously in chapter 2. Plants were transplanted in bunch of 3 plants wrapped with sponge rubber and transferred to 10-L plastic buckets (16 bunches bucket^{-1}) filled up with 1/2–strength Hoagland-Arnon solution for 2 days. After that, the plants were

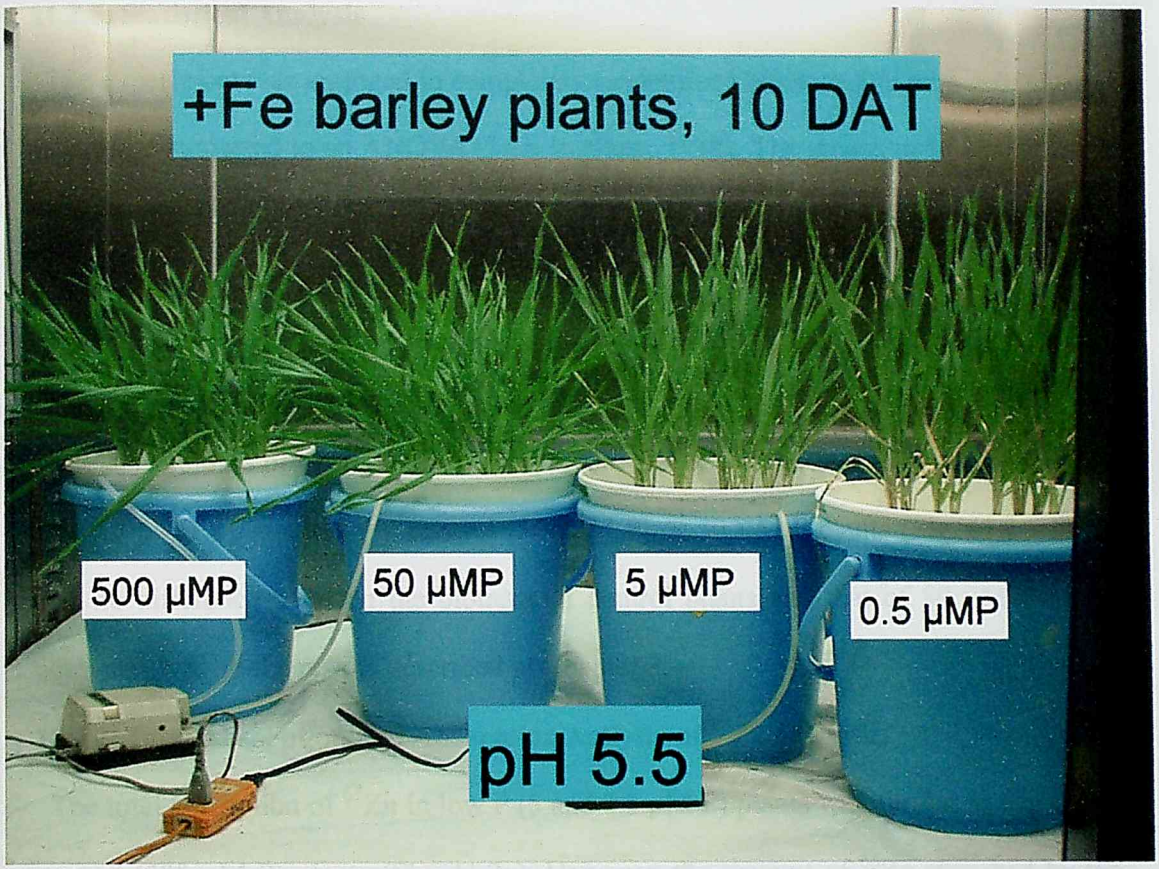
transferred to the 1/2–strength modified Hoagland-Arnon solution (Takagi 1993) with 4 different levels of P, 500 (control), 50, 5, and 0.5 μMP. Phosphorus was supplied as $NH_4H_2PO_4$. The plants were grown in the phytotron as described in Chapter 3. The pH of the nutrient solutions was daily monitored and adjusted to 5.5. The nutrient solutions were weekly renewed. The plants were allowed to grow for 10 days after treatment (DAT) prior to ^{65}Zn feeding experiment. Plants are shown in Photograph 11.1 with the typical P deficiency symptoms in the low P (5 and 0.5 μMP) plants.

11.2.2. Source of used PS and ^{65}Zn

Mugineic acid, one of the major PS released by the barley cultivar, was collected from the root washings of –Fe barley as described in chapter 8. The ^{65}Zn-radionuclide was given by Dr. S. Enomoto from RIKEN.

11.2.3. Feeding with ^{65}Zn

At 10 DAT, plant roots were washed with deionized water. Subsequently, plants were transferred to feeding solution containing macronutrients and Zn with the 4 P levels, where ^{65}Zn as $^{65}ZnCl_2$ (0.2 μM) was added with or without PS (10 μM). Plants were fed with ^{65}Zn in triplicate for 4 hours in time, when PS were not released by roots, in beakers wrapped with aluminum foil containing 100 mL of feeding solution similarly to those shown earlier in Photograph 8.1 (Chapter 8). The starting time of the feeding of ^{65}Zn of plants was also 8 hours after the onset of light in the phytotron. The radioactivity of ^{65}Zn in each beaker was 50 KBq. The apoplastic ^{65}Zn of roots was solubilized and removed after the feeding time by the modified method of Bienfait et al. (1985) where bipiridyl was omitted and EDTA (100 μM) was added. After that, the plants were completely washed with tap water, divided into shoots and roots, oven dried at 70°C for 1 d, and weighed.



Photograph 11.1. Barley plants as affected by 4 different P levels in the media at pH 5.5

11.2.4. Measurement of ^{65}Zn

Dried shoots and roots of the plants were digested in concentrated nitrate as described by Zarcinas et al. (1987). The radioactivity of ^{65}Zn of the digested plant solutions or the root washing containing apoplastic ^{65}Zn was determined by using the methods described in chapter 8. The amount of the extracellular ^{65}Zn in the root apoplast was not included in the ^{65}Zn content in roots. The total absorption represents the sum of the amount of ^{65}Zn in shoots and roots, and the absorption activity per root dry weight (DW) represents the total amount of ^{65}Zn in plant divided by root dry weight. The translocation per plant represents the shoot content of ^{65}Zn, and the translocation per shoot DW was also calculated. The relative translocation rate represents the percentage of the ^{65}Zn translocated from roots to shoot to the total absorption of ^{65}Zn per plant.

11.2.5. Statistical Analysis

The experiment was arranged in completely randomized block design with 3 replicates. Data were subjected to an ANOVA (SAS Institute, 1988). The means of treatment were compared according to Duncan's Multiple Range Test ($P<0.05$) using the computer "Origin 5" in Iwate University.

11.3. RESULTS AND DISCUSSION

11.3.1. Dry Weight of the Plants

The dry biomass of the roots was higher while that of the shoots was lower in low P (5 and 0.5 μM P) plants than in control (500 μM P) or 50 μM P plants (Fig. 11.1). There was no difference between the biomass of control plants and that of 50 μM P plants consistently with the results described in Chapter 3.

11.3.2. Total Absorption and Absorption Activity of ^{65}Zn of Plants

The total absorption of ^{65}Zn in low P (5 and 0.5 μM P) plants was similar to that of the control (500 μM P) plants (Fig. 11.2a). However, the amount of ^{65}Zn absorbed by the plants fed in medium containing 50 μM P was higher than that of the low P (5 and 0.5 μM P) plants. It is suggested that in medium with 10 times lower P concentration, the uptake of Zn may be enhanced while not affected in medium with lower P (5, 0.5 μM) concentration. The supply of PS to the media enhanced ^{65}Zn absorption at all the P levels. In the presence of PS, there was no significant difference between the absorption amounts of ^{65}Zn of the low P (5, 0.5 μMP) plants and that of the control (500 μMP) plants, though 50 μMP plants absorbed higher amount of ^{65}Zn than the other plants.

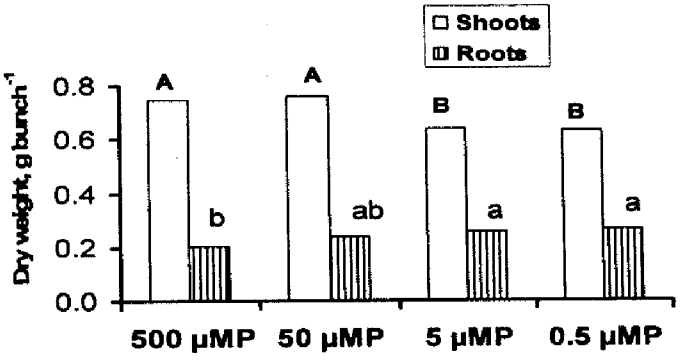


Figure 11.1. Dry weight of barley plants grown with 4 different P levels under pH 5.5. Different letter at the top of each bar indicates significant differences ($P<0.05$) among P levels according to Duncan Multiple Range Test.

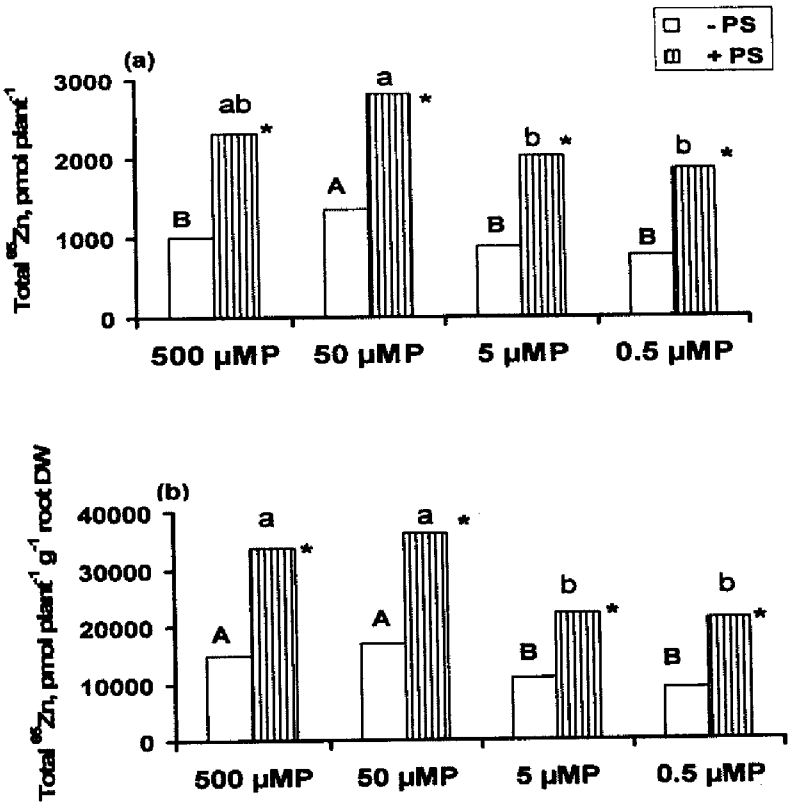


Figure 11.2. Absorption and absorption activity of ^{65}Zn of barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

The total absorption of ^{65}Zn in plants per gram root DW was lower in low P (5 and 0.5 μMP) plants than in control (500 μMP) plants or in 50 μMP plants (Fig. 11.2b) either with or without PS in the medium. The supply of PS to the medium enhanced the root absorption activity of plants regardless of P concentration of the medium. It was indicated that low P (5 and 0.5 μMP) conditions of the medium decreased slightly the root absorption activity of Zn in plants. The Zn-P antagonistic relationship did not occur in this hydroponical experiment.

11.3.3. Translocation and Relative Translocation rate of ^{65}Zn to Shoots in Plants

When PS was not added to the feeding media, the translocation amount of ^{65}Zn from roots to shoots was higher in 50 μMP plants than control (500 μMP) plants (Fig. 11.3a). The translocation amounts of ^{65}Zn in the lower P (5 and 0.5 μMP) plants were lower than that in control plants. The translocation of ^{65}Zn per gram root DW (Fig. 11.3b) followed similar pattern to that of the translocation amount per plant. The supply of PS to plants enhanced the translocation of ^{65}Zn per plant and per root DW at all P levels of the media. The amount of ^{65}Zn translocated to the shoots of the low P (5 and 0.5 μMP) plants was also lower than that of control plants or 50 μMP plants when PS was added to the media. The translocation amount of ^{65}Zn of the 50 μMP plants was similar to that of the control plants when PS was added to the media. It was indicated that PS efficiency on ^{65}Zn translocation was higher in 500 μMP plants than in 50 μMP plants. The translocation of ^{65}Zn per gram shoot DW followed similar pattern to the translocation of ^{65}Zn per plant both in the media with and without PS. It was indicated that low P (5 and 0.5 μMP) condition of the medium depressed the translocation of Zn. The addition of PS to the media increased Zn translocation at all P levels. The relative

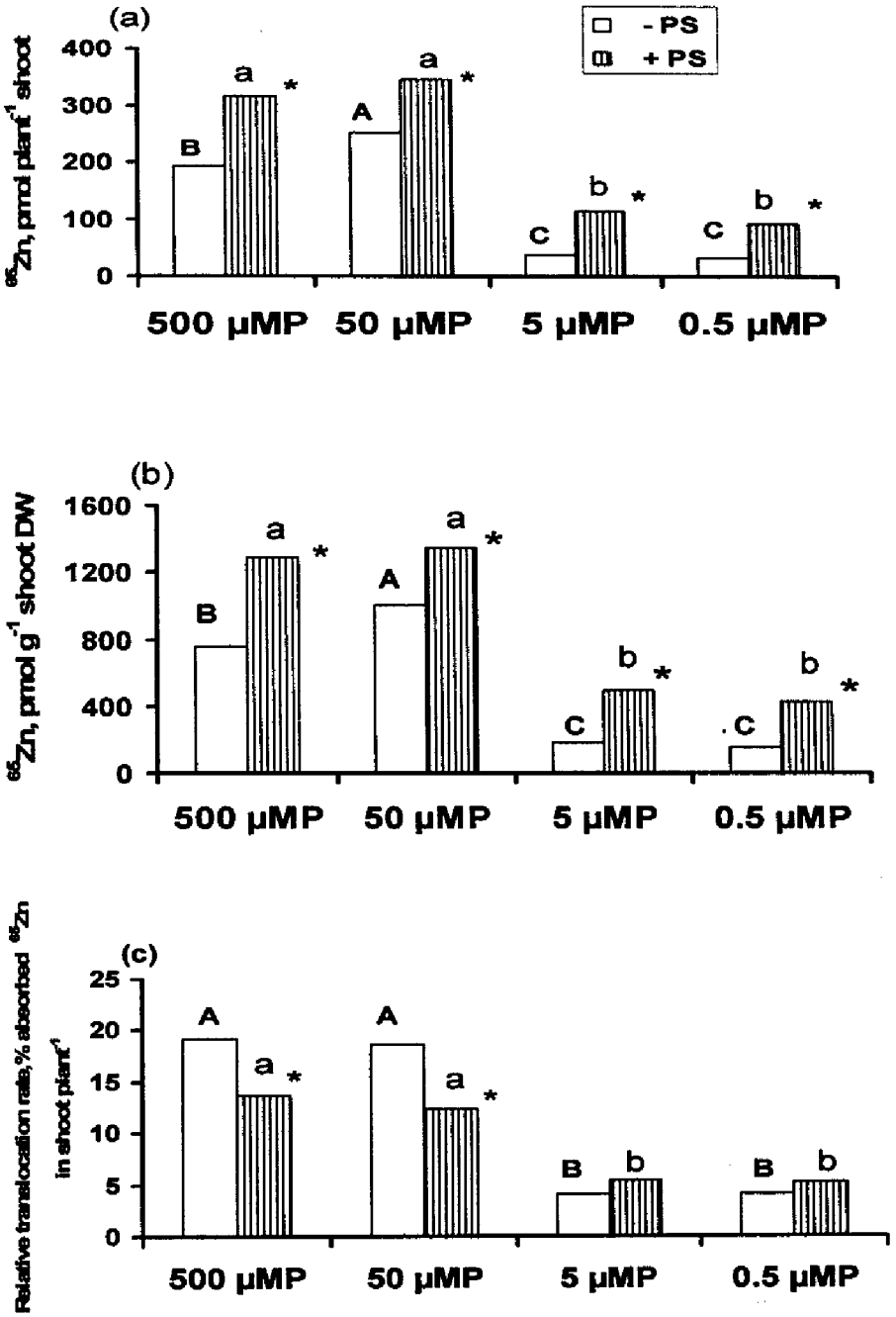


Figure 11.3. Translocation and relative translocation rate to shoots of ^{65}Zn in barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

translocation rate of ^{65}Zn was lower in low P (5 and 0.5 μMP) plants than in control (500 μMP) plants (Fig. 11.3c). This parameter was similar for control (500 μMP) plants and 50 μMP plants. The supply of PS did not affect the relative translocation rate in low P (5 and 0.5 μMP) plants, and decreased it in control (500 μMP) or 50 μMP plants. It was suggested that in higher P (500 and 50 μMP) condition of the medium, PS depressed the relative translocation rate of Zn of plants.

11.3.4. Apoplastic ^{65}Zn in Roots

The effect of low P (50, 5, and 0.5 μMP) on the accumulation of ^{65}Zn in root apoplast

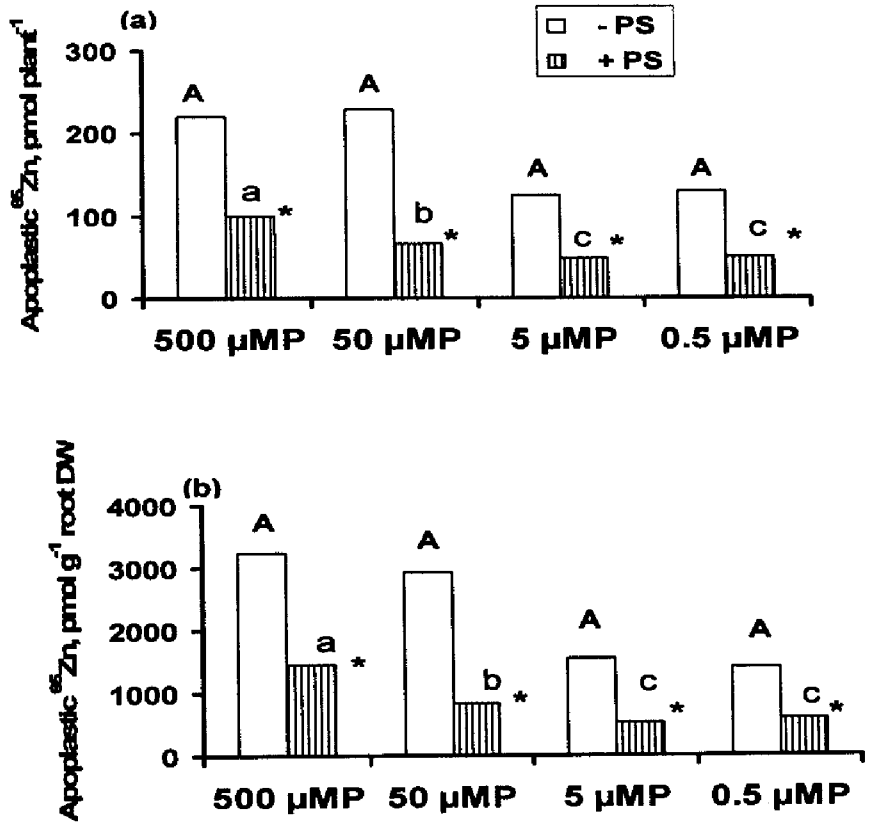


Figure 11.4. Apoplastic ^{65}Zn in roots of barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

of the plants was not significant as compared to that of the control (500 μMP) plants (Fig.11.4a). Addition of PS lowered the amount of apoplastic ^{65}Zn in roots of the plants. In the presence of PS, the amount of apoplastic ^{65}Zn in roots decreased with decreasing P level of the medium. Apoplastic ^{65}Zn per root DW followed similar pattern to that of the accumulation of ^{65}Zn in plant root apoplast as shown in Figure 11.4b. It was indicated that PS depressed accumulation amount of ^{65}Zn in root apoplast was more lowered in low P (5, and 0.5 μMP) plants than in higher P plants. This may be related with the higher Zn translocation to shoots of the plants in the presence of PS.

11.4. SUMMARY

When PS was not added, it was shown that low P (5 and 0.5 μMP) conditions did not affect much the total absorption of ^{65}Zn while low P (5 and 0.5 μMP) conditions depressed the translocation and the relative translocation of ^{65}Zn to shoots. It was considered that low P (5 and 0.5 μMP) conditions lowered ^{65}Zn translocation to the shoots resulting in more ^{65}Zn remained in roots.

The supply of PS enhanced the absorption and translocation of ^{65}Zn in plants regardless of the P concentration in the rhizosphere. The presence of PS in the media decreased apoplastic ^{65}Zn of plant roots at all P levels. Though PS decreased much the accumulation of ^{65}Zn in the apoplast of the low P (5 and 0.5 μMP) plants than in that of the high P (500 and 50 μMP) plants, ^{65}Zn translocation was still lower in the former plants than in the latter plants when PS was fed.

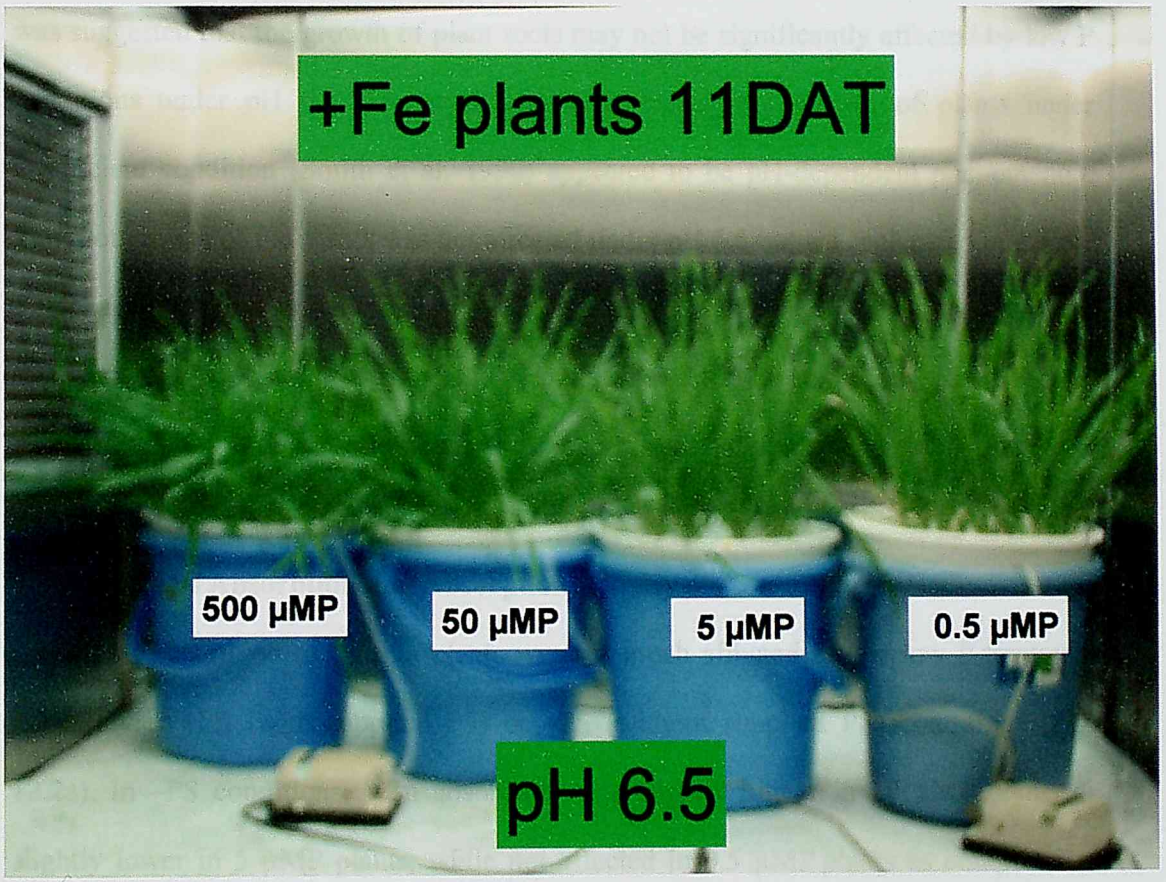
CHAPTER 12

ABSORPTION AND TRANSLOCATION OF ^{65}ZINC IN LOW PHOSPHORUS BARLEY PLANTS AS AFFECTED BY PHYTOSIDEROPHORES UNDER pH 6.5

12. Absorption and translocation of ^{65}Zinc in low phosphorus barley plants as affected by phytosiderophores under pH 6.5

12.1. INTRODUCTION

The experiment reported in chapter 11 with barley plants grown and fed with ^{65}Zn under pH 5.5 was also conducted similarly with barley plants grown with the 4 P levels for 11 DAT under pH 6.5. Plants are shown in photograph 12.1. The objectives of this experiment were to examine the effect of PS on Zn absorption and translocation in plants under low P and pH 6.5 conditions.



Photograph 12.1. Barley plants as affected by 4 different P levels in the media at pH 6.5

12.2. MATERIALS AND METHODS

The materials and methods of the experiment were generally similar to those described in chapter 11. Phosphorus was supplied as NaH_2PO_4 and the plants were grown with the 4 P levels (500 (control), 50, 5, and 0.5 μMP) for 11 DAT under pH 6.5, and subsequently fed ^{65}Zn alone or together with PS under pH 6.5 for 4 hours.

12.3. RESULTS AND DISCUSSION

12.3.1. Dry Weight of the Plants

The DW of the shoots of the low P (5 and 0.5 μMP) plants was lower than that of control plants (500 μMP) or that of 50 μMP plants (Fig. 12.1). The DW of the low P (50, 5 and 0.5 μMP) plant roots was similar to that of the control (500 μMP) plant roots. It was suggested that the growth of plant roots may not be significantly affected by low P conditions under pH 6.5. The well known increased root growth of plants under P-deficient condition (Smith et al. 1990) appeared to be pH-dependent phenomenon. The root growth of the plants was not affected under pH 6.5 but was enhanced under pH 5.5 (chapter 11) by low P condition. The effect of the deficiency of P on shoot growth of the plants was consistent between pH 6.5 and 5.5 (chapter 11) conditions. However, the response of plant roots to P-deficiency was inconsistent between plants grown under pH 6.5 and those grown under pH 5.5 (chapter 11).

12.3.2. Total Absorption and Absorption Activity of ^{65}Zn of Plants

The total absorption of ^{65}Zn in plants was not much affected by the low P treatment either under +PS (with supplied PS) or –PS (without supplied PS) conditions (Fig. 12.2a). In –PS conditions, ^{65}Zn absorption was slightly higher in 50 μMP plants or slightly lower in 5 μMP plants, while not affected in 0.5 μMP plants as compared to

control (500 μMP) plants. In +PS conditions, the pattern of the absorption of ^{65}Zn by plants was more or less

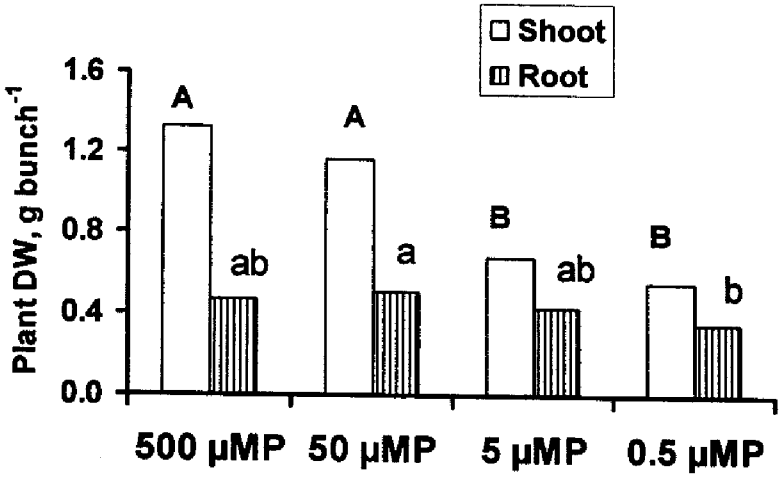


Figure 12.1. Dry weight of barley plants grown with 4 different P levels under pH 6.5. Different letter at the top of each bar indicates significant differences ($P<0.05$) among P levels according to Duncan Multiple Range Test.

similar to those of the plants fed with ^{65}Zn in –PS conditions either in low P media or in control medium. The absorption of ^{65}Zn of the plants was not affected by PS under control (500 μMP) or 50 μM P conditions. The supply of PS enhanced slightly ^{65}Zn absorption in 5 μM P plants or slightly lowered it in 0.5 μM P plants as compared to –PS plants.

The total absorption of ^{65}Zn in plants per gram root DW was not affected by low P (50, 5, and 0.5 μMP) treatment as compared to control (500 μMP) treatment in –PS conditions (Fig. 12.2b). In presence of PS, the total absorption of ^{65}Zn in plants per gram root DW was not affected by the low P (50 and 5 μMP) treatment, but was enhanced slightly by 0.5 μM P treatment as compared to that of the plants of the control treatment. This results suggested that the absorption of Zn by plants may not be affected

much by low P conditions of the medium neither under pH 6.5 nor under pH 5.5 (Chapter 11). It was indicated that PS may not be effective for Zn absorption in plants under pH 6.5, though PS was effective for Zn absorption of plants under pH 5.5 (Chapter 11). The activity of PS for Zn absorption in plants seemed to be pH-dependent.

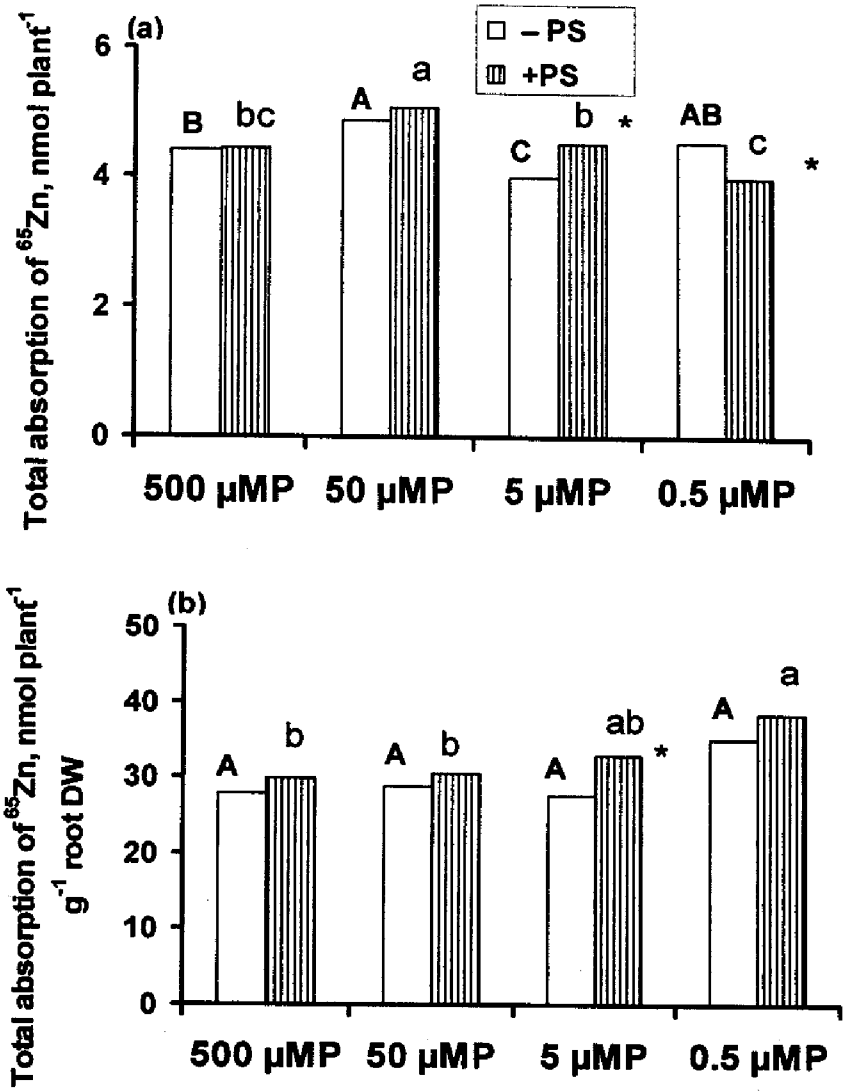


Figure 12.2. Absorption and absorption activity of ^{65}Zn of barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

12.3.3. Translocation and Relative Translocation rate of ^{65}Zn to Shoots in Plants

The translocation of ^{65}Zn from roots to shoots in plants was lower in low P (5, and 0.5 μMP) media than in control (500 μMP) medium either under –PS or +PS conditions (Fig. 12.3a). The effect of PS on the translocation of ^{65}Zn in plants was not clearly observed as compared to –PS plants. Though the translocation of ^{65}Zn was slightly decreased in control plants and slightly increased in the lowest P (0.5 μMP) plants by PS. The translocation of ^{65}Zn in plants per gram shoot DW (Fig. 12.3b) followed almost similar pattern to that of the translocation per plant. This effect of low P condition on the translocation of ^{65}Zn in plants was consistent with that described in chapter 11, suggesting that low P may reduce Zn translocation in plants either under pH 6.5 or 5.5. The effect of PS on ^{65}Zn translocation in plants grown under pH 6.5 was inconsistent with that of PS in plants under pH 5.5 (chapter 11) at each P level. It was suggested that the effectiveness of PS for the enhancement of the translocation of Zn in plants may be pH-dependent.

The relative translocation rate of ^{65}Zn (Fig. 12.3c) in plants followed similar pattern to that of the translocation of ^{65}Zn in plants either under –PS or +PS condition. The effect of PS on the relative translocation rate of ^{65}Zn in plants at each P level was not clearly observed. The low P conditions affected similarly the relative translocation of ^{65}Zn in plants under pH 6.5 or pH 5.5 (chapter 11). It was indicated that a pH variation from 6.5 to 5.5 of low P media may not affect the relative translocation rate of Zn in plants. The supply of PS affected consistently the relative translocation rate of ^{65}Zn in plants under both pH values 6.5 and 5.5 suggesting that PS activity may be independent on the variation of pH from 6.5 to 5.5.

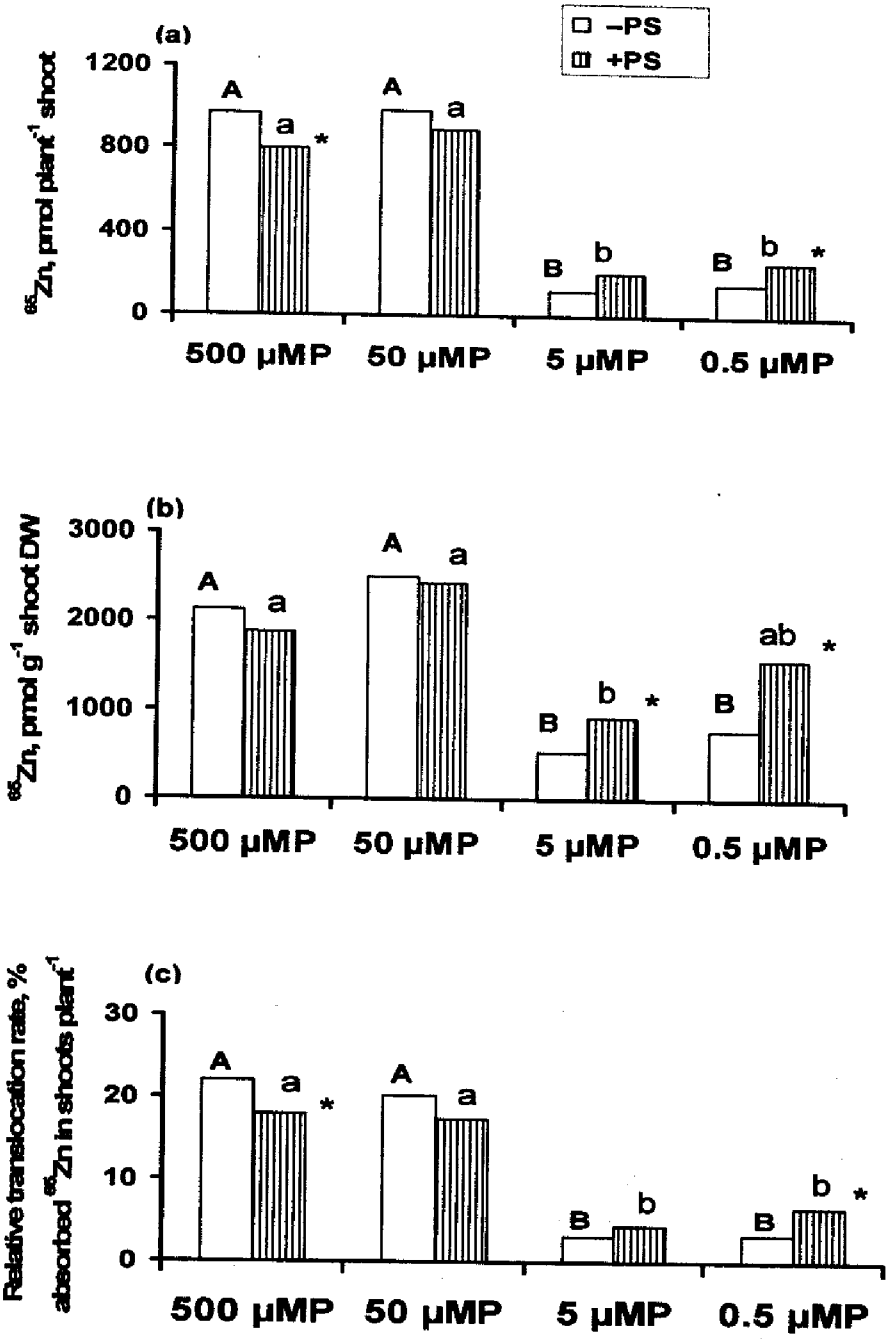


Figure 12.3. Translocation and relative translocation rate to shoots of ^{65}Zn in barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

12.3.4. Apoplastic ^{65}Zn in Roots

The apoplastic ^{65}Zn per plant roots of the low P (5 and 0.5 µMP) plants was similar to that of the control (500 µMP) plants under –PS or +PS condition (Fig. 12.4a).

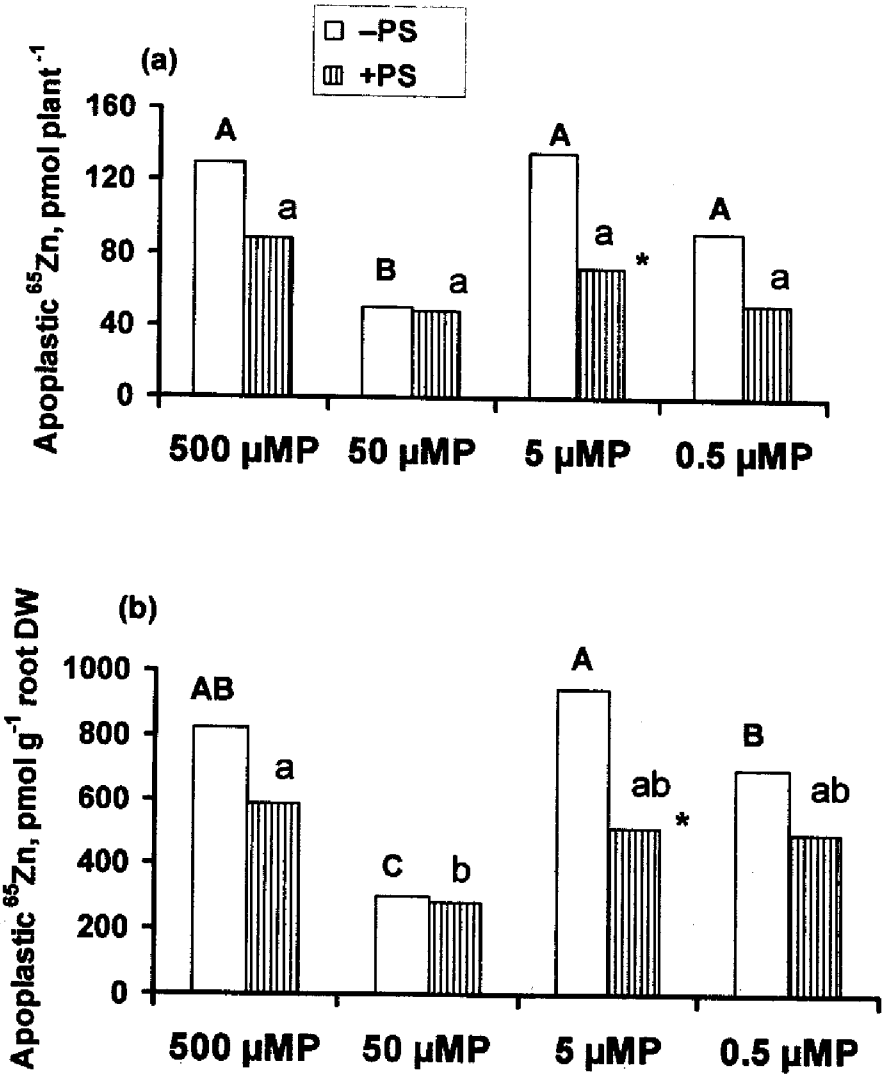


Figure 12.4. Apoplastic ^{65}Zn in roots of barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

Apoplastic ^{65}Zn per plant roots, however, was lower in 50 μM P plants than in control plants under –PS condition. The supply of PS did not affect the formation either in control plants or in low P (50, and 0.5 μMP) plants. The formation of apoplastic ^{65}Zn in roots per plant, however, was lower in 50 μM P plants than in control plants. The pattern of the formation of apoplastic ^{65}Zn in plants per root DW was similar to that in roots per plant either under –PS or +PS condition (Fig. 12.4b). The formation of apoplastic ^{65}Zn in roots of the plants fed at pH 6.5 was consistent with that of the plants fed at pH 5.5 (chapter 11) under –PS condition regardless of the level of P. Apoplastic ^{65}Zn in roots of plants was reduced by PS at all of the P levels under pH 5.5 (chapter 11) while being reduced by PS only in 5 μM P plants and not in other P levels. It was suggested that low P condition may not affect much the formation of apoplastic Zn in plant roots. The effect of PS on the reduction of apoplastic Zn in plant roots may be dependent on pH level.

12.4. SUMMARY

The growth of the plants in roots was not affected by low P conditions under pH 6.5 while being increased by low P conditions under pH 5.5 (chapter 11). The effect of low P condition on root growth may be pH-dependent. The absorption of ^{65}Zn by plants was not much affected by low P conditions either under pH 6.5 or 5.5 (chapter 11). The translocation of ^{65}Zn in plants was decreased by low P conditions either under pH 6.5 or 5.5. The relative translocation rate of ^{65}Zn in plants showed similar pattern as that of the translocation of ^{65}Zn at both pH values 6.5 and 5.5. The effect of low P on Zn absorption and translocation in plants may not be affected by variation of pH from 6.5 to 5.5. The formation of apoplastic ^{65}Zn in plants was not much affected by low P conditions at both pH values 6.5 and 5.5 (chapter 11).

The effect of PS on the absorption and the translocation of ^{65}Zn in plants under pH 6.5 was not clearly observed. The supply of PS enhanced these parameters in plants at all the P levels under pH 5.5 (chapter 11). The application of PS reduced the formation of apoplastic ^{65}Zn in plants only at 5 μM P level under pH 6.5 and at all the P levels under pH 5.5 (chapter 11). The ability of PS to reduce the formation of apoplastic Zn in roots of +Fe plants may be pH-dependent. The effectiveness of PS for enhancing the absorption and translocation of Zn, or reducing the formation of apoplastic Zn in roots seemed to be pH-dependent. The role of PS on Zn absorption and translocation in plants needs deeper investigation.

CHAPTER 13

ABSORPTION AND TRANSLOCATION OF ^{65}ZINC IN LOW PHOSPHORUS AND IRON-DEFICIENT BARLEY PLANTS AS AFFECTED BY PHYTOSIDEROPHORE UNDER pH 6.5

13. Absorption and translocation of ^{65}Zinc in low phosphorus and Fe-deficient barley plants as affected by phytosiderophores under pH 6.5

13.1. INTRODUCTION

The ability of PS of to chelate and convey Fe to plant roots in the rhizosphere was also reported for other metal micronutrients, such as Zn, Cu, and Mn, in calcareous soil conditions (Treeby et al., 1989; Singh et al., 1992). Potentially P-deficient condition may be accompanied with micronutrient deficiencies as reported by Srinivasarao et al. (2006). Our results in chapter 5 showed increased PS release from plant roots in –P medium as compared to +P medium. The results described in chapter 7 showed that the concentrations of several micronutrients including Zn of the xylem sap increased in low P and –Fe conditions. The uptake and translocation of Zn in plants under low P and –Fe conditions has not been reported in related with P concentration of the medium and PS activity in plants. This experiment was conducted to examine the absorption and translocation of Zn in low P and –Fe barley plants.

13.2. MATERIALS AND METHODS

13.2.1. Plant Culture and Growth

Seedlings of barley plants were cultivated by the method described previously in chapter 2. Plants were transplanted in bunch of 3 plants wrapped with sponge rubber and transferred to 10-L plastic buckets (16 bunches bucket^{-1}) filled up with 1/2–strength Hoagland-Arnon solution for 2 days. Subsequently, the plants were transferred to the –Fe 1/2–strength modified Hoagland-Arnon solution (Takagi, 1993) with 4 different levels of P, 500 (control), 50, 5, and 0.5 μMP. Phosphorus was supplied as NaH_2PO_4. The plants were grown in the phytotron as described in Chapter 2. The pH of the nutrient solutions was daily monitored and adjusted to 6.5. The nutrient solutions were

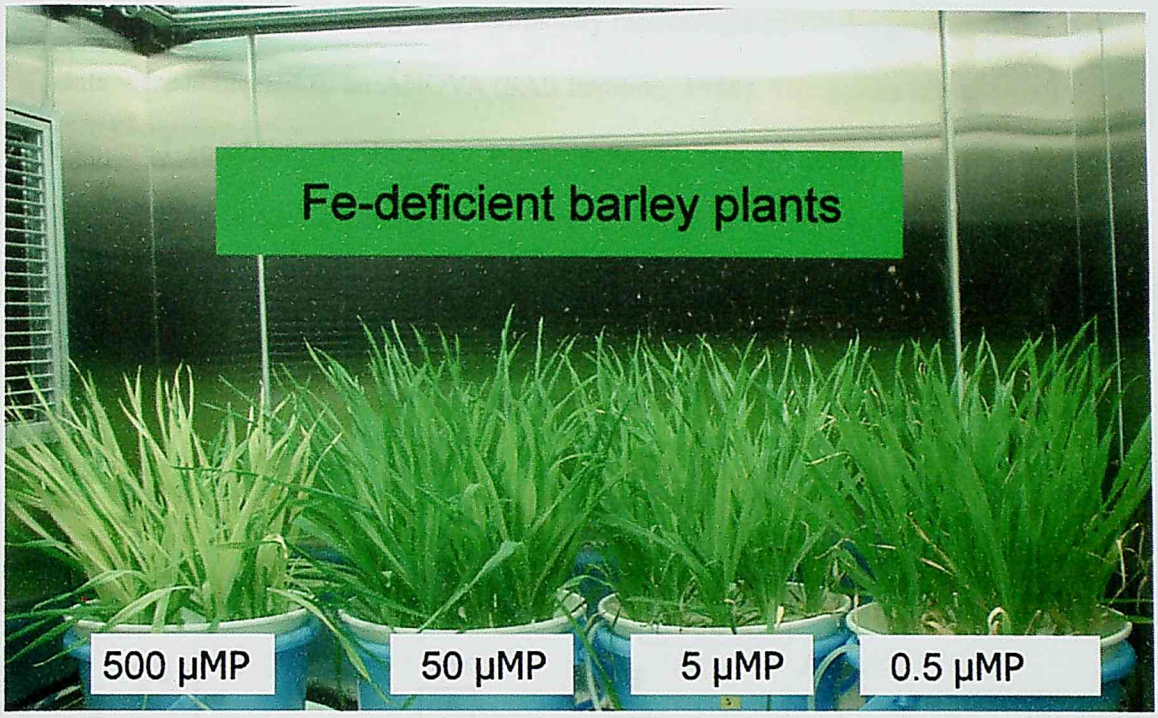
weekly renewed. The plants were allowed to grow for 14 days after treatment (DAT) prior to ^{65}Zn feeding experiment in medium added PS. Plants are shown in Photograph 13.1 with the typical P-deficiency symptoms in the low P (5 and 0.5 μMP) plants and the usual Fe chlorosis in leaves of the control (500 μMP) plants.

13.2.2. Source of used PS and ^{65}Zn

Phytosiderophore was collected from the root washings of –Fe barley as described earlier in chapter 8. The origin of the purchased ^{65}Zn-radionuclide was the same as that described in chapter 11.

13.2.3. Feeding with ^{65}Zn

At 14 DAT, plant roots were washed with deionized water. Subsequently, plants were transferred to feeding solution containing macronutrients and Zn with the 4 P levels, where ^{65}Zn as $^{65}ZnCl_2$ (0.2 μM) was added with or without PS (10 μM). Plants were fed with ^{65}Zn in triplicate for 4 hours, when PS were not released by roots, in beakers wrapped with aluminum foil containing 100 mL of feeding solution. The starting time of the feeding of ^{65}Zn of plants was also 8 hours after the onset of light in the phytotron. The radioactivity of ^{65}Zn in each beaker was 50 KBq. The apoplastic ^{65}Zn of roots was solubilized and removed after the feeding time by the modified method of Bienfait et al. (1985) as described in Chapter 11. After that, the plants were completely washed with tap water, divided into shoots and roots, oven dried at 70°C for 1 d, and weighed.



Photograph 13.1. Barley plants as affected by 4 different P levels in Fe-deficient medium under pH 6.5.

13.2.4. Measurement of ^{65}Zn

Dried shoots and roots of the plants were digested in concentrated nitrate as described by Zarcinas et al. (1987). The radioactivity of ^{65}Zn of the digested plant solutions or the root washing containing apoplastic ^{65}Zn was determined by using the methods described in chapter 8. The amount of the extracellular ^{65}Zn in the root apoplast was not included in the ^{65}Zn content in roots. The total absorption of ^{65}Zn in shoots and roots, the absorption activity of ^{65}Zn per root DW, the translocation per plant of ^{65}Zn, and the translocation of ^{65}Zn per shoot DW were calculated and defined as described previously in Chapter 11. The relative translocation rate of ^{65}Zn to shoot was also measured as previously described.

13.2.5. Statistical Analysis

The experiment was arranged in completely randomized block design with 3 replicates. Data were subjected to an ANOVA (SAS Institute, 1988). The means of treatment were compared according to Duncan's Multiple Range Test ($P<0.05$) using the computer "Origin 5" in Iwate University.

13.3. RESULTS AND DISCUSSION

13.3.1. Dry Weight of the Plants

Dry weights of shoots and roots were higher in low P (50, 5, and 0.5 μMP) plants than in control (500 μMP) plants (Fig. 13.1) consistently with the results of chapter 4.

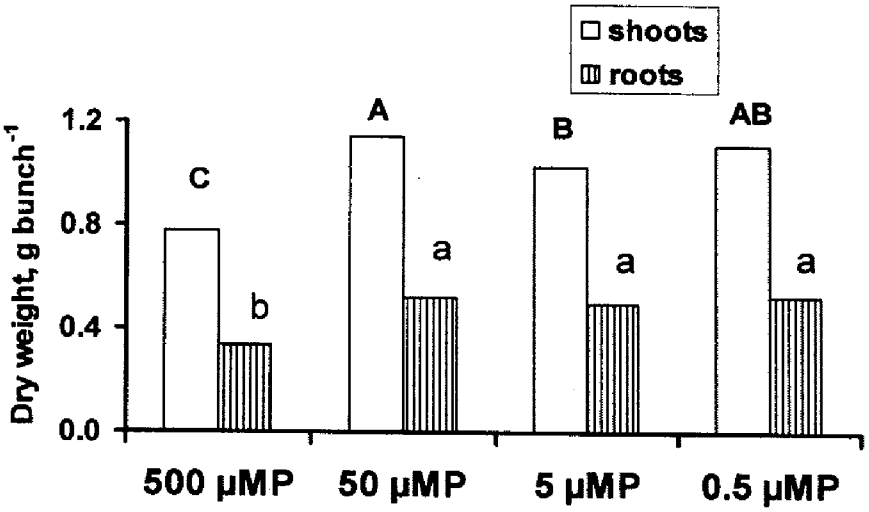


Figure 13.1. Dry weight of barley plants grown with 4 different P levels under –Fe and pH 6.5 conditions. Different letter at the top of each bar indicates significant differences ($P<0.05$) among P levels according to Duncan Multiple Range Test.

13.3.2. Total Absorption and Absorption Activity of ^{65}Zn in Plant

The low P (50 and 5 μM P) condition of the medium did not affect plant absorption of ^{65}Zn (Fig. 13.2a). However, at the lowest P (0.5 μM P) concentration, plants absorbed slightly lower ^{65}Zn than at control (500 μM P) concentration of the medium. The supply of PS to the plants did not affect the absorption of ^{65}Zn regardless of the P levels. The absorption activity of ^{65}Zn per root DW (Fig. 13.2b) was not affected by the presence of

PS in the rhizosphere. The root absorption activity of ^{65}Zn was lower in low P (50, 5, and 0.5 μM P) plants than in control (500 μM P) plants. This result did not support the

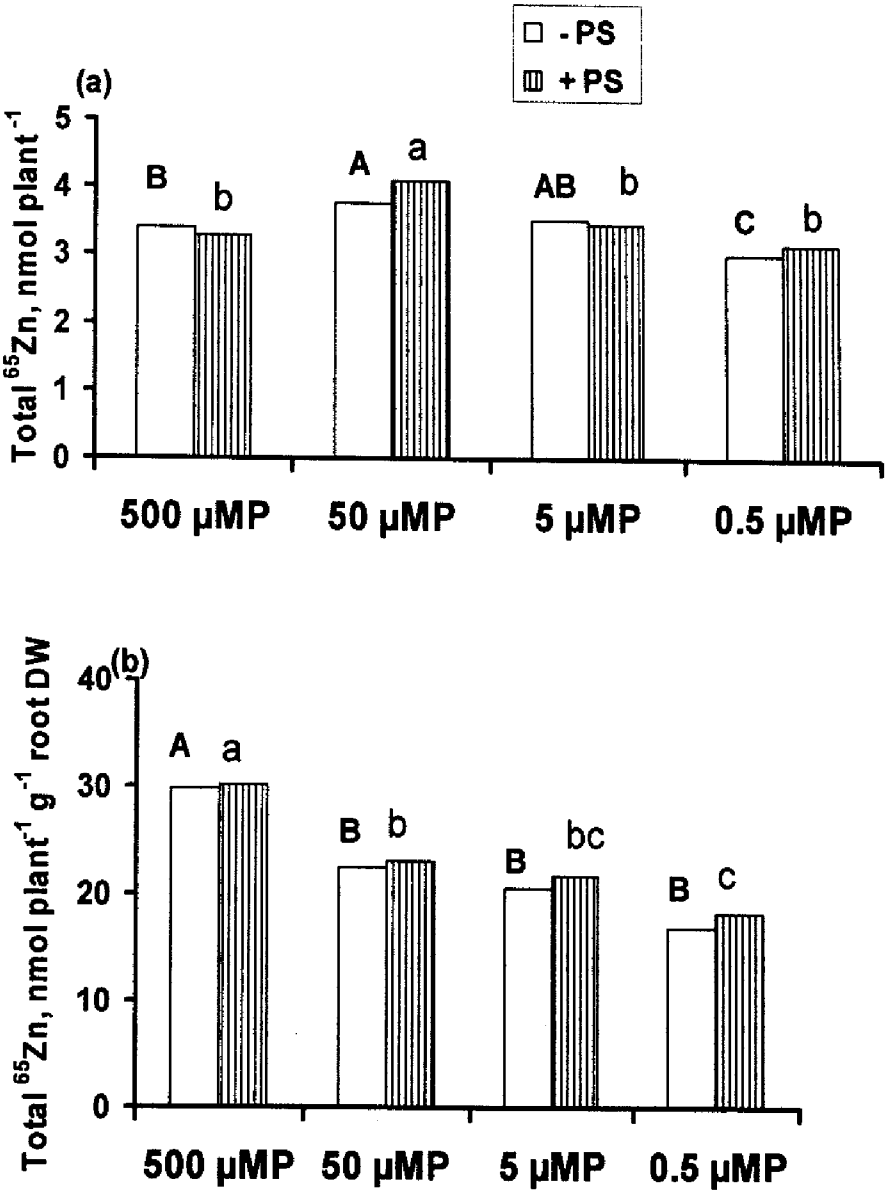


Figure 13.2. Absorption and absorption activity of ^{65}Zn of Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

discussion of the Zn-P antagonistic interaction (Loneragan et al., 1979) or the chelation of Zn by PS in calcareous soils (Treeby et al., 1989; Singh et al., 1992). Our results suggested that PS is ineffective for Zn absorption by –Fe plants.

13.3.3. Translocation and Relative Translocation rate of ^{65}Zn to Shoots in Plant

The translocation per plant (Fig. 13.3a), the translocation per root DW (Fig. 13.3b), and the relative translocation (Fig. 13.3c) of ^{65}Zn from roots to shoots were not affected by PS as compared to plants without fed PS. It was suggested that PS was ineffective for Zn translocation in –Fe plants. The translocation and the concentration of ^{65}Zn of shoots were decreased with decreasing P concentration of the feeding solution either with or without PS similarly to the relative translocation rate of ^{65}Zn. It was indicated that low P (50, 5, and 0.5 μMP) conditions of the medium depressed the translocation of Zn from roots to shoots. The absorption of ^{65}Zn (Fig. 13.2a) was not much affected in low P (50, 5, and 0.5 μMP) plants as compared to control (500 μMP) plants. It is known that P toxicity induced Zn deficiency in plants (Boawn and Leggett, 1964; Cakmak and Marschner, 1986; Loneragan et al., 1979). Our results showed synergistic interaction between P and Zn in regard to the translocation of Zn under low P and –Fe conditions. High P concentration of the medium induced higher translocation of Zn to shoots. To our knowledge, there is no report of such synergistic Zn-P interaction in –Fe plants. The mechanisms of Zn absorption and translocation in plants under low P and –Fe conditions need to be investigated.

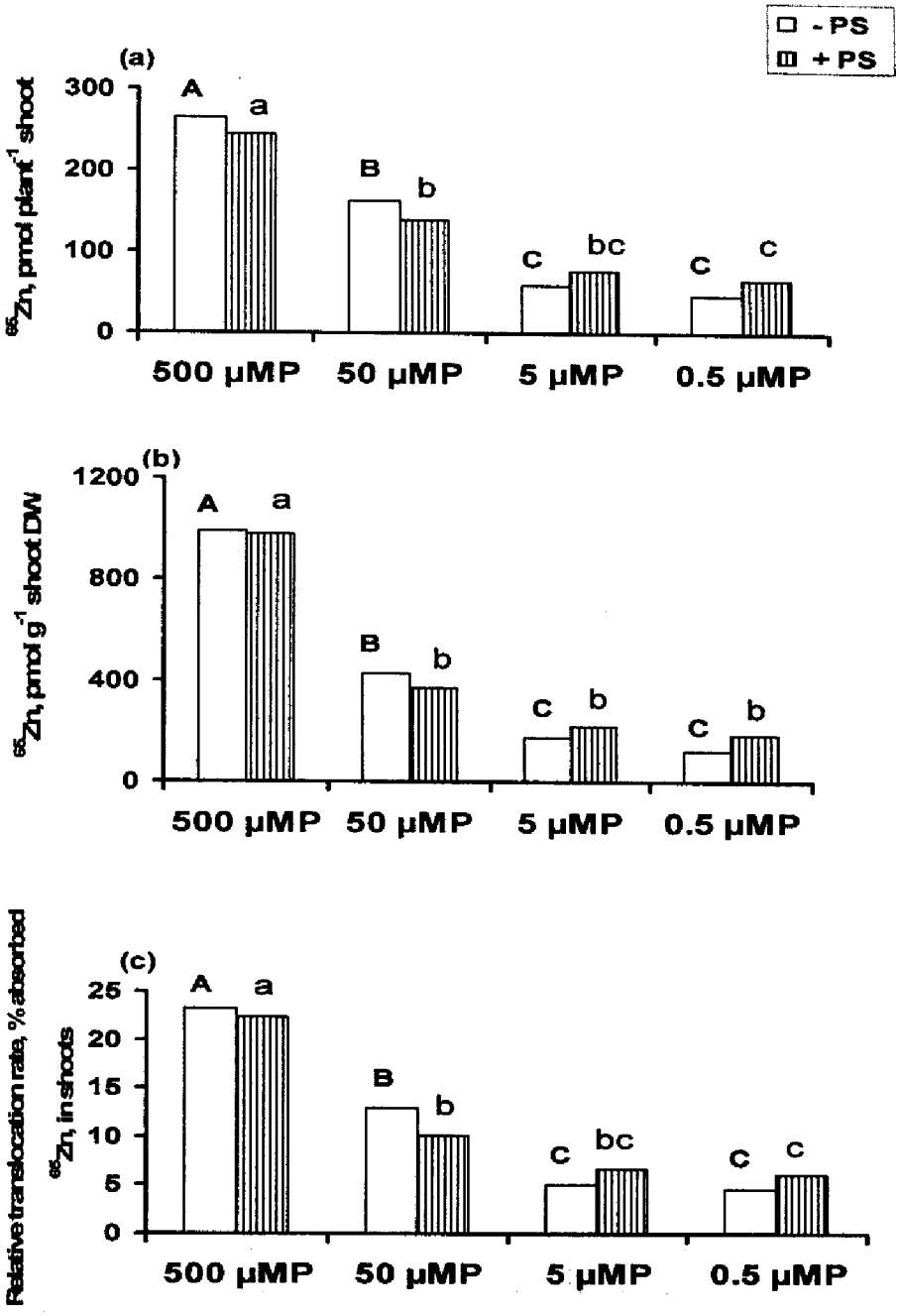


Figure 13.3. Translocation and relative translocation rate to shoots of ^{65}Zn in Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

13.3.4. Apoplastic ^{65}Zn in Roots

The effect of low P (5 and 0.5 μMP) on the accumulation of ^{65}Zn in root apoplast of the plants was not significant as compared to that of the control (500 μMP) plants (Fig. 13.4a). However, apoplastic ^{65}Zn in roots of 50 μMP plants was significantly lower than

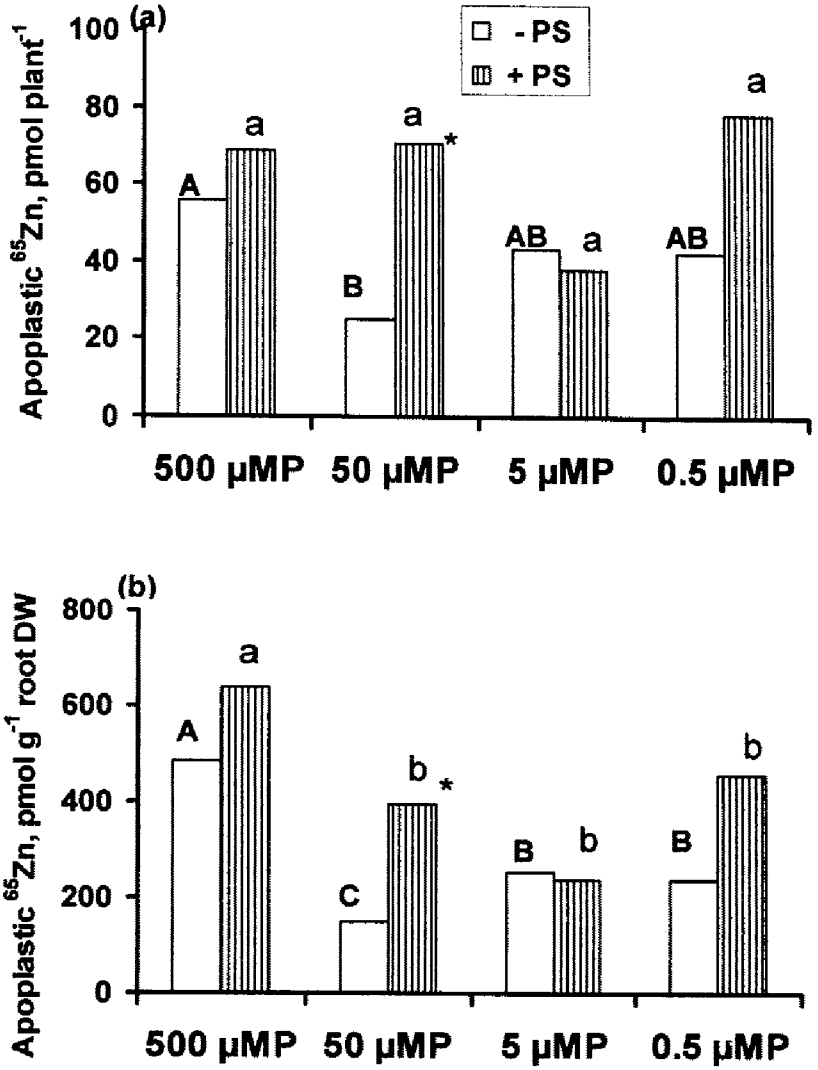


Figure 13.4. Apoplastic ^{65}Zn in roots of Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 6.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

that in roots of control (500 μMP) plants. Except an increase in apoplastic ^{65}Zn accumulation in roots of 50 μMP plants by PS, the addition of PS to the medium did not affect significantly the amount of apoplastic ^{65}Zn. When PS was added to the medium, the concentration of apoplastic ^{65}Zn in roots was statistically similar for low P (50, 5, and 0.5 μMP) plants and control (500 μMP) plants.

Apoplastic ^{65}Zn per root DW was lower in low P plants than in control (500 μMP) plants both in presence and absence of PS in the media (Fig.13.4b). This may probably due to dilution effect because of the higher root growth of low P (50, 5, and 0.5 μMP) plants than control (500 μMP) plants. The supply of PS increased apoplastic ^{65}Zn per root DW of the 50 μMP plants but did not affect it in the other P levels.

13.4. SUMMARY

The results showed that in –Fe plants, the absorption of ^{65}Zn was not significantly affected by low P (50, 5, and 0.5 μMP) conditions. It was suggested that Zn absorption in –Fe plants may not be dependent on the concentration of P of the rhizosphere. The translocation and the relative translocation of ^{65}Zn to shoots were largely reduced in low P (50, 5, and 0.5 μMP) plants. It was indicated that under low P (50, 5, and 0.5 μMP) and –Fe conditions, Zn translocation to shoots was hampered by low P status of plants. Our results showed that Zn concentration in shoots might be reduced under low P and –Fe conditions, not because of lower absorption of Zn, but because of the reduction of its translocation to shoots. The supply of PS to the plants was ineffective for ^{65}Zn absorption and translocation under –Fe condition regardless of the P concentration of the media. The mechanism of the reduction of Zn translocation of plants under –Fe condition by P deficiency needs to be clarified in the future.

CHAPTER 14

ABSORPTION AND TRANSLOCATION OF ^{65}ZINC IN LOW PHOSPHORUS AND IRON-DEFICIENT BARLEY PLANTS AS AFFECTED BY PHYTOSIDEROPHORE UNDER pH 5.5

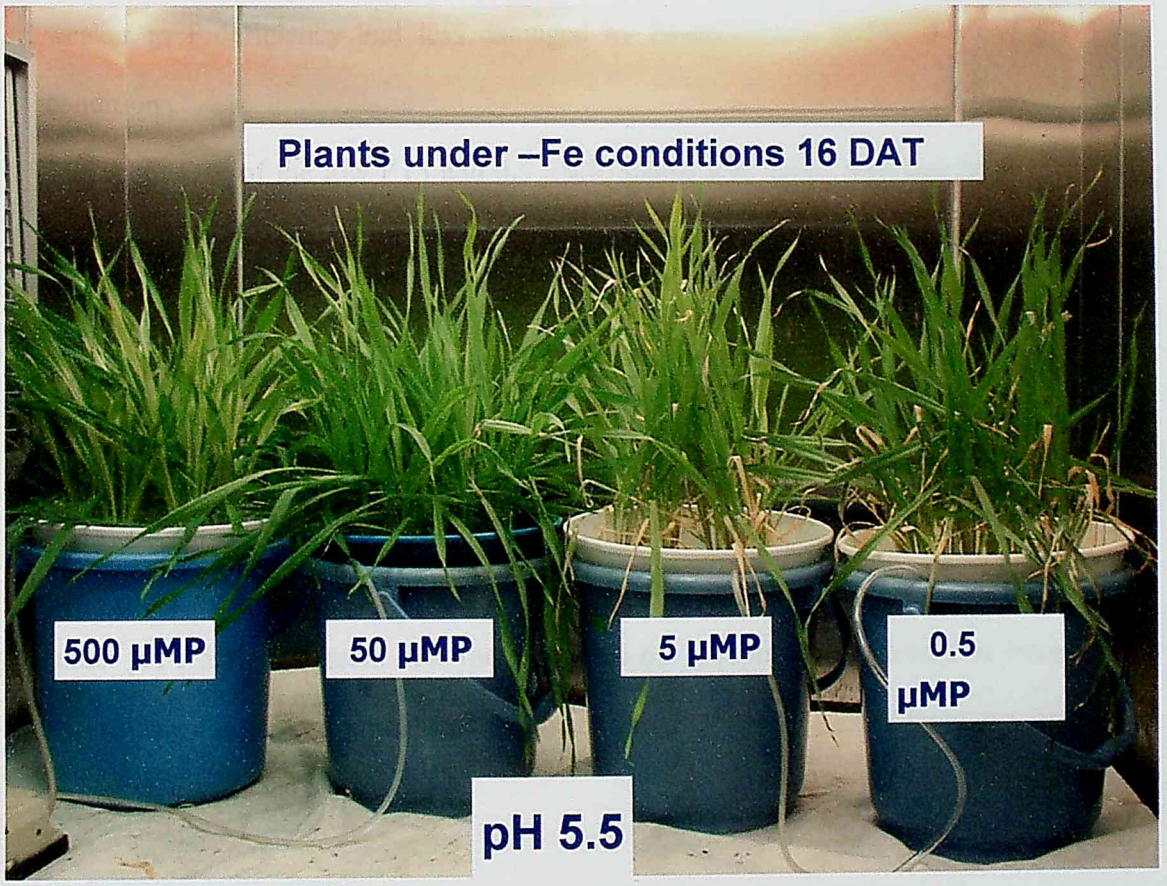
14. Absorption and translocation of ^{65}Zinc in low phosphorus and Fe-deficient barley plants as affected by phytosiderophores under pH 5.5

14.1. INTRODUCTION

The experiment described in chapter 13 with barley plants grown with 4 P levels under –Fe condition and fed with ^{65}Zn under pH 6.5 was repeated similarly under pH 5.5. The objectives were to examine the effect of PS on Zn absorption and translocation in plants in low P and –Fe media under pH 5.5.

14.2. MATERIALS AND METHODS

The materials and methods of the experiment were similar to those described in chapter 13, except a decrease of the pH to 5.5. The plants were grown with the 4 P



Photograph 14.1. Barley plants as affected by 4 different P levels in Fe-deficient media under pH 5.5.

levels (500 (control), 50, 5, and 0.5 μMP) under –Fe conditions for 16 DAT under pH 5.5 as shown in photograph 14.1. Subsequently, plants were fed in nutrient solutions containing the 4 P levels with ^{65}Zn alone (–PS) or together with PS (+PS) under pH 5.5 for 4 hours in a phytotron.

14.3. RESULTS AND DISCUSSION

14.3.1. Visual Symptoms and Dry Weight of the Plants

The symptoms of the deficiency of Fe were weaker in control (500 μMP) plants and 50 μMP plants under pH 5.5 (photograph 14.1) than those of these plants when grown under pH 6.5 (photograph 13.1). The expression of the symptoms of P deficiency were more severe in the low P (5 and 0.5 μMP) plants under pH 5.5 (photograph 14.1) than those of these plants under pH 6.5 (photograph 13.1). It was shown that plants may be weaker to P-deficiency and less damaged by Fe-deficiency under –Fe and pH 5.5 conditions.

The shoot DW of the low P (5 and 0.5 μMP) plants was lower than that of the control (500 μMP) plants (Fig. 14.1). The DW of the shoots of 50 μM P plants was similar to that of the control plants. The root DW of the low P (50, 5, and 0.5 μMP) plants was similar to that of control plants. This result may be explained by a higher resistance to Fe-deficiency and a lower resistance to low P (5 and 0.5 μMP) conditions of the plants under pH 5.5. It was suggested that the effect of low P on growth of plants under –Fe conditions may vary differently depending on the medium pH as the growth pattern under pH 5.5 was different than that under pH 6.5 (chapter 13). It would be interesting to evaluate the release amount of PS under varied P and –Fe conditions at varied pH values in the future.

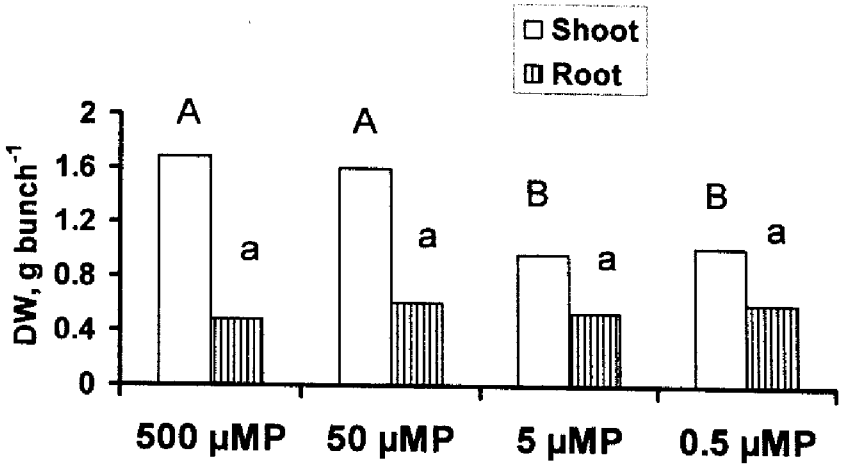


Figure 14.1. Dry weight of barley plants grown with 4 different P levels under –Fe and pH 5.5 conditions. Different letter at the top of each bar indicates significant differences ($P<0.05$) among P levels according to Duncan Multiple Range Test.

14.3.2. Total Absorption and Absorption Activity of ^{65}Zn in Plant

The low P (50 and 0.5 µMP) condition of the media did not affect the absorption of ^{65}Zn per plant though ^{65}Zn absorption of plants was slightly lower under 5 µM P condition as compared to that in control (500 µMP) condition (Fig.14.2a) in the absence of PS. In +PS condition, the total absorption of ^{65}Zn of low P (50 and 5 µM P) plants was slightly higher while that of 0.5 µM P plants was slightly lower than that of control (500 µM P) plants. In fact, the absorption of ^{65}Zn by plants was not much affected by low P conditions either in –PS or +PS under pH 5.5. This result was consistent with that of the plants fed with ^{65}Zn at pH 6.5 under –PS or +PS condition (chapter 13). The supply of PS was ineffective for the absorption of ^{65}Zn in plants either under pH 5.5 or pH 6.5 (chapter 13) regardless of the P level. The absorption activity of ^{65}Zn per gram root DW (Fig. 14.2b) was not affected by the low P treatment of the media neither under –PS nor +PS condition, except a decrease of this parameter by the lowest P (0.5 µMP)

treatment under +PS condition. The application of PS did not affect this parameter at all of the P levels in plants.

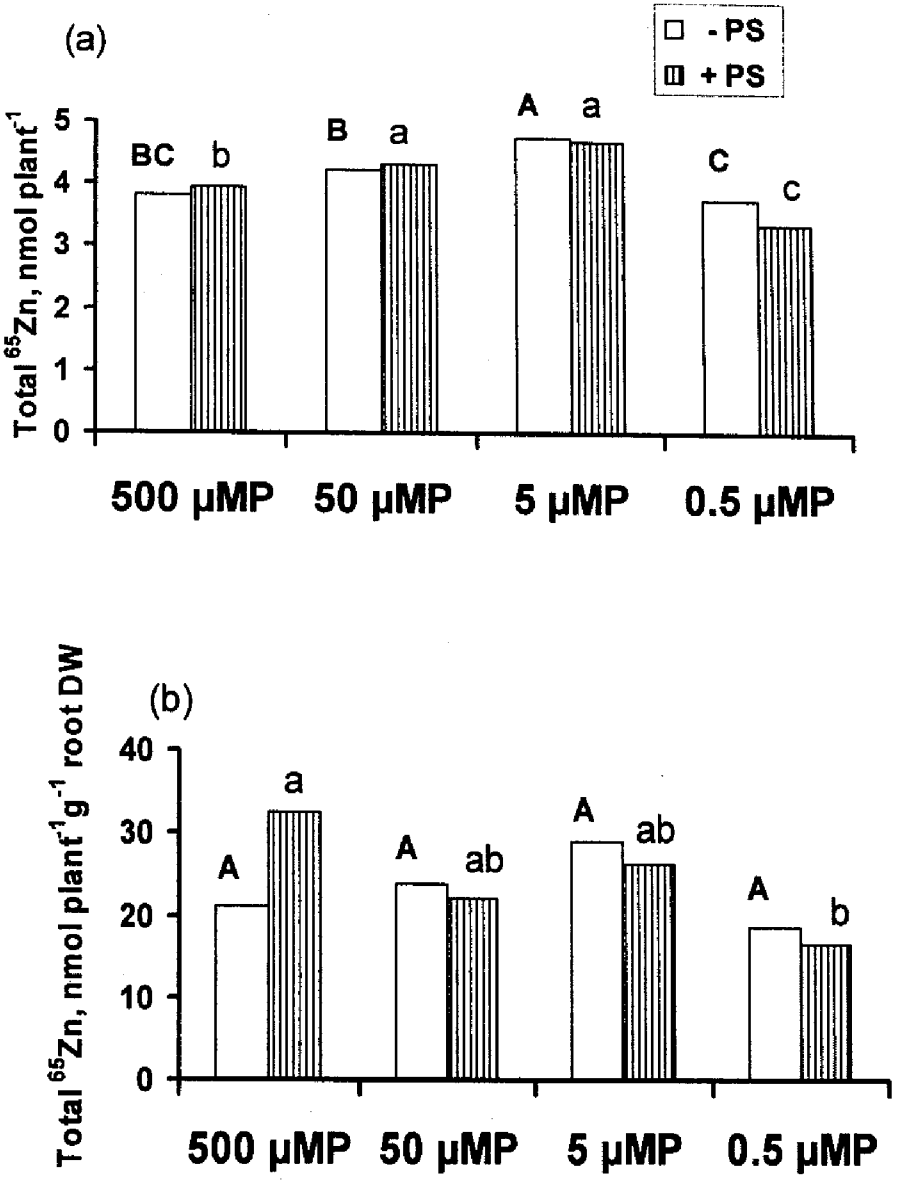


Figure 14.2. Absorption and absorption activity of ^{65}Zn of Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

14.3.3. Translocation and Relative Translocation rate of ^{65}Zn to Shoots in Plant

The translocation per plant (Fig. 14.3a), the translocation per gram root DW (Fig. 14.3b), and the relative translocation (Fig. 14.3c) of ^{65}Zn from roots to shoots were not affected by PS as compared to plants without PS under the low P (50, 5, and 0.5 μMP) conditions. However, the above parameters were all reduced by PS in plants under control (500 μMP) condition. It was suggested that PS was ineffective for enhancing Zn translocation in plants regardless of the P level under –Fe condition either at pH 5.5 or 6.5 (chapter 13). In control (500 μMP) condition, PS reduced the translocation of ^{65}Zn in plants under pH 5.5 and not under pH 6.5 (chapter 13). It was indicated that the depressive effect of PS on Zn translocation in plants under P-sufficient condition may be a pH-dependent phenomenon. The translocation and the concentration of ^{65}Zn of shoots were similar between control plants and 50 μM P plants either under –PS or +PS conditions. The translocation per plant of ^{65}Zn was lower while the concentration of ^{65}Zn in shoots of 5 μM P plants was similar to those of the control plants under –PS condition. In presence of PS, the translocation and the concentration of ^{65}Zn in shoots were similar between 5 μM P plants and control (500 μMP) plants. These parameters were found lower in plants of the lowest P (0.5 μMP) media than in plants of the control (500 μMP) medium either under –PS or +PS condition. The relative translocation rate of ^{65}Zn in plants decreased with decreasing P concentration of the media under –PS or +PS condition. Except that 50 μM P plants showed similar relative translocation rate of ^{65}Zn to that of the control plants. It was shown that in low P (0.5 μMP) and –Fe medium, the translocation of Zn in plants may decrease either under –PS or +PS conditions at both pH values 5.5 or 6.5 (chapter 13). However, the effect of 50 μM P under –Fe

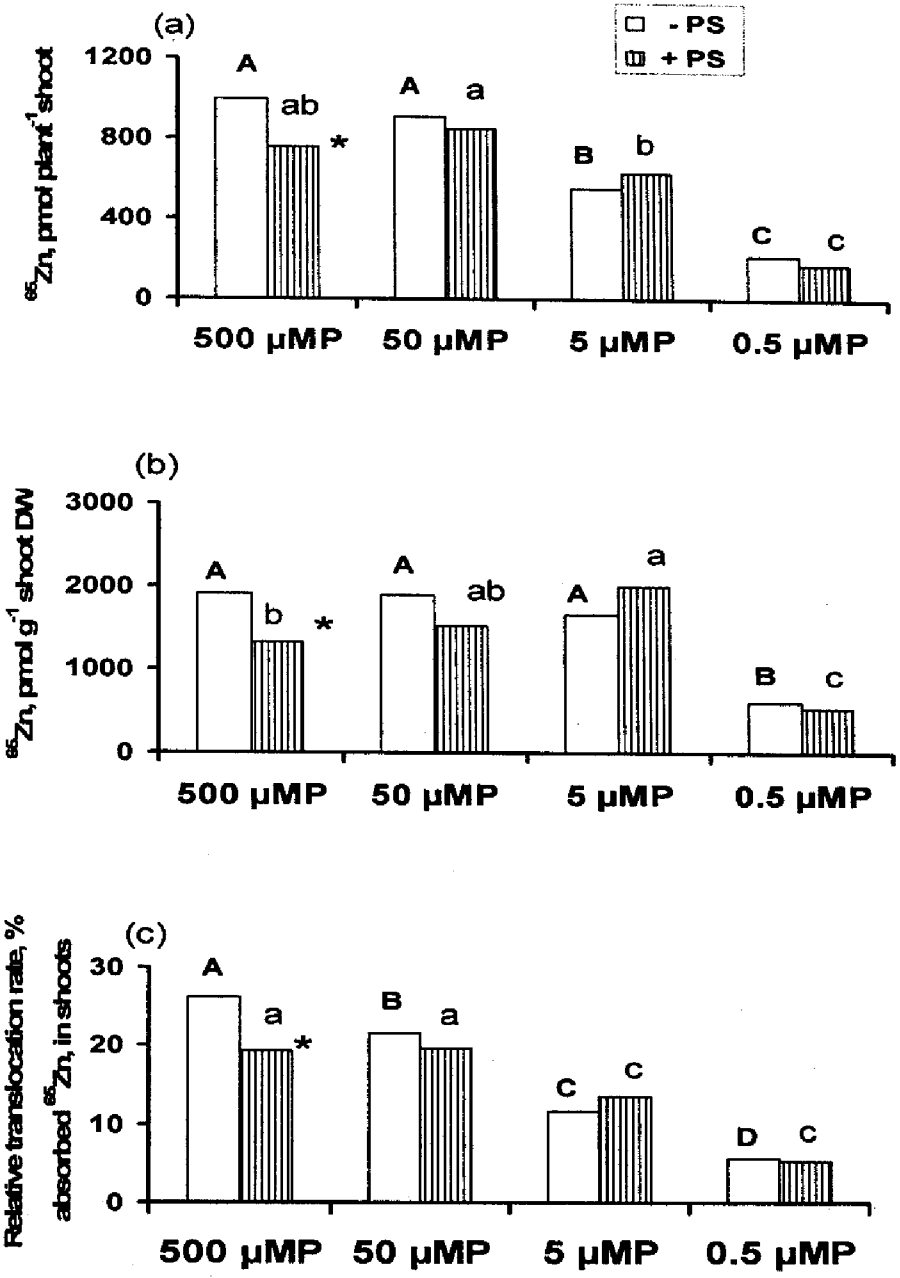


Figure 14.3. Translocation and relative translocation rate to shoots of ^{65}Zn in Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

conditions on the translocation of Zn in plants may vary inconsistently depending on the medium pH level. The translocation of ^{65}Zn in plants was not affected at medium pH 5.5 while it was decreased at pH 6.5 (chapter13) under 50 μM P condition as compared to control (500 μMP) condition.

14.3.4. Apoplastic ^{65}Zn in Roots

The formation of apoplastic ^{65}Zn in roots per plant (Fig. 14.4a) and per plant root DW (Fig. 14.4b) were not affected significantly by PS neither in low P (50 and 5 μMP) media nor under control (500 μMP) medium as compared to –PS conditions. However, these parameters were reduced by PS in plants under the lowest P (0.5 μMP) condition of the medium as compared to those in plants under control (500 μMP) condition. It was suggested that PS may reduce the formation of apoplastic Zn in plants under severe P-deficient and –Fe conditions. This effect of PS on the formation of apoplastic ^{65}Zn in plants in 0.5 μM P and –Fe medium under pH 5.5 was inconsistent with that in plants under pH 6.5 (chapter 13). Apoplastic ^{65}Zn in plants was not affected by PS under 50 μM P and –Fe conditions at pH 5.5 while being increased by it at pH 6.5 (chapter 13). The effect of PS on the formation of apoplastic Zn in plants under low P and –Fe seemed to be pH-dependent. The low P (50 and 5 μMP) and –Fe conditions of the media did not affect significantly the formation of apoplastic ^{65}Zn neither per plant roots (Fig.14.4a) nor per plant root DW (Fig.14.4b) as compared to control (500 μMP) medium under –PS or +PS conditions. However, apoplastic ^{65}Zn in roots per plant of 0.5 μMP plants was higher than that of control plants under –PS or +PS conditions. The apoplastic ^{65}Zn per root DW in 0.5 μM P plants was higher under –PS and similar under +PS condition to that of the control plants.

The effect of low P and –Fe conditions on the formation of apoplastic ^{65}Zn in roots under pH 5.5 or pH 6.5 (chapter 13) was not clearly observed. Though apoplastic ^{65}Zn, in roots of 0.5 μM P plants was increased under pH 5.5 and decreased in roots of 50 μM P plants under pH 6.5 (chapter 13).

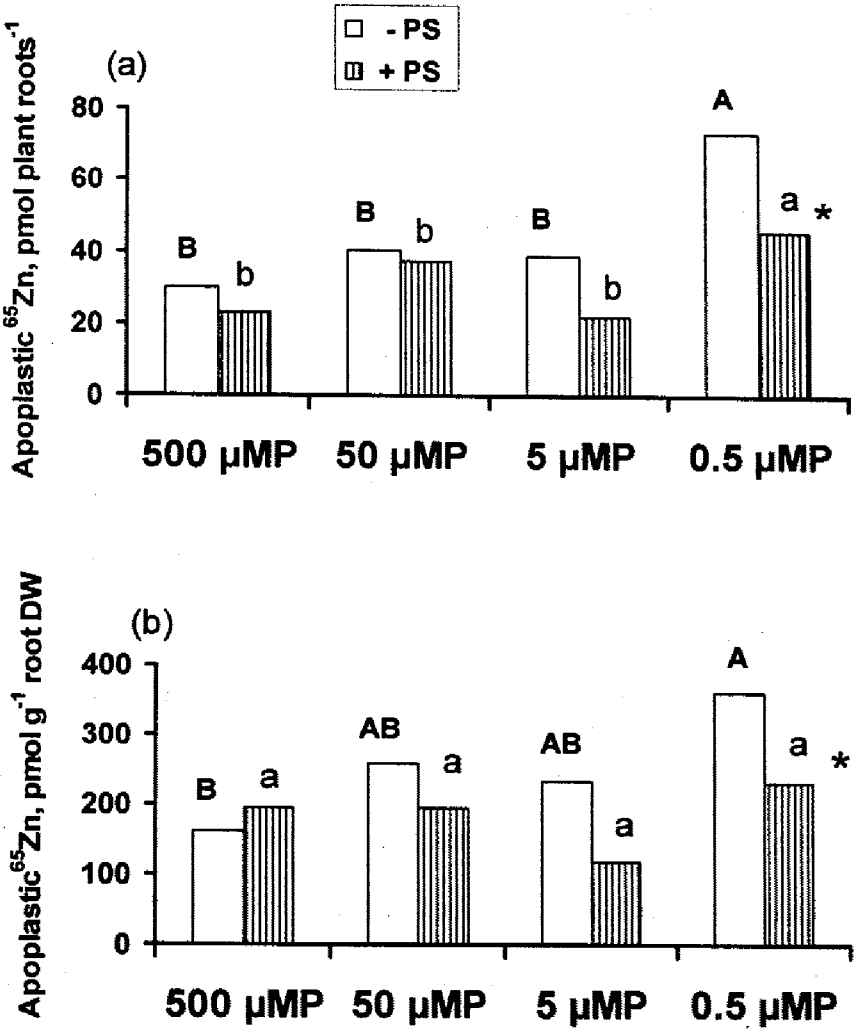


Figure 14.4. Apoplastic ^{65}Zn in roots of Fe-deficient barley plants as affected by different P levels (capital letter for –PS plants and small letter for +PS plants) and by PS in each P level under pH 5.5. Different letter or asterisk * at the top of each bar indicates significant differences ($P<0.05$) between with and without PS in each P level, respectively, according to Duncan Multiple Range Test.

14.4. SUMMARY

The results of this experiment led to the conclusion that low P and –Fe conditions may not affect much the absorption of ^{65}Zn by plants neither in –PS nor +PS medium. The absorption pattern of ^{65}Zn by plants did not vary with decreasing medium pH from 6.5 (chapter 13) to 5.5 under –PS or +PS condition. The supply of PS was ineffective for the absorption of ^{65}Zn in plants either under pH 5.5 or pH 6.5 (chapter 13) regardless of the P level.

The translocation of ^{65}Zn in plants under low P (0.5 μMP) and –Fe conditions was decreased either under –PS or +PS conditions. This effect of low P (0.5 μMP) on ^{65}Zn translocation was expressed either under pH 5.5 or 6.5 (chapter 13). However, the translocation of ^{65}Zn in plants under less critical low P (50 μMP) and –Fe conditions varied inconsistently depending on the medium pH level. The translocation of ^{65}Zn in plants was not affected at pH 5.5 while decreased at medium pH 6.5 (chapter13) under 50 μM P condition as compared to control (500 μMP) condition. The application of PS was ineffective for ^{65}Zn translocation in plants in low P and –Fe media either under pH 5.5 or pH 6.5 (chapter13). However, PS reduced translocation and relative translocation rate of ^{65}Zn in plants in P-sufficient (500 μMP) and –Fe medium under pH 5.5 in this chapter but not under pH 6.5 (chapter 13). The reduction of Zn translocation in plants by PS under P-sufficient and –Fe conditions seemed to be a pH-dependent phenomenon. The effect of low P and –Fe conditions on the formation of apoplastic ^{65}Zn in roots under pH 5.5 or pH 6.5 (chapter 13) was not clearly observed. Though apoplastic ^{65}Zn, in roots of 0.5 μM P plants was increased under pH 5.5 and decreased in roots of 50 μM P plants under pH 6.5 (chapter 13). The application of PS did not affect the formation of apoplastic ^{65}Zn in roots under P-sufficient and –Fe conditions either at pH 5.5 or pH 6.5

(chapter 13). The uptake and translocation of Zn in plants as affected by PS should be investigated under wide range of pH values in the future.

CHAPTER 15

GENERAL DISCUSSION AND CONCLUSION

15. General Discussion and Conclusion

Our results showed that 10 times reduction of P concentration of the control (500 μMP) medium may not affect growth, chlorophyll index, and the general status of the nutrition of the plants (chapter 3). The deficiency of P (5 and 0.5 μMP) decreased growth without significant effect on the concentrations of mineral nutrients, such as K, Ca, Mg, Cu, and Zn, in shoots and roots, and those of Fe and Mn in shoots of the plants. The concentrations of Mn and Fe in plant roots were higher in lowest P (0.5 μMP) condition. The low P (50, 5, and 0.5 μMP) condition in –Fe rhizosphere alleviated Fe chlorosis, reduced PS release, and increased the growth of the plants (chapter 4).

A treatment of depletion of P in the –Fe medium, after the induction of Fe chlorosis in leaves and the activation of PS synthesis and release in the roots, caused the greening of Fe chlorotic plant leaves and an abrupt increase of the amount of PS released from roots (chapter 5). Chlorophyll index was not reliable for predicting Fe status and amount of PS released, since +P and –P plants, with similar Fe concentration of the shoots, displayed large differences in regard to the amount of released PS from roots and the chlorophyll index of the leaves. The depression of chlorophyll synthesis and loss of chlorophyll of the plants under –Fe conditions were not due to low concentration of Mg or Fe, but to the high P concentration that may repress the translocation of Fe from roots to shoots. The concentrations of the macronutrients other than P, such as K, Ca, and Mg, and those of the micronutrients, such as Fe, Mn, Zn, and Cu, in shoots were not significantly different in –P plants from +P plants under –Fe conditions. Furthermore, the –P treatment may induce a mobilization of Fe within the plants resulting in higher Fe content in roots and old leaves (chapter 6). The higher PS release amount of the –P plants may increase the Fe uptake activity of the sparingly contaminated Fe of the –Fe

medium. It was also considered that the lower Fe/P ratio of the plants grown under control (500 μMP) and –Fe conditions may be a major factor for the induction of Fe chlorosis. Low P physiological status of roots, old and new leaves of –Fe plants may enhance the remobilization of P and Fe resulting in higher Fe/P ratio within the plants organs.

The flow of xylem sap from roots to shoots was largely decreased when plants experienced P deficiency in –Fe medium (chapter 7). Consequently, the concentrations of macronutrient, such as K, Ca, and Mg, and those of the micronutrients were higher in the xylem sap of low P plants than in that of control (500 μM P) plants under –Fe conditions. The concentration of PS of the xylem sap increased in low P (0.5 μMP) and –Fe medium. The concentrations of organic acids such as citrate and malate in the xylem sap of the plants increased also under low P (0.5 μMP) and –Fe conditions. This was probably due to concentration effect by reduction of water flow in xylem tube. It was noticeable that PS concentration of xylem sap was not reduced in low P (50, 5, and 0.5 μM) plants without showing Fe chlorosis. It is known that PS is translocating in +Fe plants (Kawai and Alam, 2006).

In feeding experiment of –Fe plants with ^{59}Fe and PS, control (500 μMP) plants with Fe chlorosis had higher absorption activity of ^{59}Fe than the low P (50, 5, and 0.5 μMP) plants without Fe chlorosis with or without added PS to the media (chapter 8). The absorption of ^{59}Fe of –Fe plants with Fe chlorosis (control (500 μMP) plants) was higher than that of –Fe plants without Fe chlorosis (50, 5, and 0.5 μMP plants) when Fe was supplied. Phytosiderophore enhanced the absorption of ^{59}Fe and its translocation from roots to shoots regardless of the P level of the media. Though the absorption of ^{59}Fe was higher in control (500 μMP) plants, its translocation amounts to shoots were

similar for both low P (50, 5, and 0.5 μMP) and control (500 μMP) plants because the relative translocation was higher in the former plants. The relative translocation of ^{59}Fe to shoots of the plants was enhanced by PS in the lowest P (0.5 μMP) condition. It was suggested that much of the Fe of plants under control (500 μMP) and –Fe condition might accumulate in roots with the low relative translocation of the metal nutrient to shoots. The lower relative translocation rate of Fe in control (500 μMP) plants may be induced by the physiological inactivation of Fe in the roots.

By contrast, in the feeding experiment of +Fe plants, the low P (50, 5, and 0.5 μMP) plants absorbed higher amounts of ^{59}Fe than control (500 μMP) plants, but the relative translocation rate of ^{59}Fe to shoots remained similar for both control (500 μMP) and low P (50, 5, and 0.5 μMP) plants (chapter 9). It was suggested that low P (50, 5, and 0.5 μMP) plants might accumulate higher amounts of ^{59}Fe in roots than control (500 μMP) plants. The presence of PS in the rhizosphere enhanced the absorption of ^{59}Fe and its translocation in +Fe plants at all the P levels of the media. Apoplastic ^{59}Fe content in roots of +Fe plants in the low P media were reduced by PS. It was indicated that PS was effective for Fe absorption and Fe translocation not only in –Fe plants but also in +Fe plants. The feeding of PS to other crops should also be examined in the future.

Differently from PS, the presence of citrate or EDTA in the media was not effective for the absorption and translocation of ^{59}Fe in plants either under control (500 μMP) or low P (50 μMP) conditions (chapter 10). However, citrate enhanced the relative translocation rate of ^{59}Fe of the low P (50 μM) plants as compared to that of control (500 μMP) plants. It was suggested that citrate might be also involved in Fe translocation in plants under low P (50 μMP) and –Fe conditions. It is important to clarify the form of Fe in xylem sap of low P plants and the mechanism for the

enhancement of relative translocation rate of absorbed Fe under low P (50 μMP) condition.

The results of the feeding experiments with ^{65}Zn fed with or without PS to +Fe plants (chapter 11 and 12) or –Fe plants (chapter 13 and 14) with varied P concentrations of the media showed that low P (50, 5, and 0.5 μMP) condition of the medium did not affect the absorption of ^{65}Zn as compared to control (500 μMP) regardless of the Fe status of the plants. Our results showed no relationship of the P concentration of the medium with Zn absorption in plants and followed the discussion of a few researchers such as Pasricha et al. (1996) that the antagonistic interaction between P and Zn may not always occur.

Low P status depressed the translocation of ^{65}Zn and the relative translocation of ^{65}Zn to shoots of both +Fe plants and –Fe plants. It was indicated that P deficiency may induce high accumulation of Zn in roots resulted from reduced Zn translocation to shoots. These results have not been reported. The results may be out of the common understanding that P toxicity induced low Zn translocation to shoots (Loneragan et al., 1979). It is important to examine the mechanism of the P-Zn interaction in plants under P-deficient conditions.

The responses of +Fe plants and those of –Fe plants to the presence of PS in the media were different:

The absorption and translocation of ^{65}Zn, and the apoplastic ^{65}Zn content in roots of the +Fe plants were enhanced by PS regardless of the P concentration of the media under pH 5.5 and not under pH 6.5. By contrast, the above parameters were not affected by PS in –Fe plants either under pH 5.5 or 6.5. It was suggested that PS activity on Zn absorption and translocation in plants may vary depending on Fe status of the plants and

the pH of the medium. The reasons of these phenomena are not known. The release of PS occurs when plant experiences –Fe condition, but our results showed that PS was effective on Zn uptake and translocation in +Fe plants. It is known that PS-Fe can be absorbed both in +Fe and –Fe conditions. However, it seemed that absorption of PS-Zn is affected by Fe status of plants. The mechanism by which PS enhanced Zn absorption in +Fe plants needs to be investigated.

This study of barley plants pointed out several phenomena and new insights regarding the interactions of P with Fe and Zn. The relationship between P and micronutrients in graminaceous plants needs further investigation.

CHAPTER 16. ABSTRACT

16. Abstract

The knowledge of the beneficial balances among minerals under nutrient-deficiency-stress may reduce the adverse effects of mineral deficiency in crops and increase the tolerance of plants to adverse conditions. Several hydroponical experiments were conducted with barley (*Hordeum vulgare L.* cv. Minorimugi) in phytotron or green house. The interactions between P and Fe or Zn on mineral nutrition and physiology of plants under –Fe and +Fe conditions were studied. The objectives of this study were to examine the effect of : (1) low P on phytosiderophores (PS) release from roots, PS accumulation in roots, (2) PS on absorption and translocation of Fe or Zn in plants under low P and +Fe or –Fe condition. (3) low P on the distribution of minerals in –Fe plants.

The results showed that P deficiency (5 and 0.5 μMP) decreased growth without significant effect on the concentrations of minerals, such as K, Ca, Mg, Cu, and Zn, in shoots and roots, and those of Fe and Mn in shoots. The concentrations of Mn and Fe in roots were higher in lowest P (0.5 μMP) condition. The low P condition (50, 5, and 0.5 μMP) in –Fe media alleviated Fe chlorosis, reduced PS release, and increased the growth of the plants. A short-term –P treatment in the –Fe medium, after the induction of Fe chlorosis in leaves and the activation of PS release by the roots, caused the greening of chlorotic leaves and increase of PS release by roots. Chlorophyll index of leaves was not reliable for predicting Fe status and PS release, because PS release of +P and –P plants was largely different in spite of similar Fe concentration of the shoots. These results suggested a physiological competition between P and Fe in plant tissues. Chlorosis of the plants under –Fe conditions were not due to low concentration of Mg or Fe, but to the high P condition (500 μMP) that may repress the translocation of Fe from

roots to shoots. The concentrations of the macronutrients, such as K, Ca, and Mg, and those of the micronutrients, such as Fe, Mn, Zn, and Cu, in shoots were not significantly affected in –P plants as compared to +P plants under –Fe conditions. However, the –P treatment may induce a mobilization of Fe within the plants resulting in higher Fe content in roots and old leaves. It was considered that the lower Fe/P ratio of the plants grown under 500 μM P (control) and –Fe conditions may be a major factor in the induction of Fe chlorosis. Low P status of roots and leaves of –Fe plants may enhance the remobilization of Fe which may be resulted in higher Fe/P in the plant tissues.

The flow of xylem sap from roots to shoots was largely decreased in plants under –P and –Fe conditions. Consequently, the concentrations of macronutrient, such as K, Ca, and Mg, and those of the micronutrients were higher in the xylem sap of low P plants than in that of high P (500 μMP) plants under –Fe conditions. The results showed that metal micronutrients, such as Fe, Mn, and Zn, translocated more under lower P (5, 0.5 μM) condition which may affect the greening of the leaves.

In feeding experiment with ^{59}Fe and PS, plants grown under high P (control) and –Fe condition with Fe chlorosis had higher absorption activity of ^{59}Fe than the plants of the low P and –Fe media without Fe chlorosis in the presence and absence of fed PS.

Phytosiderophore enhanced the absorption of ^{59}Fe and its translocation from roots to shoots regardless of the P level of the media. Though the absorption of ^{59}Fe was higher in high P (control) plants, its translocated amounts to shoots were similar for both low and high P plants because the relative translocation rate was higher in the low P plants. The relative translocation of ^{59}Fe to shoots was enhanced by PS in low P (0.5 μMP) condition. It was suggested that much of the Fe of plants under high P and –Fe

conditions might accumulate in roots. The lower relative translocation rate of Fe in high P (control) plants may be induced by the physiological inactivation of Fe in the roots.

By contrast, the low P and +Fe plants absorbed higher amounts of ^{59}Fe than high P (control) and +Fe plants, but the relative translocation rate of ^{59}Fe to shoots was similar for both high and low P plants. The presence of PS in the rhizosphere enhanced the absorption of ^{59}Fe and its translocation in +Fe plants at all the P levels of the media. Apoplastic ^{59}Fe content in roots of +Fe plants in low P media were reduced by fed PS. It was indicated that PS effectiveness on Fe chelating and transporting activities occurred in –Fe and +Fe plants. The response of other crops to PS should also be examined in the future.

The presence of citrate or ethylene diamine-tetraacetic-acid (EDTA) in the –Fe media did not affect the absorption and translocation of ^{59}Fe in plants either under control (500 μMP) or low P (50 μMP) conditions. However, citrate enhanced the relative translocation rate of ^{59}Fe of the low P (50 μMP) plants as compared to that of control plants. It was suggested that citrate might also be involved in Fe translocation under low P and –Fe conditions. The form of Fe in xylem sap and the mechanism of the enhanced relative translocation rate of Fe by citrate in plants under low P and –Fe conditions need to be clarified.

Feeding experiments with ^{65}Zn with or without PS were conducted using +Fe or –Fe plants grown with varied medium P concentrations. Low P condition of the media did not affect the absorption of ^{65}Zn as compared to control condition regardless of the plant Fe status. These results showed no relationship between medium P concentration and Zn absorption.

However, low P status depressed the translocation of ^{65}Zn and its relative translocation rate to shoots of both +Fe and –Fe plants. Therefore, P deficiency may induce high Zn accumulation in roots resulting from reduced Zn translocation to shoots. The decreased Zn translocation in plants by P deficiency has not been reported and out of the common understanding that higher P condition induced low Zn translocation to shoots. The mechanism of the P-Zn interaction in plants under P-deficient condition needs to be examined.

Addition of PS to the media affect +Fe and –Fe plants differently in ^{65}Zn absorption and translocation. In +Fe plants, the absorption and translocation of ^{65}Zn, and the apoplastic ^{65}Zn content in roots were enhanced by PS regardless of the P concentration of the media. The above parameters were not affected by PS in –Fe plants. It was suggested that PS activity on Zn absorption and translocation in plants may vary depending on the Fe status of the plants. Though PS is known to convey Fe, function of PS for carrying Zn has not been much documented. The mechanism by which PS enhanced Zn absorption in +Fe plants needs to be clarified. Further investigation about the relationship between P and micronutrients are necessary.

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