

**ROLE OF ROOT MORPHOLOGY AND IRON SOURCE IN ENHANCEMENT
IN NUTRIENT USE BY POLYASPARTIC ACID**

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THESIS

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Dedication

This thesis is dedicated to my family in Albania. My professional accomplishments and the completion of this thesis would not have been possible without their love and support. I feel so blessed for having the best loving family and I only hope that my accomplishments will make them proud. Kjo teme i dedikohet familjes time te dashur, per mbeshtetjen, dashurine dhe te qeshurat qe me kane shoqeruar gjate gjithë jetes, e sidomos gjate ketyre viteve larg shtepise. Pa ju, kjo teme dhe çdo arritje tjetër profesionale apo personale s'do te ishte e mundur. Ju dua shume.

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Preface

The main body of this thesis is written in the form of a literature review and one manuscript.

The manuscript follows the guidelines suggested for contributors to Crop Science.

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Chapter 1. Roots and their Importance to Plant Growth and Productivity

Root systems, known also as 'the hidden half', have been the subject of much less research than above-ground plant parts. Historically, this lack of attention was partially due to assumptions that root systems were passive absorbers of nutrients and water, and therefore were not challenging enough to be investigated. However, because of their unique underground growth habitat, investigating root development is extremely difficult. The early methods for studying roots were laborious, tedious, and time consuming, and were usually destructive (Böhm, 1979; Box, 1996). Today, root research is utilizing video cameras combined with sophisticated computers.

Roots have long been known to be responsible for anchorage and absorption of water and mineral nutrients. They are also the site of synthesis for some important plant hormones, and can play a major role in assimilate storage (Torrey, 1976; Itai and Birnbaum, 1996). Root systems develop and function in an intricate environment, where they establish complex relationships with the biotic and abiotic components of the soil, as well as the shoot. The ability to study and understand root function depends on a thorough knowledge of these interrelationships and the external factors that influence root metabolism (Box, 1996). The goal of this review is to summarize previous work on roots and root functions in order to better understand their role in plant growth and productivity.

TYPES OF ROOT SYSTEMS

Although all plants have the capacity to develop an effective and dynamic root system, the type and the extent depends on the plant and the soil environment (Taiz and Zeiger, 1991). Root systems differ in their anatomical and morphological features, both between and within species. The reason for these differences can be genetic, environmental, or both (Fitter, 1991).

Many attempts have been made to classify various types of root systems beginning with William Cannon's classification in 1949 (Cannon, 1949). In this system, the first distinction among root systems is based on roots that originate from the seed embryo, and those that originate from the stem base (Fitter, 1991). Other ways to characterize root systems include developmental and topological models, which are based on the potential of roots to produce lateral branches. However, the most widely used classification of root systems is based on whether the plant is a monocotyledonous or dicotyledonous species (Klepper, 1991; Taiz and Zeiger, 1991).

Monocotyledonous plants, like wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), develop what is known as a fibrous root system. Most fibrous-rooted plants exhibit two distinct phases; the seminal root system, and the nodal root system. The seminal root system (also known as seed roots) is made up of the radical and three to six roots originating from the embryo (MacKey, 1979; Rickman et al., 1985; Barlow 1986; Waisel and Eshel, 1991). The nodal root system, also called crown roots, originates not from the embryonic axis, but from crown primordia or lower stem nodes (Klepper et al., 1984; Barlow, 1986).

The seminal root system emerges first and plays a major role in water and nutrient uptake in young seedlings. Unlike seminal roots, nodal roots branch extensively, thereby ultimately becoming the main root system of the plant. After the development of nodal roots, seminal roots appear to support mainly the primary shoot (Waisel and Eshel, 1991). Although both root types can remain active for long periods, it is believed that nodal roots support the plant during most of its life (Klepper et al., 1984).

The extent of nodal root development can vary widely as these roots are affected by numerous environmental factors and cultural practices. In contrast, seminal root number is more constant, and is primarily influenced by cultivar and seed size (Tennant, 1976; MacKey, 1979; Belford et al., 1987; Wang and Below, 1992). For example, in wheat, N application has been shown to alter (enhance) the growth and branching of nodal but not seminal roots (Belford et al., 1987; Vincent and Gregory, 1989a, b).

In contrast to monocotyledonous plants, the root system of dicotyledoneous species develops from a main single root (the radical), which after elongation forms the taproot. A characteristic of some dicotyledoneous plants is secondary root growth, whereby the taproot thickens due to activity of the lateral cambium. This secondary cambium is formed around the xylem star and gives rise to secondary xylem and phloem (Bidwell, 1979; Klepper, 1991). Secondary root growth, however, does not occur in herbaceous dicotyledoneous species that do not possess a secondary cambium. Monocotyledonous species, with the exception of members of the *Palmae* family, also do not possess a secondary cambium and therefore lack secondary root thickening.

An obvious result of secondary growth is an increase in root diameter. When thickening occurs in the outer cortical layer, a heavy lignified parenchyma is formed which functions as a supporting tissue. In other cases, the secondary growth results in swollen tissues which serve as storage organs. This function is of particular importance for commercially valuable root crops such as carrots (*Daucus carota*), turnips (*Brassica rapa*), and potatoes (*Solanum spp.*). In either case, the presence of secondary growth explains the wide range of root sizes observed among dicots, from tiny laterals, to woody or fleshy taproots (Klepper, 1991).

Lateral roots can develop from the primary roots of both monocots and dicots. These lateral roots referred to as secondary roots initiate from the pericycle and increase the surface area of the plant's root system (Peterson and Peterson, 1986). A special type of lateral roots known as proteoid roots (Purnell, 1960) emerge simultaneously along lateral roots, and in such close proximity to each other that they form dense clusters (Peterson and Peterson, 1986; Louis et al., 1990). For this reason, they are better known as cluster roots. These roots were first described for the *Proteaceae* family, but have also been observed on plants of the *Leguminosae*, *Miriaceae*, *Betulaceae*, and *Casuarinaceae* families (Lamont, 1982; Gardner et al., 1982a, b; Louis et al., 1990). Although the function of cluster roots is not fully

understood, one theory is that they help plants to grow in soils with low levels of mineral nutrients, especially phosphorus (P) (Jeffrey, 1967; Gardner et al., 1982a, b).

In addition to the primary root system that originates from the embryonic root, many plants also have roots that originate from parts of the shoot and are known as adventitious roots (Barlow, 1986). These roots are often found above ground and may have special functions; such as brace roots of maize which develop from above-ground stem nodes and seem to increase shoot stability (Fitter, 1991). Others adventitious roots called stilt and prop roots are frequently found in waterlogged soils where they are thought to aid in plant support. Another example of specialized roots are buttresses or tabular roots, which are the result of secondary thickening between the trunk and the tap root. These roots are found in trees native to rain forests where it is thought they play a role in reelevating trunks downed by heavy winds (Barlow,1986).

Root of some plants are associated with sheaths of soil particles which are collectively called rhizosheaths. Rhizosheaths result from an interaction between the soil and the plant, including soil particles, root hairs, and the mucilage from both the root and the associated bacterial flora (Duell and Peacock, 1985; Watt et al., 1994). Soil particles are bound to the root surface via hydrogen bonds between the neutral sugars of the mucilage and the soil (Watt et al., 1993).

The first descriptions of rhizosheaths were on desert grasses, which led to the incorrect assumption that they are confined to only a few species grown under xeric conditions. However, in reality, rhizosheaths are found on both annual and perennial grasses grown in either high or low fertility soils (Duell and Peacock, 1985). While the physiological significance of rhizosheaths is not known, it is thought that they enhance nutrient uptake by putting the root and the soil in better contact. Another theory is that they conserve water by acting as a barrier to water flow from the root into the soil (Nambiar, 1976; Bristow et al., 1985; Watt et al., 1994). This mechanism could be viewed as an adaptive feature, especially for mesophytic grasses that grow under dry conditions. In any event, the presence of

rhizosheaths demonstrates that root systems play a role not only in ion absorption and anchorage, but also in establishing complex relationships with the soil.

GROWTH FUNCTIONS OF ROOTS

The main function of roots is the acquisition of nutrients and water from the soil, and transportation of these assimilates to the rest of the plant. Roots play an active role in the acquisition of nutrients by changing the chemical status of the soil around them, and therefore the availability of many nutrient elements. Roots also synthesize phytohormones, thereby playing an important role in the regulation of plant growth and development. While considered to be of secondary importance compared to the acquisition of water and nutrients, other root function such as storage and anchorage are also important to plant development.

Acquisition of Nutrients

Water and nutrient acquisition is one of the most important functions of root systems. It is part of a universal and continuous cycle which involves living organisms and their environment (Taiz and Zeiger, 1991). In order for the absorption process to be effective, root systems should provide: (1) a large enough surface area for soil exploration, (2) the least resistance to transport of materials to the rest of the plant and, (3) an ability to change the availability of mineral nutrients in the rhizosphere (Fitter, 1991; Van Noordwijk et al, 1994; Marschner, 1995; Marschner and Rohmeld, 1996).

Soil Exploration

As mentioned earlier, the growth of plant roots is continuous and dependent on the soil environment. In any case, the volume of soil in contact with the roots plays an important role in the acquisition of mineral nutrients (Taiz and Zeiger, 1991). In order for roots to have access to soil nutrients, they must come in close contact with them (Jungk, 1996). For this

reason, plants alter the size and architecture of their root systems by developing new branches, increasing root length, and by changing other structural properties such as root diameter and the formation of root hairs.

Lateral roots, or branches, arise endogenously from main or primary roots and increase the root's absorption area (Peterson and Peterson, 1986; Peterson, 1992). Even though there are genotypic differences in branching pattern, plants have adapted to various habitats. In soils infested with mycorrhizal fungi, plants facilitate their nutrient uptake by means of a symbiotic association without the need of an intensive root system; while plants that are not dependent on mycorrhizal fungi develop a well branched system of fine roots (Peterson, 1992). Other plants grown in nutrient-deficient soils, exhibit a branching pattern called proteoid roots (Lamont, 1972). Proteoid roots are thought to modify the soil environment by increasing the concentration of chelators and reductants in the root rhizosphere (Gardner et al., 1982a, b). Since most proteoid roots are produced under non-sterile conditions, their formation is thought to be a result of an interaction between rhizosphere microorganisms and the root system (Gardner et al., 1982a, b). However, for white lupin (*Lupinus albus*), microorganisms do not appear to be necessary for proteoid root formation, but they can enhance their development (Gardner et al., 1982).

Phosphorus is one of the mineral elements that plants acquire principally by diffusion (Itoh and Barber, 1983a, b). Since the diffusion coefficient for P movement is low, morphological characteristics such as root hairs and root length play an important role in P uptake (Barley, 1970; Föehse et al., 1991; Eissenstat, 1992). The importance of root length also seems to depend on the P status in the rooting zone. Experiments conducted in solution culture with maize showed that P uptake was related to root length (Jungk and Barber, 1974). However, subsequent data showed that the relationship between root length and P uptake was significant only in high-P soils, and that different mechanisms were employed for P uptake from low P-soils (Otani and Ae, 1996). In contrast, Romer et al. (1988) showed that total root

length was the most important for nutrient uptake by wheat in low-P soil, while uptake efficiency per unit length was more important in high-P soil.

Root diameter varies widely between species, from fine branches of grasses to extremely coarse roots of many trees. When nutrient supply is low, the production of a finer root system is often observed (Fitter, 1991). Thin roots are more efficient in ion absorption because of issues of ion mobility and the volume of soil occupied by the root (Robinson and Rorison, 1983). A decrease in root diameter increases the root surface area, and the volume of soil that the roots occupy. Therefore, the thinner the roots, the greater the potential availability of nutrients; assuming the influx per unit root surface does not change (Barber, 1984). The higher efficiency of thin roots in nutrient absorption is why root fineness is a principle selection criteria in wheat breeding programs seeking plants with efficient P uptake (Blair, 1993). However, it is still unclear why plants invest in coarse roots when grown in soils containing an adequate supply of mineral nutrients.

Root hairs originate from epidermal cells and vary in number and length depending on their location on the root, and the plant species (Farr, 1928; Dittmer, 1949; Cormack, 1949; Hofer, 1991). Usually root hairs on the secondary or higher order laterals are shorter than ones on the main root (Dittmer, 1949; Tanaka and Woods, 1972). Root hairs are believed to be short lived, with a life span of a few days to a few weeks. However, in some species they can become suberized or lignified and last for several months (Dittmer, 1949; Tanaka and Woods, 1972). The size of root hairs varies with pH, ion concentrations, soil texture, relative humidity, and microorganism population (Cormack, 1949; Tanaka and Woods, 1972; Reynolds, 1975; Bowen and Rovira, 1976; Ewens and Leigh, 1985). Although root hairs constitute a small portion of the root system, they play an important role in the function of the whole root system, and are considered to be the main site for the uptake of both water and mineral nutrients (Bowen and Rovira 1969; Reynolds, 1975; Itoh and Barber, 1983a). Early estimates showed that the total nutrient influx across root hair surfaces is three to ten times

greater than across the surface of the root cylinder (Bouldin, 1961). Root hairs also increase root surface area by increasing total root length (Green et al., 1991).

Root hairs are especially important in facilitating nutrient absorption when the element moves to the root by diffusion. For example, the presence of root hairs on rye grass (*Lolium multiflorum*) roots has been shown to increase K uptake up to 77 % compared to plants without root hairs (Drew and Nye, 1969). When grown in low P soils, the contribution of root hairs can be even greater, accounting for up to 90% of the total P uptake (Föehse et al., 1991). Root hairs have also been shown to be more efficient than whole roots in the uptake of P per unit of root radius (Lewis and Quirk, 1967). Experiments studying the P depletion around roots of onion (*Allium cepa* L.) (Bhat and Nye, 1974a, b), rape (*Brassica napus*), and cotton (*Gossypium hirsutum*) (Misra et al., 1988) showed an increase in width of the depletion zone due to the presence of root hairs. This depletion zone may also be related to root hair length, as longer root hairs have been implicated in the greater efficiency in P uptake of a modern wheat cultivar (Cosir) compared to an older one (Peragis) (Horst et al., 1993).

Itoh and Barber (1983a) measured P uptake by six plant species [including tomato (*Lycopersicon esculentum* Mill.), lettuce (*Lactuca sativa* L.), russian thistle (*Salsola kali* L.), wheat, carrot, and onion] which differed in root hair length, and found that P uptake per unit of root length was highest in species with longer root hairs. The same authors used a simulation model for P uptake by different plants and compared the measured values with those predicted by the model (Itoh and Barber, 1983b). For species with extensive root hairs (tomato, lettuce, russian thistle), the two estimates agreed fairly well only when the root hairs were taken into account. When the root hairs were not taken into account, the model only worked well for species with no, or few root hairs, such as carrot or onion. Data from experiments using simulation models for P uptake by spinach (*Spinacia oleracea*), rape, and other species also support this conclusion (Föehse et al., 1991). According to these authors the large contribution of root hairs to P uptake is based on their small radius and their perpendicular growth pattern, which allows them to contact a higher volume of soil.

Root hairs also function in anchorage and adhesion of roots to the soil by excreting mucilages on their outer surface (Farr, 1928; Itoh and Barber, 1983a, b; Hofer, 1991). In addition, root hairs are important in establishing symbiotic associations between roots and soil microorganisms by adhering them on their surface. For legumes such as soybean (*Glycine max* L.), root hairs are the site of infection by *Bradyrhizobium* bacteria, which leads to nodule formation and symbiotic N₂ fixation (Bauer, 1981; Selker et al., 1988; Peterson, 1992).

Most plant species, including the major crops (Smith et al., 1993), facilitate their uptake of nutrients through symbiotic associations with fungi or other microorganisms called mycorrhizas. These associations appear to be particularly important for nutrient uptake under low nutrient conditions (Lamont, 1982; Jungk, 1996; Taiz and Zeiger, 1991). Thus, root hairs and mycorrhiza hyphae both act as alternative means for obtaining nutrients that are sparingly available, especially P (Baylis, 1970). Studies with white clover (*Trifolium repens*) showed that longer root hairs were of little value in low-P soils, because of the ubiquitous presence of mycorrhizal fungi (Caradus, 1981). Root hair development and the colonization by mycorrhiza are also related. Roots with no or few root hairs are more dependent on mycorrhizal fungi for nutrient uptake, whereas ones with numerous root hairs are less dependent on mycorrhizal associations (Baylis, 1970; Lamont, 1982; Meisner and Karnok, 1991). The presence of mycorrhiza seems to account for a larger surface area for nutrient absorption and soil exploration than roots alone (Smith et al., 1993). It has also been suggested that variation in root diameter is related to the extent of mycorrhizal dependence, with larger diameter plants being more mycotrophic (Baylis, 1975). Interestingly, plants that form proteoid roots are not mycorrhizal, implying that proteoid roots may be an alternative mechanism for P uptake (Gardner et al., 1981). When such alternative strategies are well developed, mycorrhizal associations might be less important (Smith et al., 1993).

Transport

Another function of roots that is closely related to nutrient acquisition is the transport of absorbed ions to the rest of the plant. It seems reasonable that natural selection would favor root patterns that offer the least resistance and lowest cost to nutrient transport. Energy needs related to transport of materials include construction and maintenance costs as well as those related to transport. Root length and root branches are both extremely important to transport efficiency, and as Fitter (1991) has described, their high cost can often be compensated for by a greater absorbing surface area. Transport efficiency also seems to be related to the distribution of branches within the root system (Fitter, 1991).

Root-induced Changes in the Availability of Micronutrients

Root surface area is especially important for the uptake of nutrients with low mobility (such as P), and plants increase their root surface area by branching or by producing root hairs. Another strategy used by plants to acquire nutrients is to change their availability in the rhizosphere (Bar-Yosef, 1991; Bowen and Rovira, 1991; Marschner and Romheld, 1996). Root-induced changes in the rhizosphere can effect the availability of nutrients by: (1) altering the rhizosphere pH, (2) changing the reducing capacity of the roots or, (3) by releasing root exudates.

The rhizosphere is the zone of soil that is in contact with and influenced by roots (Bowen and Rovira, 1991). The chemical composition and the pH of the rhizosphere is different from that of the bulk soil for a number of reasons (Lewis and Quirk, 1967; Marschner and Romheld, 1996). For example, roots absorb ions selectively, excrete or reabsorb ions (H^+ or HCO_3^-), evolve CO_2 , and release low-molecular weight compounds. The main changes in rhizosphere pH are caused by excretion of H^+ and HCO_3^- in response to an imbalance in the cation-anion uptake ratio (Marschner and Romheld, 1996). For example, N can be absorbed by plants as either nitrate (NO_3^-) or ammonium (NH_4^+); and the influence that

each form has on rhizosphere pH is different. Nitrate uptake is associated with lower rates of H^+ excretion and an increase in rhizosphere pH, while NH_4^+ uptake is associated with higher rates of H^+ excretion and rhizosphere acidification (Marschner and Romheld, 1996).

An increase in H^+ excretion and acidification of the rhizosphere also occurs in response to deficient levels of certain nutrients, such as Zn, Fe, and P (Grinsted et al., 1982; Marschner et al., 1987; Romheld, 1987). For nongraminaceous monocots and most dicots, the rhizosphere acidification induced by Fe-deficiency is part of a complex root response called 'Strategy I' for Fe acquisition (Marschner et al., 1986; Romheld 1987). This strategy is a combination of different components (Hughes et al., 1992) involving: (1) an enhancement in the reduction of Fe^{3+} at the root surface by a plasmalemma bound reductase (Bienfait et al., 1983), (2) lowering of the rhizosphere pH as a result of an active ATPase pump (Brown, 1978) and, (3) the release of reductants and organic acids such as citrate. Fe-deficiency can also bring about changes in root morphology, such as formation of rhizodermal transfer cells which are the site of root response for Strategy I (Marschner, 1995).

For dicots and nongramineous monocots, Fe-deficiency changes the reducing capacity of the root (Romheld and Marschner, 1986; Bienfait, 1988), not only for Fe^{3+} -compounds but also for other natural and artificial oxidants such as Mn- and Cu-chelates (Norvell et al., 1993). Other nutrient deficiencies can also change the reducing capacity of roots as Norvell et al. (1993) showed that pea (*Pisum sativum* L.) and soybean roots grown with limited Cu or Zn were able to reduce Cu- and Zn-chelates as well as Fe. Thus, root reductases may play a non-selective role in nutrient uptake.

Proteoid roots of white lupin also use rhizosphere acidification to make P more available, but they accomplish it by the release of citrate. An agar film technique has been used to show that soil associated with proteoid roots exhibits a higher reducing and chelating activity than the bulk soil (Gardner et al., 1982). Johnson et al. (1996) recovered substantially higher amounts of citrate, malate, and succinate in the exudates of P-deficient plants than in P-

sufficient ones. Formation of proteoid roots in white lupin was enhanced in response to P deficiency and the roots constituted up to 50% of the root dry weight of P-sufficient plants (Dinkelaker et al., 1989; Johnson et al., 1994). Although the mechanism and the control of development of proteoid roots under P-deficiency is still not clear, it has been suggested that secreted citrate mobilizes Fe, Al, or Ca from the soil in a polymeric form with PO_4^{2-} ; which is then degraded by reducing agents at the root surface (Gardner et al., 1983 a; Marschner et al., 1986; Dinkelaker et al., 1989). Gardner et al. (1983 a) proposed that this degradation is in conjunction with an Fe^{2+} uptake mechanism that is balanced by H^+ secretion.

Similar results have also been reported for other species such as eucalyptus (*Eucalyptus gummiifera*), oat (*Avena sativa*), rape, and alfalfa (*Medicago sativa* L.). Eucalyptus is able to grow in soils with insoluble P sources such as AlPO_4 and FePO_4 (Mullette et al., 1974), presumably because the root exudates remove Al^{3+} and Fe^{3+} by chelating them. Rape plants are able to solubilize and utilize rock phosphate as their source of P by exuding organic acids such as malate and citrate to acidify the rhizosphere (Hoffland et al., 1989). Citrate, malate, and succinate have also been shown to be released in greater quantities from P-stressed seedlings of alfalfa than ones grown under P-sufficient conditions (Lipton et al., 1987).

Another interesting response to nutrient deficiency is observed in pigeonpea (*Cajanus cajan* L.) (Ae et al., 1993). Pigeonpea is known to respond poorly to P fertilization even under conditions where P is unavailable because of binding with Fe. In contrast to other legumes such as chickpea (*Cicer arietinum* L.) and peanut (*Arachis hypogaea*) that respond to P deficiency by releasing high amounts of organic acids (citric acid and fumaric acid, respectively), pigeonpea roots exude a compound called piscidic acid (p-hydroxybenzyl) (Marschner, 1995). Piscidic acid is able to chelate Fe^{3+} from Fe-P in iron-rich soils, thereby facilitating the uptake of P by the roots. At the same time, the chelated complex piscidic acid- Fe^{3+} is excluded from the rhizosphere, thereby preventing excess uptake of Fe. Collectively, these studies show that an increase in organic acid exudation, especially citric acid, serves as an

adaptive mechanisms by which nutrient-stressed plants can mobilize nutrients in the rhizosphere. In addition to the release of organic acids in response to mineral nutrient deficiencies, exudation of other low-molecular weight organic compounds such as sugars, amino acids, and phenolics has also been reported. For example, some dicotyledonous plants, such as tomato, release caffeic acid or other phenolics in order to increase Fe availability (Olsen and Brown, 1980; Olsen et al., 1981).

While nongramineous monocots and most dicots respond to Fe deficiency by employing 'Strategy I', grasses use a different mechanism known as 'Strategy II'. Takagi (1976) showed that washings of oat roots contain a chelating compound which solubilizes Fe^{3+} under high pH conditions. Later evidence showed this response is based on the release of nonproteinogenic amino acids called phytosiderophores (such as mugineic acid) which play an important role in mobilizing sparingly soluble Fe^{3+} , and making it available to the plant (Takagi, et al., 1984; Marschner et al., 1986; Romheld and Marschner, 1986). The chelated Fe^{3+} -phytosiderophore is then taken in by a specific transport system located in the membranes of root tips which is activated under Fe deficiency.

In conclusion, roots play an active role in the uptake of mineral nutrients by actively modifying their rhizosphere by selectively absorbing ions, and by releasing H^+ , HCO_3^- and low-molecular-weight exudates. These modifications can have a large impact on nutrient uptake, and may also play a role in plant adjustment to adverse soil conditions.

Synthesis of Plant Growth Regulators

Another important function of root systems is the synthesis of phytohormones (Torrey, 1976; Itai and Birnbaum, 1996). Early evidence indicated the presence of five types of plant hormones in the roots; auxin, cytokinins, abscisic acid, gibberellins, and ethylene (Torrey, 1976). Additional studies suggest that roots may actually produce all known phytohormones (Itai and Birnbaum, 1996). While auxin is generally thought to be transported from the shoot to the root, there is also evidence for its production by roots (Taiz and Zeiger, 1991). For

gibberellins, even though they occur in the roots, there are indications that roots might not be the primary site of synthesis, but rather a site of conversion from one form to another (Torrey, 1976). In this regard, gibberellin synthesized in the shoot moves to the root where it is converted to another active form and then transported back to the shoot.

In contrast to other phytohormones, cytokinins are mainly synthesized in the apical meristem of the root and transported passively to the shoot via the xylem (Skene, 1975; Carmi and Van Staden, 1983). Abscisic acid (ABA), is one of the most ubiquitous plant hormones, and is found in both the roots and root exudates (Itai and Birnbaum, 1996). Robertson et al. (1985) noticed a higher level of ABA in root apices of sunflower (*Helianthus annuus* L.) following desiccation, suggesting that roots are able to synthesize ABA when water potential decreases. Most of the evidence shows an increase in ABA synthesis in response to various kinds of stresses such as floods, salinity, and desiccation (Itai and Birnbaum, 1996). Ethylene is the only phytohormone found in a gaseous form and is synthesized in most plant organs and tissues, including roots.

In conclusion, root systems play a crucial role in plant growth and development. Their most important function, the uptake of nutrients and water, is vital for plants to grow and complete their life cycles. By actively changing rhizosphere conditions, roots help create conditions for nutrient uptake, and for survival under adverse soil conditions. As the site of synthesis of important phytohormones, especially cytokinins, roots also play an important role in shoot development and the physiology of the whole plant.

INTERNAL AND EXTERNAL FACTORS AFFECTING GROWTH MORPHOLOGY AND FUNCTIONS OF ROOTS

Root systems develop in a unique environment and are involved in complex interactions with the soil. Because of the heterogeneous nature of the root environment, both in space and time, root systems need to possess a high phenotypic plasticity (Fitter, 1991). Root system

architecture is susceptible to environmental changes, especially to localized nutrient supply, or other chemical, physical, or microbiological factors in the environment (Marschner, 1995).

Light

Since light is the energy source for photosynthesis, it is an important factor in growth of roots, especially when plant development is no longer dependent on seed storage reserves. Under low light intensity, the production and translocation of photosynthates to the root decreases, affecting root length and the acquisition of nutrients (Marschner, 1995). High light intensity can also have adverse effects on the root development, by altering the balance of phytohormones. The formation of lateral roots in Norway spruce (*Picea abies* L. Karst.) seedlings was inhibited as a result of high levels of cytokinins induced by high light intensity (Bollmark and Eliasson, 1990).

Phytohormones

Roots are capable of synthesizing all known phytohormones (Itai and Birnbaum, 1991), which are important for regulation of root functions and for the initiation of primary, lateral, and adventitious roots. Auxins play an important role as the primary trigger in the initiation and development of lateral and adventitious roots. Exogenous application of auxins (IAA and NAA) can increase the formation of lateral roots in seedlings, excised roots in culture, or roots of mature plants for a number of plant species (Torrey, 1986). Depending on the concentration and the ratio with supplied auxin, cytokinins application can inhibit (at high concentration) or stimulate (at low concentration) lateral root initiation. A study on lateral root formation in pea showed that exogenous auxins promote, while exogenous cytokinins inhibit initiation and emergence of lateral roots (Wightman et al., 1980). Zeadan and MacLeod (1984a) showed that exposure to IAA stimulates lateral root emergence of excised pea roots by suppressing meristematic activity of the root apex. In intact (attached) roots of maize, IAA treatment stimulated primordium initiation and increased lateral root emergence (Hurren et al.,

1988). However, auxin and cytokinin might not interact directly to affect lateral root initiation, as kinetin inhibited both the NAA-stimulated production of lateral roots, and the spontaneous lateral root development which was not dependent on NAA level. (MacIsaac et al., 1989). The limited information which is available on the role of gibberellins in lateral root initiation shows either an inhibitory effect, or no effect (Torrey, 1986).

The use of NAA to stimulate adventitious rooting from stem cuttings is one of the first uses of synthetic auxins (Oosterhuis and Zhao, 1994). Studies with exogenous applications of auxins to non-woody stem cuttings show that maximum rooting occurs when auxin concentrations are just below the threshold level which inhibits root formation (Jarvis, 1986). A number of compounds called 'auxin synergists' and phenolics are able to influence auxin levels, and therefore have an effect on adventitious root formation (Jarvis, 1986).

Cytokinins and gibberellic acid (GA_3) are generally considered to have an inhibitory effect on adventitious root formation. However, when applied at low concentrations and when in the proper ratio with auxin, cytokinins often stimulate lateral root initiation (Jarvis, 1986). Treating leaf cuttings with GA_3 also influences rooting, presumably by affecting auxin transport from the leaf blade to the site of root regeneration.

Ethylene is necessary for organization of the root primordium during the later stages of root development, but only in the presence of auxin which initiates cell division during the early stages. However, during the early stages of root growth, ethylene appears to be inhibitory. In addition, ethylene effects on root growth are concentration dependent as low concentrations enhance, while high concentrations inhibit root elongation (Marschner, 1995)

The role of abscisic acid (ABA) on root development is not clear. Torrey (1976) showed that ABA applications promoted the growth of excised root tips of pea, while Watts et al. (1981) showed that ABA treatment promoted root growth in maize. There is also evidence showing that ABA mediates the response of root hairs (abnormally short and swollen) to water stress (Schnall and Quatrano, 1992). When treated with ABA the root hairs of wild-type

Arabidopsis thaliana became short and swollen, compared to those of an ABA-insensitive mutant.

Synthetic growth regulators such as PGR-IV, which consist mainly of giberellic acid and indolebutyric acid have been shown to increase root length and the number of lateral branches in cotton (Oosterhuis and Zhao, 1994). Similarly, two synthetic auxins, N-phenyl indolyl-3-butyramide and phenyl indole-3-thiolobutyrate, can enhance adventitious rooting in common bean (*Phaseolus vulgaris* cv.) and jack pine (*Pinus banksiana* Lamb.) (Haissig, 1983).

Exogenous application of phytohormones have demonstrated their important role in root growth and morphology. Of the plant growth regulators, auxin appears to be the primary trigger for lateral root initiation, and for initiation of root regeneration (in cuttings) (Jarvis, 1986; Torrey, 1986). Furthermore, the effect of a given phytohormone is the result of an interaction with other plant hormones which might enhance or negate the effect of the primary trigger and modify the plant response (Torrey, 1986).

Soil Chemical Factors

Mineral nutrient supply

The size, morphology, and growth pattern of roots are strongly affected by nutrient supply. This effect depends on the nutrient, its concentration, and the environment (Sattelmacher et al., 1993). Studies on root morphology of perennial grasses from different habitats showed that the proportion of photosynthates invested into roots was positively associated with the habitat's N fertility (Boot and Mensink, 1990). The form of N (NH_4^+ or NO_3^-) absorbed by the root can also have a large influence on root growth (Robinson and Rorison, 1983). A number of investigations have shown that high levels of soil NH_4^+ inhibits root growth, especially root elongation. Possible explanations are that NH_4^+ induces deficiencies of other ions, causes depletion of sugars from NH_4^+ detoxification, or results in

low pH of the root rhizosphere (Sattelmacher et al., 1993). For maize, NH_4^+ nutrition promoted the initiation of higher order laterals but restricted the elongation of primary and nodal roots (Teyker and Hobbs, 1992). In contrast, NO_3^- -nutrition resulted in plants with a finer primary root system and with no restriction on root elongation.

The effects of nitrogen nutrition depend significantly on the environmental conditions such as pH, and the plant nutrient status (Sattelmacher et al., 1993). While the external pH affects the form of N that is absorbed, at the same time it is also affected by it. The uptake of NH_4^+ results in rhizosphere acidification, which can also result in poor root growth. Moreover, the changes in the rhizosphere pH alters the availability of other nutrient elements, which in turn affects root growth and morphology (Sattelmacher et al., 1993). Plant nutrient status is also important, as plants with higher K status are less susceptible to high levels of NH_4^+ because of the role that K plays in NH_4^+ detoxification.

The influence of mineral nutrition on changes in root morphology can be effectively demonstrated by altering the location of available nutrients. The most extensive development of roots in a soil occurs around areas with a high content of humus, or particles and bands of fertilizers (Wiersum, 1958; Sattelmacher et al., 1993). Drew et al., (1973) showed that lateral root growth of barley (*Hordeum vulgare*) was locally stimulated only when the root system was deprived of N. The extension of seminal axes was not influenced by N concentration in solution, but rather by the plant's N status. Barley plants grown in sand culture with localized zones of low and high levels of N had a greater number of lateral roots, and the extension of their branches was higher in the nutrient-rich zones (Drew, 1975).

Other nutrient elements like P and K, have also been shown to have a strong influence on root morphology (Hallmark and Barber, 1984). For maize, P deficiency resulted in roots with a smaller radius (Schenk and Barber, 1979), while semiarid grasses grown under low P increased their specific root length (ratio of root length to the root dry weight) (Christie and Moorby, 1975). The P supply has also been shown to stimulate the second-order branching in pea roots (Wiersum, 1957). Similarly, Drew and Saker (1978) observed an increase in the

growth of lateral roots of barley in response to a localized supply of P. The portion of the root system enriched with P had an increase in the number and extension of laterals, partially compensating for the rest of the root system which was deficient in P.

Under K deficiency, a delay in development of lateral roots has been observed in barley (Hackett, 1968), while under K fertilization root length of soybean is increased (Mengel and Foster, 1973). However, conflicting results have been reported by Hallmark and Barber (1984) and Rosolem et al. (1993) who observed that the effect of K on root length of soybean varies among cultivars. Hallmark and Barber (1984) found that P and K have a positive interaction on the secondary root radius of soybean, as addition of K decreased the root radius in a high P-soil while it was increased in a low-P soil.

Plant response to nutrient deficiency involves both morphological and physiological changes. Physiological changes such as the release of root exudates were discussed earlier. Soil nutrient concentration and composition affects the production of proteoid roots (Lamont, 1971), which are mainly formed under conditions where P is limiting (Gardner et al., 1982a; Marschner et al., 1987; Dinkelaker et al., 1989). The most accepted view is that proteoid root formation occurs in response to a low P status of the plant, rather than the P status of the soil (Louis et al., 1990); and that they are involved in rhizosphere modification by increasing citrate exudation and therefore P mobilization by roots (Gardner et al., 1983).

The development of root hairs is also strongly influenced by the nutrient supply, with examples published for N, K, and Ca (Tanaka and Woods, 1972, 1973; Bhat and Nye, 1974a, b; Föehse and Jungk, 1983). Root hair formation of oat plants is decreased under Ca deficiency, while the opposite phenomena occurs when K is deficient (Tanaka and Woods, 1972, 1973). The effect of Ca on root hairs is thought to be the result of the presence of other ions (Tanaka and Woods, 1972; Ewens and Leigh, 1985). For example, when Ca is lacking in the nutrient solution the decrease in root hair length is due to toxicity from other ions. It has also been shown that Ca is important for the differentiation of rhizodermal cells into trichoblasts (root hair forming cells) and atrichoblasts (hairless cells) (Jaunin, and Hofer

1988), and that Ca promotes the formation and growth of lateral roots (Poovaiah and Reddy, 1991).

Other studies with rape showed that length of the root hairs increases with a decrease in P concentration in the soil solution (Bhat and Nye, 1974a, b). Föehse and Jungk (1983) also reported a strong effect of P supply on the formation of root hairs in tomato, rape, and spinach. However, similar to proteoid roots, experiments with spinach grown in a split-root system have shown that P status of the plant has a more direct effect on root hair formation than does the P concentration of the nutrient solution. Thus, root hair formation must be regulated by mechanisms localized within the plant, and not by the external solution (Föehse and Jungk, 1983). Boot and Mensnik (1990) showed that grass species from infertile habitats respond to low N nutrition by increasing the length and density of root hairs. Other authors were not able to demonstrate a direct effect of P and K on root hair length in wheat, and concluded that it was the lack of these nutrients that actually stimulated root hair formation (Ewens and Leigh, 1985)

Soil pH

A slightly acidic soil pH (5.5-6.5) is generally considered optimal for root growth, with higher or lower values having an inhibitory effect (Taiz and Zeiger, 1991). High pH can directly inhibit root growth by affecting electropotential and pH gradients, and is also associated with ammonia toxicity. High pH (> 7.0) has been shown to decrease root hair length of wheat by effecting the H⁺ flux at the growing tip, which is necessary for maintaining gradients within the cell (Ewens and Leigh, 1985).

Low pH values (≤ 3.8) can also have an inhibitory effect on root development and root hairs as has been reported for *Deschampsia flexuosa* (L.) (Balsberg Pålsson, 1995). Root growth inhibition at low pH values is often associated with Al toxicity and high levels of heavy metals (Mn, Fe, Cd) (Marschner, 1995). Aluminum toxicity limits plant growth by inhibiting root elongation (Slaski, 1995). Al concentrations higher than 2.2 μM decrease root hair

development in white clover (Care, 1995). The difference in root hair development and Al tolerance between species has made possible the use of root hair measurement as a selection criteria. In order to overcome Al toxicity, a higher molar ratio of Ca to total cations is needed, which results in the practice of soil liming (Marschner, 1995).

When present in excessive amounts, heavy metals such as Cd and Cu have a negative effect on root growth and morphology (Arduini et al., 1994), particularly on root elongation (Breckle, 1991). Arduini et al, (1994) showed that Cd and Cu toxicity decreased tap root elongation, and lateral root length in two varieties of pine seedlings. Even though cell elongation and cell division were both affected, cell elongation was more sensitive to heavy metal toxicity (Breckle, 1991).

Aeration

Adequate soil aeration is essential for meeting the respiratory needs for both plant roots and soil microorganisms (Marschner, 1995). Although plants can usually obtain sufficient O₂ directly from the soil, under flooded conditions (especially under high temperatures) dissolved O₂ is rapidly depleted from the soil solution (Drew and Stolzy, 1991). Different species exhibit a wide range in sensitivity to low O₂ concentrations. Stems and roots of species adapted to anoxic conditions such as rice (*Oryza sativa*), have developed gas spaces known as aerenchyma for the internal diffusion of O₂ from leaves to roots. Rice roots also contain suberized walls in their exodermis, presumably to preserve O₂ from radial leakage (Clark and Harris, 1981). In contrast, for mesophytic (nonwetland) plants the internal transport of O₂ from the shoot is not an important source of O₂ for the roots, which absorb most of their O₂ from the rhizosphere (Saglio et al., 1984). In some mesophytic species such as maize, lowering the O₂ concentration from 21 to 10% did not effect respiration, but still inhibited root growth; indicating the involvement of other nonrespiratory oxidative processes in root elongation (Saglio et al., 1984). CO₂ can have a stimulatory effect on root growth at low

concentrations and an inhibitory effect at high concentration. Some species (*Agave deserti*, Norway spruce) are sensitive to CO₂ values as low as 0.5% (Marschner, 1995).

In poorly aerated soil, phytohormones such as ethylene play an important role in plant adaptation, as high ethylene levels (either diffused from the root or produced by microorganisms) are trapped within the soil (Drew et al., 1979). High levels of ethylene can also promote adventitious rooting and formation of cortical air spaces, serving as an adaptive mechanism to flooding.

Organic Solutes

Substances released from plants or plant roots which have a negative effect on plant growth are referred to as allelochemicals. Allelochemicals which inhibit growth of other plants are mainly phenolic substances (Vaughan and Ord, 1990). Phenolic acids such as vanillic, ferulic, and coumaric acids have been shown to inhibit growth of pea roots by affecting both root elongation and cell division (Vaughan and Ord, 1990). Although, their mode of action is still unclear, the negative effects are thought to be the result of an interaction with plant growth regulators such as IAA (Vaughan and Ord, 1990).

Microorganisms

Soil microorganisms can have either a positive effect (rhizobia, micorrhizae) or a negative effect (pathogens) on root growth (Bowen and Rovira, 1991). Pathogens (different genera of bacteria and fungi) can impair root functions (such as cytokinin production), or root growth, due to the production of toxins. Certain bacteria known as plant-growth-stimulating-bacteria (PGPB), can enhance root growth by producing phytohormones such as IAA and cytokinines, or by suppressing the inhibitory effect of soil pathogens (Kapulnik, 1991; Marschner, 1995). Mycorrhiza development occurs at the same time as root growth, and influences not only root anatomy and function, but also root morphology (Wilcox, 1991).

Roots of mycorrhiza-infected seedlings of *Trifolium parviflorum* had longer lateral roots and more branches than the uninfected ones (Wilcox, 1991).

Soil Physical Factors

Moisture

The supply of soil water is crucial for plant productivity in arid or semiarid areas where water is the major factor limiting plant growth (Taiz and Zeiger, 1991). A soil water deficit usually has a more drastic effect on shoot growth (inhibition of leaf elongation rate) than root growth, resulting in a lower shoot/root ratio (Marschner, 1995). Under drought conditions, roots tend to grow into the soil areas that still contain available moisture, and usually proliferate into deeper layers of soil depending on the carbohydrate supply from the shoots. The effects of water stress on root development are often combined with other stresses such as mechanical impedance (in dry soils), lack of aeration (in poorly aerated soils), and salinity (Marschner, 1995).

Mechanical impedance

Growing plant roots exert a pressure on the soil and the resistance of soil particles against deformation by growing roots is called mechanical impedance (Marschner, 1995). Thus, the process of root elongation depends on the extent to which root pressure exceeds mechanical impedance. Reductions in root length, increases in root thickening, and enhanced formation of lateral roots occurs when plants experience compacted soils and mechanical stress (Lachno, 1983). Experiments with pea showed that soil compaction delays the early stages of root development, reduces root proliferation, and decreases the diameter and length of the tap root (Dawkins et al., 1983). The mechanism by which plant roots response to mechanical impedance is not clear but likely involves plant hormones as impeded plants often evolve more ethylene (Dawkins et al., 1983). Exposure of maize seedlings to ethylene ($1 \mu\text{l L}^{-1}$) results in the same morphological changes in roots as those caused by mechanical impedance (Sarquis et

al., 1991). Similarly, the inhibition of ethylene synthesis (or action) by inhibitors that block ACC synthase, or ethylene receptor sites, partially reestablished the growth of impeded plants.

Morphological symptoms associated with impedance are not always a sign of inhibited nutrient uptake. Root systems often compensate for a decrease in root length by increasing their uptake rate per unit root length (Marschner, 1995). However, this approach can cause a rapid depletion of the soil nutrients around the roots and may make plants more susceptible to nutrient deficiencies or water stress (Bennie, 1991).

Temperature

The optimum temperature for root growth is around 20° to 25°C with minimum (about 5°C) and maximum ranges (around 40°C) that vary according to plant species (Bowen, 1991). Even though roots are subject to less fluctuations in temperature than shoots, temperature is still an important environmental influence on their growth. The metabolic rate of root tissues, as well as the uptake of nutrients and water, are all affected by soil temperature (Klepper, 1991).

Soil temperature especially affects root length, which has a direct impact on the accumulation of ions acquired by diffusion such as P. A longer length of corn roots under optimum temperature (25°C) was associated with greater uptake of soil P than under cool temperature (18°C) (Mackay and Barber, 1984). Other experiments showed that root growth of pea, radish (*Raphanus sativus*) and spinach seedlings was decreased by soil temperatures of 10°C or lower. For warm season crops, such as maize, eggplant (*Solanum melongena*), cucumber (*Cucumis sativus*), and watermelon (*Citrullus vulgaris*), root growth was decreased at 12.3°C and 14.5°C (Wilcox and Pfeiffer, 1990). A decrease in water uptake was also observed at these cool soil temperatures due to a decrease in the root growth (Wilcox and Pfeiffer, 1990). The number of lateral roots also increases with higher soil temperatures (Bowen, 1991). Since lateral roots are part of and influenced by the whole root system,

changes in temperature affect roots directly as well as indirectly by altering the shoot growth, which then in turn affects the roots.

Another role of soil temperature on root function is the effect on the symbiotic relationships with the root and microorganisms such as mycorrhizae and nitrogen fixing bacteria. This role is especially important in those areas where mycorrhizae are ubiquitous, and for those plants that are largely dependent on symbiosis to acquire nutrients like N and P (Bowen, 1991). For onion grown at low temperatures, the rates of infection by endophyte mycorrhizae as well as plant growth decreased compared to the rates at high temperature (Krikun, 1991).

ROOT GROWTH AND PLANT PRODUCTIVITY

The importance of root systems to plant productivity lies not only in their role in water and nutrient uptake, phytohormone production, or anchorage, but also in their control of the size and growth of the shoot (Leskovar and Stoffella, 1995). Root development, either when it is optimal or under stress, is manifested in the shoot's development and ultimately in plant yield. Since many plants are subject to abiotic stresses and nutrient limitations (uneven distribution of resources and localized depletion), soil exploration for nutrient and water resources is of primary importance for plant productivity (Lynch, 1995). Changes in root architecture primarily determines the ability of the root system to explore the soil and to respond to localized changes in supply (Lynch, 1995).

Root distribution varies with species, plant age, soil characteristics, and plant competition (Brown and Scott, 1984). Root competition for water and nutrients within plants of the same species (intraspecific) or different species (interspecific) can have a large effect on crop growth, especially if it occurs during the reproductive stage. Field experiments with soybean showed that when roots are close together the volume of soil explored is limited and roots develop more primary branches under the row and in the furrow (Raper and Barber,

1970). Maximum yields are generally associated with uniformly distributed plants where the competition for water and nutrients between roots is minimal (Brown and Scott, 1984).

The relationship between roots and shoots is a functional equilibrium which means that changes in root growth are transmitted to and effect the shoot (Brown and Scott, 1984). Sanders and Brown (1976) used a grafting technique to study the effect of variations in shoot:root ratio on the growth of soybean and reported an increase in growth, nutrient uptake, and yield when the number of roots was increased from one to three relative to the number of shoots (decreasing the shoot:root ratio). Other work with different common bean genotypes showed a nutrient-efficient genotype had a highly developed root system, while a nutrient-inefficient one did not (Lynch and Van Beem, 1993). Differences in root systems are often reflected in shoot architecture where the more vigorous and branched the root system the more developed and branched the shoot system.

For soybean, an association between stages of root development and shoot growth was observed by Hoogenboom et al. (1987). Roots develop rapidly during vegetative stage, branch extensively during reproductive growth, and cease growth during seed maturation. For wheat, there are indications that development of root axes occurs in a predictable manner with regard to shoot development, such that the number of axes relates to the number of culms at maturity (Klepper et al., 1984).

The equilibrium between shoots and roots can be easily demonstrated by examinations of biological stresses and their effects on root growth. An environmental or biotic stress on the root system, depending on the severity and duration, has a strong influence on shoot development, and on whether or not the plant achieves its potential yield (Brown and Scott, 1984). Experiments with common bean have shown that drought resistance is related to the depth of rooting (White et al., 1990), likely because a longer and more extensive the root system is better able to extract water and thereby overcome the negative effects of water stress (Huck et al., 1986). Robertson et al. (1980), showed that limited rooting of maize plants under water stress was reflected in low grain yields. Other work showed that differences in

dry matter of irrigated tomato was related to changes in root weight and their distribution (Bar-Yosef et al., 1980).

In conclusion, the distribution, growth rate, and longevity of roots is related to, and largely determines shoot growth and plant yield (Brown and Scott, 1984). Since, root growth varies with soil properties, genetic properties of the plant, and the climate, it is difficult to definitively relate root parameters with plant productivity. However, situations where roots are subject to various environmental or biological stresses have demonstrated the importance of the functional equilibrium between roots and shoots in the determination of final yield. Maximizing yield potential largely depends on removing or minimizing stress on the roots.

RATIONALE AND OBJECTIVES OF RESEARCH PROJECT

Based on the previous review one of the most important functions of roots is their active role in nutrient acquisition. Plants can respond to changes in nutrient supply by altering the size and architecture of their root systems, by developing new root branches, by increasing root length or decreasing root diameter, or by forming root hairs (Bhat and Nye, 1974a, b; Wiersum, 1957; Christie and Moorby, 1975; Schenk and Barber, 1979). Roots can also change the availability of mineral nutrients in the rhizosphere by several means including: (1) altering the rhizosphere pH, (2) changing their reducing capacity or, (3) by releasing exudates (Fitter, 1991; Van Noordwijk et al, 1994; Marschner, 1995; Marschner and Romheld, 1996).

The distribution, growth rate, and surface area of the root determines nutrient uptake, which has a large impact on plant yield. Therefore, finding ways to alter root morphology which results in greater surface area should also have a positive impact on plant productivity. Humic and folic acid containing biostimulants such as Enersol and Ergostim have been shown to increase seedling emergence and enhance root and plant growth, especially under stress conditions (Sanders et al., 1990). Similarly, compounds like ACA[®] (zinc ammonia acetate)

and Asset[®] (manganese ammonium acetate) have also been reported to enhance root growth (Liu et al., 1996). However, while the number of compounds that appear to act as nutrient absorption enhancers has been increasing, in most cases their mode(s) of action have not been fully elucidated.

Our preliminary experiments with a new polymer known as polyaspartic acid (PA), have shown that in some cases it can prevent limitations in plant growth caused by a low supply of mineral nutrients. Experiments varying the macronutrient level and the PA concentration imply that PA-treated roots occupy more surface area (Wang et al., 1996). While our preliminary field trials have given mixed results, we believe this problem could be due to a lack of understanding regarding the physiological basis for PA-induced enhancement in nutrient use and plant growth. Therefore, the objectives of this research were to investigate the effects of PA on root morphology and assess the role of other nutrients, especially iron, in the growth response of plants to the addition of PA.

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Chapter 2. Source of Iron Affects Enhancement in Nutrient Use and Plant Growth by Polyaspartic Acid.

ABSTRACT

The synthetic protein polyaspartic acid (PA) has been shown to remove limitations in plant growth caused by an inadequate nutrient supply. However, the physiological basis for plant response to PA is not fully understood, and could be related to the use and source of iron. To investigate this possibility, two plant species that differ in iron acquisition [spring wheat (*Triticum durum* Desf. cv. Inbar) and soybean (*Glycine max* L. Merr. cv. Bell)] were grown hydroponically with three different iron sources (Fe^{2+} , Fe^{3+} and Fe-chelate), in nutrient solutions containing a normal (1x) or a low (0.1x) level of macronutrients with or without PA. A separate experiment used wheat to evaluate the effects of PA addition to a low-nutrient solution containing varying concentrations (0.5; 2; 4; and 6 mg L⁻¹) of Fe^{2+} -iron. Changes in plant growth and root morphological parameters such as root length, and the number and length of lateral root branches were measured at two times during an 18 d experimental period. Nutrient level had the greatest effect on plant growth for both species when the Fe-source was Fe^{2+} , and when PA was not included in the nutrient solution. For both species, the addition of PA to Fe^{2+} -plants grown with low-nutrients increased plant dry weight to levels similar to Fe^{2+} -plants grown with normal nutrients. Nutrient level and PA enhancement were confined to soybean when the Fe-source was Fe^{3+} , while there was no effect of PA addition or nutrient level on growth of either species when iron was supplied as Fe-chelate. PA addition had no effect on root morphology of either species when the iron source was Fe-chelate, but increased it to levels similar to chelate-plants when iron was supplied as Fe^{2+} or Fe^{3+} . The presence of PA increased nutrient accumulation in the shoots of both species grown with Fe^{2+} or Fe^{3+} , making them similar to levels contained in plants grown with Fe-chelate. PA addition had no effect on the nutrient accumulation by Fe-chelate-grown plants. When wheat plants were

grown with low nutrients, increasing the concentration of Fe^{2+} to excess levels (greater than 2 mg L^{-1}) decreased the dry weight of roots and shoots, the length and number of primary root branches, and the concentration of phosphorus in the shoot, and these effects were ameliorated by the addition of PA. The data shows that changes in root morphology and increases in plant growth and nutrient accumulation induced by PA are influenced by the iron source, but not by the mechanism used by different plant species to acquire iron. Both PA and Fe-chelate appear to act as nutrient absorption enhancers by inducing changes in root morphology that result in increased nutrient uptake, and ultimately in better plant growth.

INTRODUCTION

Providing an adequate supply of the essential minerals needed by the crop is crucial for optimal plant growth and yield. Since roots are the site of nutrient uptake, their distribution, growth rate, and surface area, should have a large impact on nutrient accumulation and crop yield. Morphological characteristics such as length and diameter of primary roots, and the length and number of branches determines the root surface area, and as such plays an important role in the acquisition of mineral nutrients (Barber, 1992). When root growth is impeded, nutrient uptake, and as a result shoot growth, is limited which can be reflected in low grain yields (Robertson et al. 1980). Therefore, technologies which increase the uptake and accumulation of nutrients, or that improve the efficiency of nutrient use, could benefit crop growth and yield; and are becoming a new trend in modern crop production (Wani et al., 1990).

Several products such as plant growth regulators, soil conditioners, and biostimulants have been used to enhance nutrient availability by a variety of means (Kinnersley et al., 1990; Chang et al., 1996). Humic substances have been shown to enhance plant growth by inducing changes in root morphology that have a positive effect on the uptake of nutrients (Chen and Aviad, 1990; Van De Venter et al., 1991; David et al., 1994). Humic and folic acid containing

biostimulants such as Enersol and Ergostim have also been shown to increase seedling emergence, and to enhance root development, especially under conditions of stress (Sanders et al., 1990). Similarly, additives like ACA (zinc ammonia acetate) have been reported to enhance root growth in maize, which in some cases is associated with higher yields (Liu, et al., 1996). Other products purported to enhance the availability of mineral nutrients are available commercially, but in many cases their chemistries are not known, and/or their mode(s) of action have not been fully elucidated (Kelling and Schulte, 1991).

Our previous experiments with hydroponically grown wheat and tomato showed that the synthetic thermal protein polyaspartic acid ((copoly-[(3-carboxypropanamide) (2-carboxymethylacetamide)]), sodium salt; Amilar International, Bedford Park, IL), hereafter abbreviated as PA, prevents limitations in plant growth caused by low nutrient supply, presumably by enhancing the availability of mineral nutrients (Wang et al., 1996). Plants grown with low nutrients in the presence of PA exhibited enhanced mineral accumulation and more growth than untreated plants, with the magnitude of response being greater for the dicot species (tomato) than monocots. Although the physiological basis for PA-induced increases in nutrient use are not clear, experiments varying nutrient level and PA concentration have suggested that changes in root morphology may be involved (Zeko et al., 1996). The growth response from PA also seems to be related to the source of iron, as our preliminary work has shown less growth enhancement from PA when plants were grown with Fe-chelate than with Fe^{2+} as the iron source (unpublished data).

The main forms of iron commonly used in solution culture are chelates (especially ferric-chelates), FeSO_4 (Fe^{2+}), and ferric salts (Fe^{3+}). Ferric-chelates are organic compounds and are widely used as an iron source because of their ability to complex iron and keep it from becoming unavailable (Papastyliou, 1990). Reduction of Fe^{3+} to Fe^{2+} is a prerequisite to iron uptake from ferric-chelates (Romheld and Marschner, 1983; Holden et al., 1991), which can occur via the release of reducing substances, or by enzymatic reduction (Chaney, 1972; Brown and Ambler, 1973; Olsen et al., 1981; Bienfait et al., 1982). Either case results in a weaker

form of chelated iron (ferrous chelate) which can readily release Fe^{2+} for plant uptake (Bienfait, 1988). While ferric salts are sparingly soluble, and therefore do not provide the necessary free Fe^{3+} concentration for plant growth (Lindsay and Schwab, 1982), the Fe^{2+} ion is readily taken up by plant roots (Crawford, 1982). The main problem with using Fe^{2+} as an iron source in nutrient solution is its rapid oxidation to Fe^{3+} (especially at high pH's) which makes it less available for plant uptake (Hageman et al., 1961).

For a number of reasons, including: 1) low solubility of ferric salts, 2) unavailability of Fe^{2+} at high pH's and, 3) the need for Fe-chelate reduction prior to uptake, plant species have developed at least two different mechanisms by which they acquire iron under conditions of low availability (Romheld and Marschner, 1986; Marschner et al., 1986). Most dicots and nongramineous monocots exhibit a mechanism known as Strategy I (Marschner and Romheld, 1994), which is characterized by enhanced reduction of Fe^{3+} via a plasmalemma-bound reductase, a lowering of the rhizosphere pH, and in some cases, the release by roots of reductants or chelates (Olsen et al., 1981; Masaoka et al, 1993). The importance of each aspect of the Strategy I mechanism varies both across plant species, as well as within genotypes of the same species (Marschner and Romheld, 1994). In contrast to dicots, grass species exhibit what is known as Strategy II, which is characterized by root secretion of iron-solubilizing compounds called phytosiderophores, that form chelates with Fe^{3+} , and prior to absorption are taken up by a high affinity transport system on the plasma membrane of the roots (Takagi, 1976; Romheld and Marschner, 1986; Awad et al., 1994; Marschner and Romheld, 1994).

Based on this review, the activity of PA as a nutrient absorption enhancer, may be related to the source and /or form of available iron, and possibly by the iron acquisition strategy used by a particular plant species. Therefore, the objectives of this study were to: 1) determine the effect of iron source on plant growth and root morphology, 2) verify that PA acts as a nutrient absorption enhancer and, 3) determine the role of iron in PA-induced enhancement in nutrient use and plant growth. To accomplish these goals, plants of two species which differ in their mechanism for iron uptake (wheat, soybean) were grown with variable iron sources

(Fe²⁺, Fe³⁺, Fe-chelate) in nutrient solutions containing a low or normal level of nutrients, both with or without PA.

MATERIALS AND METHODS

Plant Materials, Treatments, and Growth Conditions

Two approaches were taken to investigate the effects of nutrient level and iron on PA-induced changes in plant growth and root morphology. Plants were grown hydroponically to allow for the precise control of nutrient level (including iron form) and to facilitate the quantitative collection of root samples. One experiment evaluated the response of two different plant species, spring wheat (*Triticum durum* Desf. cv. Inbar) and soybean (*Glycine max* L. Merr. cv. Bell) to PA addition when grown with three different iron sources. These species were selected to represent a monocot and a dicot, which have inherently different root systems, and which acquire iron by different mechanisms. Iron sources used were FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate (sodium ferric diethylene-triamine penta-acetate) to supply iron at a rate of 1 mg L⁻¹ for wheat, and 2 mg L⁻¹ for soybean. Both species were grown for up to 18 DAP (days after planting) in nutrient solutions containing a normal (1x) and a low (0.1x) level of macronutrients, with or without 50 mg L⁻¹ of PA. The concentration of macronutrients in the normal (1x) and the low (0.1x) nutrient solutions are shown in Table 1. Only the concentration of macronutrients was varied, while the supply of micronutrients was kept at the level considered optimal for plant growth. Treatments were arranged as a 3 x 2 x 2 factorial in a randomized complete block design with five replications.

Another experiment used wheat to examine the effect of Fe-level and PA addition on plant growth, root morphology, and P status. Treatments consisted of plants grown with a low level of nutrients and varying concentrations (0.5; 2; 4; and 6 mg L⁻¹) of Fe²⁺-iron (supplied as FeSO₄), with or without PA, and plants grown with normal nutrients without PA, which served as the control. Treatments were arranged in a randomized complete block design

Table 1. The concentration of macronutrient ions in the normal and low strength nutrient solutions used to grow seedlings of spring wheat and soybean in hydroponics. The normal level (1x) was considered to supply the optimal level of nutrients, while the low level (0.1x) was one that would result in suboptimal growth. Nitrogen was supplied as a mixture of NO_3 and NH_4 for wheat and as urea for soybean.

Nutrient element	Level of nutrient in solution			
	Wheat		Soybean	
	Low (0.1x)	Normal (1x)	Low (0.1x)	Normal (1x)
	-----mg L ⁻¹ -----			
Nitrogen (N)	4	42	3	28
Phosphorus (P)	1	10	1	8
Potassium (K)	4	40	17	166
Calcium (Ca)	3	33	12	120
Magnesium (Mg)	1	13	7	73
Sulfur (S)	5	47	16	160

with five replications, and plants were grown for 16 DAP.

Both experiments were conducted in the greenhouse, where temperatures were maintained as close as possible to 24° C during the light cycle, and 18° C during the dark cycle. Natural lighting was supplemented with metal halide lamps with a photosynthetic photon flux density of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the crop canopy to provide 14 hr of illumination. Under these conditions, color and morphological development of plants were similar to those grown in the field. An experimental unit consisted of an individual culture vessel (a one liter glass container covered with aluminum foil for wheat, and a two liter black plastic container for soybean), containing the respective plant species (one plant per container) and the iron/nutrient/PA treatment.

Wheat seeds were germinated in the lids of the hydroponic culture vessels using the wick and collar technique previously described by Gentry et al., (1989). For germination of soybean seeds, a metal rack was covered with tissue paper overlaid with a thin layer of vermiculite. The seeds were placed on top of this layering and covered with another thin layer of vermiculite. The rack was then placed in a 14 L plastic tray filled with germination solution (1 mM CaCl_2) up to the level of the metal rack. The moisture level of the seed was controlled by adjusting the solution level in the tray. These respective germination techniques allowed for unrestricted emergence of the new seedling and undisturbed growth of the emergent radicle. The pH of the solution during germination was monitored daily and maintained at 5.7 by appropriate titrations with diluted acid or base; and the vessels were aerated. When the majority of the radicals penetrated the solution surface (approximately 7 days after germination DAG), plants of both species were transplanted into culture vessels containing nutrient solution.

The nutrient solution for wheat supplied N as a 50:50 mixture of NO_3 and NH_4 , while for soybean N was supplied as urea. The N mixture was used for wheat because cereal plants exhibit better overall growth when N is supplied as a mixture of NO_3 and NH_4 (Wang and Below, 1992), and because simultaneous use of the two forms by the plant helps to maintain a

constant solution pH. Urea was selected as the N source for soybean in order to have the least negative impact on nodulation. Nutrient solutions were completely renewed at regular intervals (two times for wheat and three times for soybean in the course of the experiment). The solutions were aerated and the pH was maintained at 5.5-5.6 for wheat and 5.7-5.8 for soybean by including 0.5 mM (2-[N-Morpholino] ethanesulfonic acid) MES in the nutrient solution (Bugbee and Salisbury, 1985), and by daily titrations with dilute acid or base.

Sampling and Analysis

For the experiment investigating the role of iron source, plant parts were harvested and root morphology measurements were taken at two different days; at 14 and 18 DAP for wheat, and at 13 and 18 DAP for soybean. For the experiment examining the effect of Fe-level and PA addition, wheat plants were harvested for root measurements and dry weight determinations were taken at 16 DAP. For both experiments, the lengths of primary and secondary roots were measured using a PC-based Microcomp Integrated Image Analysis System (Southern MicroInstruments, Inc. Atlanta, GA) with an Optronics VI-470 camera. Roots of each plant were spread out in a tray containing a black background and filled with water, and the number of primary and secondary root branches were counted. After determination of root morphology, plants were divided into roots and shoots and the tissues dried in a forced draft oven at 80°C for a minimum of 48 hr prior to weighing to determine dry weight. The tissue nutrient concentration in 18 days old shoots was analyzed for P, K, Ca, Mg, B, Cu, Fe, Mn, Zn by a commercial laboratory (Spectrum Analytic Inc.) using the Direct Coupling Plasma technique. Unfortunately, the small amount of tissue available at 18 DAP precluded the analysis of N and S in shoots, and of all elements in the roots. For the experiment where the level of Fe²⁺ was varied, a 100 mg sample of dried, ground tissue from each plant part was digested as previously described by Heberer et al., (1985). Aliquots from the digest were analyzed for P according to Chen et al., (1956).

For the experiment evaluating iron sources, data for all parameters were subjected to

analysis of variance procedures to identify significant effects for nutrient level, Fe source, and PA treatment, and the appropriate two-way and three-way interactions. With minor exceptions, the main effects of nutrient level, iron source, and PA treatment were significant for each measured parameter at at least the 10% probability level for both species at both sampling dates (data not shown). The only main-effect exception noted at both dates was the length of soybean taproots for nutrient level. Similarly, except for seminal root and primary root number of wheat, and taproot length, primary root length, and primary branch number of soybean, the two-way interactions were also significant for all parameters at at least one sampling date. For wheat, the three-way interactions (nutrient level x iron source x PA treatment) were significant at at least one sampling date for all parameters, while for soybean this interaction was significant only for taproot length and secondary branch number (data not shown). In order to compare individual means, an LSD ($P \leq 0.05$) was calculated from the overall error term which can be used to compare any two numbers. Data from the experiment evaluating iron level was also subjected to analysis of variance and an LSD ($P \leq 0.05$) calculated to compare different treatment means within the same Fe^{2+} level, or Fe^{2+} levels within the same nutrient treatment.

RESULTS

Effect of Iron Source

Plant growth

At both sampling dates, wheat plants grown with the normal nutrient level had higher plant dry weights (29 and 76% for 14 and 18 DAP, respectively) than those grown with low nutrients when the iron source was Fe^{2+} , and when PA was not present (Table 2). With Fe^{2+} as the iron source, PA addition increased plant dry weight to levels similar to those observed for plants grown with normal nutrients. In contrast to Fe^{2+} , plant dry weight was not affected by the nutrient level when the iron source was Fe^{3+} or Fe-chelate (Table 2). Similarly, the

Table 2. Effect of iron source, nutrient level, and PA treatment on changes in whole plant dry weight for hydroponically grown wheat and soybean. Plants were grown with a low (0.1x) or a normal (1x) level of macronutrients either with or without 50 mg L⁻¹ of PA. Iron was supplied as FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate at a rate of 1 mg L⁻¹ for wheat and 2 mg L⁻¹ for soybean.

Iron source	Nutrient level	Wheat				Soybean			
		Days after planting							
		14		18		13		18	
		Polyaspartic acid							
		w/o	w	w/o	w	w/o	w	w/o	w
-----mg plant ⁻¹ -----									
Fe ²⁺	Low	81	94	132	215	224	259	410	551
	Normal	105	100	233	240	252	264	514	650
Fe ³⁺	Low	105	102	195	206	217	247	389	422
	Normal	103	90	178	225	252	273	430	606
Fe-chelate	Low	100	92	202	182	254	256	486	527
	Normal	112	106	238	261	272	259	646	648
LSD _(0.05) †		16		33		25		70	

† LSD values can be used to compare any two numbers within a species and sampling time.

addition of PA only enhanced the dry weight of 18 DAP Fe³⁺-plants grown with normal nutrients, and had no effect on dry weight of Fe-chelate grown plants at either sampling date.

For soybean plants grown without PA, differences in plant dry weight due to nutrient supply were observed at both sampling dates when plants were grown with either Fe²⁺- or Fe³⁺-iron, and only at 18 DAP when they were grown with the Fe-chelate (Table 2). For Fe²⁺- and Fe³⁺-grown plants, PA addition increased plant dry weight under low nutrients at both days, and under normal nutrients at 18 DAP. At both sampling dates, there was no effect of PA on plant dry weight when plants were grown with Fe-chelate.

Root growth and morphology

For wheat plants, there were no differences in dry weight of the roots due to nutrient supply when plants were grown with any of the iron sources (Table 3). Addition of PA increased root dry weight of Fe²⁺-grown plants with low nutrients only at 18DAP, and had no effect on the root dry weight of the plants grown with either Fe³⁺ or Fe-chelate (except at 18DAP for Fe-chelate-grown plants with low nutrients). For wheat plants grown with Fe²⁺, nutrient-induced changes in root morphology were observed at both 14 and 18 DAP (Table 3), and were absent when Fe-chelate was the iron source. The addition of PA increased the length of seminal roots (100%) and the length and number of primary root branches (85 and 56%, respectively) at 14 DAP for low-nutrient plants grown with Fe²⁺ (Table 3). At the later sampling date (18 DAP), PA effects on root morphology (the length of seminal roots and primary branches) were also observed for Fe²⁺-plants receiving normal nutrients. For Fe³⁺-grown plants, PA-induced changes in root morphology were almost absent at 14 DAP (except for the length of primary branches on low-nutrient plants), but started to appear at 18 DAP (Table 3). For both sampling dates, PA had no effect on root morphology when the iron source was Fe-chelate.

Similar to wheat there were no differences in root dry weight of soybean due to nutrient level for any of the iron sources (Table 4). Addition of PA resulted in an increase in root dry

Table 3. Effect of iron source, nutrient level, and PA treatment on changes in root growth and root morphology for hydroponically grown wheat. Plants were grown with a low (0.1x) or a normal (1x) level of macronutrients either with or without 50 mg L⁻¹ of PA. Iron was supplied as FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate at a rate of 1 mg L⁻¹.

Iron source	Nutrient level	Root length							
		Root dry weight		Polyaspartic acid				Number of primary	
				Seminal		Primary branches			
		w/o	w	w/o	w	w/o	w	w/o	w
		--mg plant ⁻¹ --		-----cm-----				-----plant ⁻¹ ----	
		<u>14 days after planting</u>							
Fe ²⁺	Low	26	26	13	26	2.0	3.7	98	153
	Normal	30	30	28	30	5.6	5.1	150	156
Fe ³⁺	Low	30	29	26	29	2.2	4.0	160	140
	Normal	29	27	23	27	2.3	3.0	170	160
Fe-chelate	Low	27	25	28	25	4.6	3.5	135	125
	Normal	30	27	29	30	3.7	3.4	168	177
LSD _(0.05) †		ns		4		0.9		36	
		<u>18 days after planting</u>							
Fe ²⁺	Low	49	63	15	41	4.2	5.8	125	281
	Normal	58	65	39	43	6.4	7.9	196	218
Fe ³⁺	Low	54	57	34	38	4.9	4.9	193	244
	Normal	40	43	26	36	2.5	4.0	215	193
Fe-chelate	Low	58	47	40	37	7.5	6.9	224	205
	Normal	46	55	40	44	5.3	6.1	180	178
LSD _(0.05) †		9.8		4		1.5		44	

† LSD values can be used to compare any two numbers.

Table 4. Effect of iron source, nutrient level, and PA treatment on changes in root growth and root morphology for hydroponically grown soybean. Plants were grown with a low (0.1x) or a normal (1x) level of macronutrients either with or without 50 mg L⁻¹ of PA. Iron was supplied as FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate at a rate of 2 mg L⁻¹.

Iron source	Nutrient level	Root length						Number of branches					
		Root dry weight		Tap root		Primary branches		Primary		Secondary			
				Polyaspartic acid									
		w/o	w	w/o	w	w/o	w	w/o	w	w/o	w		
		-mgplant ⁻¹ -		-----cm-----						-----plant ⁻¹ -----			
<u>13 days after planting</u>													
Fe ²⁺	Low	50	76	21	45	4	12	42	178	0	313		
	Normal	49	66	23	45	5	13	47	153	12	282		
Fe ³⁺	Low	48	70	21	43	5	12	37	117	0	264		
	Normal	50	72	21	45	6	14	35	114	0	229		
Fe-chelate	Low	78	75	49	47	13	14	142	140	294	295		
	Normal	69	65	46	45	14	12	121	127	188	184		
LSD _(0.05) †		9.2		2.4		1.6		27.5		72			
<u>18 days after planting</u>													
Fe ²⁺	Low	105	179	23	75	4	19	48	259	0	874		
	Normal	126	177	26	72	6	23	60	241	56	664		
Fe ³⁺	Low	96	115	21	68	5	16	32	181	0	427		
	Normal	103	162	22	68	5	19	41	185	24	597		
Fe-chelate	Low	162	182	77	76	19	2	254	239	605	696		
	Normal	182	181	71	76	20	21	245	230	735	543		
LSD _(0.05) †		25.5		4.1		3.2		22		105			

† LSD values can be used to compare any two numbers.

weight when the iron source was Fe^{2+} or Fe^{3+} at both sampling dates, and with both nutrient levels (except for Fe^{3+} -grown plants with low nutrient level at 18 DAP). When the iron source was Fe-chelate, there were no effects of PA on the dry weight of soybean roots. When PA was not present, there were no changes in root morphology of soybean due to nutrient supply (except for the number of secondary branches when grown with Fe-chelate) for any of the iron sources (Table 4). For low- or normal-nutrient plants receiving iron as either Fe^{2+} or Fe^{3+} , the addition of PA increased the length of the tap root, the length and number of primary root branches, and the number of secondary branches at both sampling dates. Similar to wheat, PA addition had no effect on root morphology of soybean when plants received Fe-chelate as their iron source. For both wheat and soybean, the addition of PA induced changes in root morphology of Fe^{2+} - and Fe^{3+} -grown plants that resulted in their being similar to when the Fe-chelate was present (Tables 3 and 4).

Nutrient use

Wheat plants grown with normal nutrients exhibited greater accumulation of all macronutrients, and some micronutrients (Cu, Mn) than plants grown with low nutrients, when iron source was Fe^{2+} , and when PA was not present (Table 5). The addition of PA increased nutrient accumulation in the shoot (except for B, and Fe) of low-nutrient plants, but not of those grown with normal nutrients (except for P). When the iron source was Fe^{3+} , increases in nutrient accumulation due to nutrient level were observed only for P, Mg, B, and Zn (Table 5). The addition of PA increased the accumulation of Mg, B, Mn, and Zn in Fe^{3+} plants, grown with low nutrients, and of K and Ca when grown with normal nutrients. Wheat plants grown with Fe-chelate without PA exhibited nutrient-induced increases in the shoot content of all the elements except for B, and Mn (Table 5). In contrast, when PA was present there were few increases in the shoot nutrient accumulation.

Soybean plants grown with Fe^{2+} and normal nutrients had higher content of all macronutrients, and of Cu and B, in their shoots than did low-nutrient plants (Table 6). The

Table 5. Effect of iron source, nutrient level, and PA treatment on nutrient accumulation in the shoots of 18 days old hydroponically grown wheat. Plants were grown with a low (0.1x) or a normal (1x) level of macronutrients either with or without 50 mg L⁻¹ of PA. Iron was supplied as FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate at a rate of 1 mg L⁻¹.

		Macronutrients						Micronutrients											
		P	K	Ca	Mg	B	Cu	Fe	Mn	Zn									
Iron source	Nutrient level	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w		
		-----mg/shoot-----						-----µg/shoot-----											
Polyaspartic acid																			
Fe ²⁺	Low	0.7	2.6	3.4	7.7	0.4	0.7	0.2	0.3	2.1	2.4	1.6	2.3	41	26	11	36	12	24
	Normal	3.1	3.8	10	10	0.8	0.7	0.4	0.3	2.4	1.9	3.4	3.1	25	28	24	21	20	17
Fe ³⁺	Low	2.1	2.3	8.0	7.6	0.5	0.5	0.2	0.4	1.8	2.3	3.2	3.9	23	22	31	42	34	53
	Normal	3.4	3.6	7.8	9.8	0.6	0.8	0.6	0.5	2.4	2.5	3.8	3.9	20	33	33	36	57	45
Fe-chelate	Low	2.4	2.2	8.1	7.0	0.5	0.4	0.2	0.4	1.8	1.5	2.7	3.6	21	18	34	38	28	33
	Normal	3.9	4.4	11	12	0.8	0.7	0.5	0.5	2.2	2.7	4.8	4.4	27	28	36	39	42	32
LSD _(0.05) †		0.6		1.7		0.2		0.1		0.5		0.7		6.1		7.8		9.7	

† LSD values can be used to compare any two numbers.

Table 6. Effect of iron source, nutrient level, and PA treatment on nutrient accumulation in the shoots of 18 day old hydroponically grown soybean. Plants were grown with a low (0.1x) or a normal (1x) level of macronutrients either with or without 50 mg L⁻¹ of PA. Iron was supplied as FeSO₄ (Fe²⁺), FeCl₃ (Fe³⁺), or Fe-chelate at a rate of 2 mg L⁻¹.

Iron source	Nutrient level	Macronutrients						Micronutrients											
		P	K	Ca	Mg	B	Cu	Fe	Mn	Zn	Fe	Mn	Zn						
		w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w	w/o	w		
		-----mg/shoot-----																	
		Polyaspartic acid																	
		-----µg/shoot-----																	
Fe ²⁺	Low	1.0	1.0	6.6	11	0.7	2.9	0.9	1.9	14	21	4.1	4.8	51	119	31	138	34	129
	Normal	3.3	5.7	12	19	2.1	5.5	1.6	2.6	19	30	5.9	7.6	41	82	29	135	54	175
Fe ³⁺	Low	0.9	0.9	7.0	8.8	0.9	3.2	1.1	3.1	16	22	4.9	5.8	32	82	42	164	43	182
	Normal	3.3	5.5	11	16	1.8	5.1	1.6	2.7	19	31	5.9	9.2	31	162	22	199	49	202
Fe-chelate	Low	0.9	1.0	9.8	11	3.1	3.6	1.7	1.8	22	22	3.0	2.9	64	54	111	154	93	53
	Normal	5.5	5.3	19	20	4.9	4.7	2.2	2.2	27	26	5.1	3.9	61	66	48	53	72	79
LSD _(0.05) †		0.5		2.6		0.5		0.4		3.4		1.2		21		50		21	

† LSD values can be used to compare any two numbers.

addition of PA generally increased the accumulation of all nutrients, regardless of whether plants were grown with low or normal nutrients. When the iron source was Fe^{3+} normal nutrient plants had higher shoot content of all macronutrients, but no micronutrients, compared to low-nutrient plants. When PA was present, these plants exhibited an increase in the shoot accumulation for most nutrients when grown with low-nutrients (except P, K, Cu), and for all nutrients when grown with normal nutrient supply. When plants were supplied with Fe-chelate, the shoot nutrient content was generally increased with the normal nutrient level but there was no increase in nutrient accumulation due to PA (except for Ca) (Table 6).

Effect of Iron Level

When the level of Fe^{2+} - iron was varied from low (0.5 mg L^{-1}) to high (6 mg L^{-1}), the dry weight of roots and shoots decreased when wheat plants were grown with low nutrients and no PA (Fig. 1). Dry weight of both roots and shoots of control plants (those grown with normal nutrients without PA) was higher than those receiving low-nutrients without PA. For control plants, Fe^{2+} level had a negative effect on shoot growth (decreased when varied from low to 4 mg L^{-1}) but had no effect on the growth of the roots (Fig. 1). When the Fe^{2+} level was increased from low to high a visible coating of brown-reddish color developed on the roots of low-nutrient plants grown without PA (data not shown). This color was absent when PA was added to the solution. Addition of PA to the low-nutrient solutions also resulted in heavier dry weights for both roots and shoots at every concentration of Fe^{2+} , and for the most part resulted in plant part dry weights which were statistically similar to the control plants (Fig. 1). For low-nutrient plants receiving PA, increasing the Fe-level from low to high slightly decreased the dry weight of shoots, but had no effect on the dry weight of roots (Fig. 1).

For wheat plants grown with the low nutrient supply, there was a significant decrease in the length and number of primary root branches when the Fe^{2+} concentration was increased from low to high level, and when PA was not included (Fig. 2B, C). Seminal root length of low-nutrient plants with no PA decreased when the Fe^{2+} level was varied from low to 2 mg L^{-1} ,

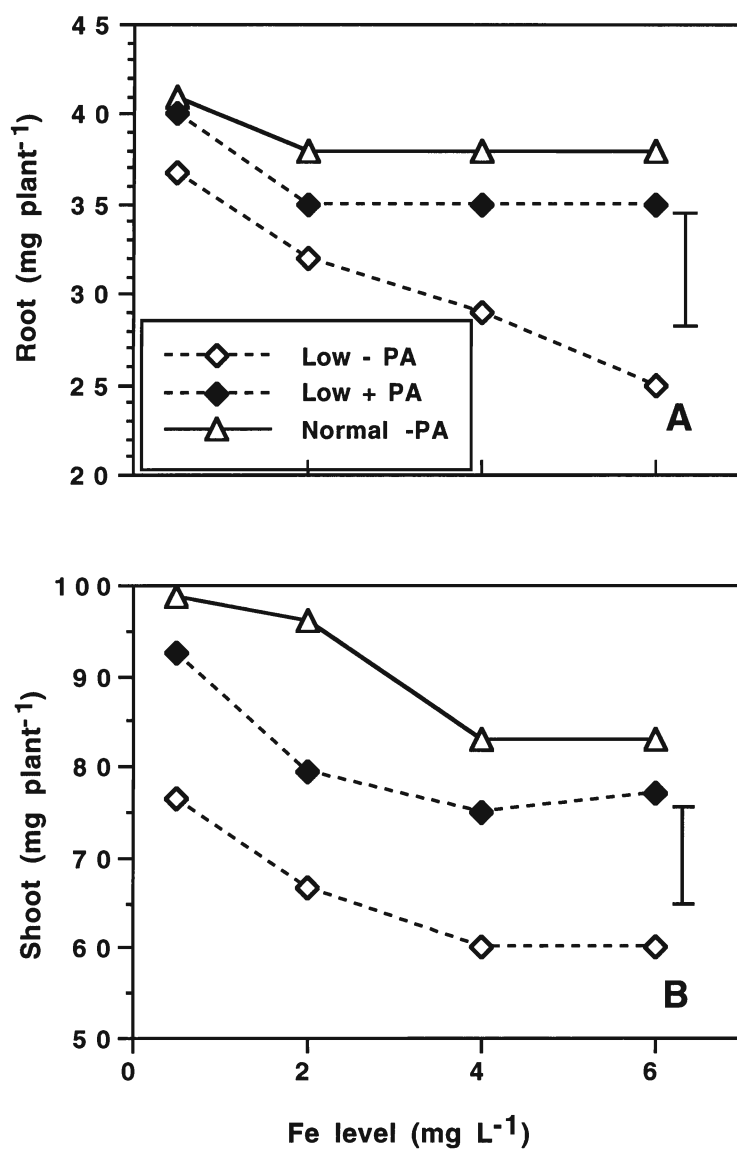


Fig. 1. Effect of Fe²⁺ concentration and PA addition on the dry weight of roots (A) and shoots (B) of wheat plants grown hydroponically for 16 days with a low (0.1x) level of macronutrients. Plants grown with a normal level of macronutrients (1x) without PA serve as the control. Bars depict the LSD (0.05) which can be used to compare different treatment means within the same Fe level or Fe levels within the same nutrient treatment.

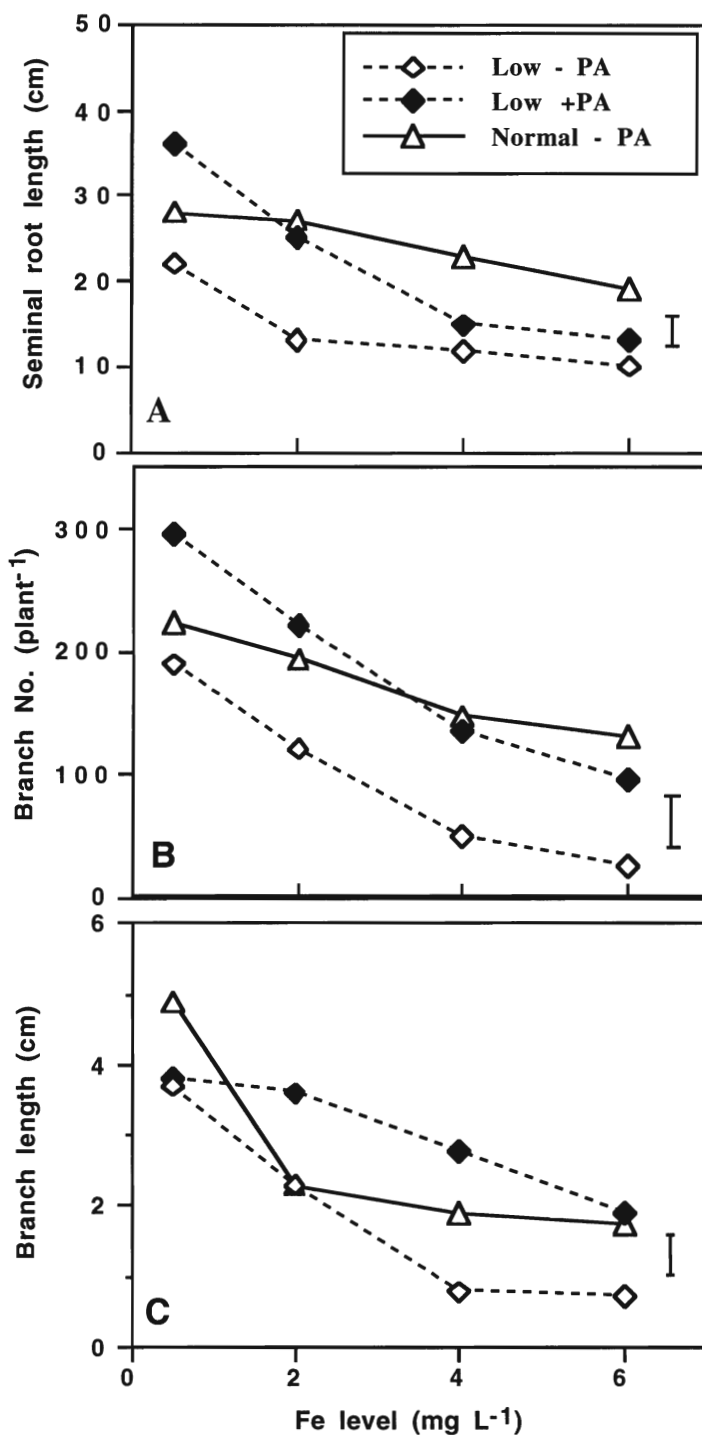


Fig. 2. Effect of Fe²⁺ concentration and PA addition on the length of seminal roots (A), the number of primary root branches (B), and the length of primary branches (C) of wheat plants grown hydroponically for 16 days with a low (0.1x) level of macronutrients. Plants grown with a normal level of macronutrients (1x) without PA serve as the control. Bars depict the LSD (0.05) which can be used to compare different treatment means within the same Fe level or Fe levels within the same nutrient treatment.

but was not affected by further increases in iron concentration (Fig. 2A). Compared to low-nutrient plants without PA, morphological parameters of control plants were significantly higher at almost every iron level (except for the length and the number of primary branches at 0.5 mg L⁻¹ and 2 mg L⁻¹ of Fe²⁺, respectively). For control plants, increasing the Fe²⁺ concentration resulted in a large decrease in the length of primary root branches (64%), and minor decreases in the length of seminal roots (33%) and in the number of primary branches (41%) (Fig. 2). The addition of PA to low-nutrient plants significantly increased all morphological parameters except for the length of primary root branches with 0.5 mg L⁻¹ of Fe²⁺. However, when the Fe²⁺ concentration was increased from low to high, low-nutrient plants with PA still exhibited decreases in the length of seminal roots (64%) and primary root branches (50%), and in the number of primary root branches (33%) (Fig. 2).

For plants grown with low nutrients, neither Fe²⁺ level nor PA addition affected the P status of the roots (Fig. 3A). Similarly, while control plants had a significantly higher concentration of root P than low-nutrient plants (3.5 fold), this value was not affected by Fe²⁺ level. The shoot P concentration of low-nutrient plants decreased 61% when iron level was varied from low to high (6 mg L⁻¹) in the absence of PA, but did not change significantly when PA was included (Fig. 3B). When PA was added to the low nutrient solution, shoot P concentration was significantly higher than similar plants grown without PA, at every Fe²⁺ level except for 0.5 mg L⁻¹. Similar to roots, the shoot P concentration of control plants was higher than the low-nutrient plants and was not affected by the increase in Fe²⁺ level, except for an 18% decrease with 6 mg L⁻¹ of Fe²⁺ (Fig. 3B).

DISCUSSION

The impact of nutrient level and PA addition on plant growth was largely dependent on the iron source. Even though wheat and soybean plants were able to utilize all three of the iron sources evaluated, plants generally grew better when supplied with the Fe-chelate (Table 2).

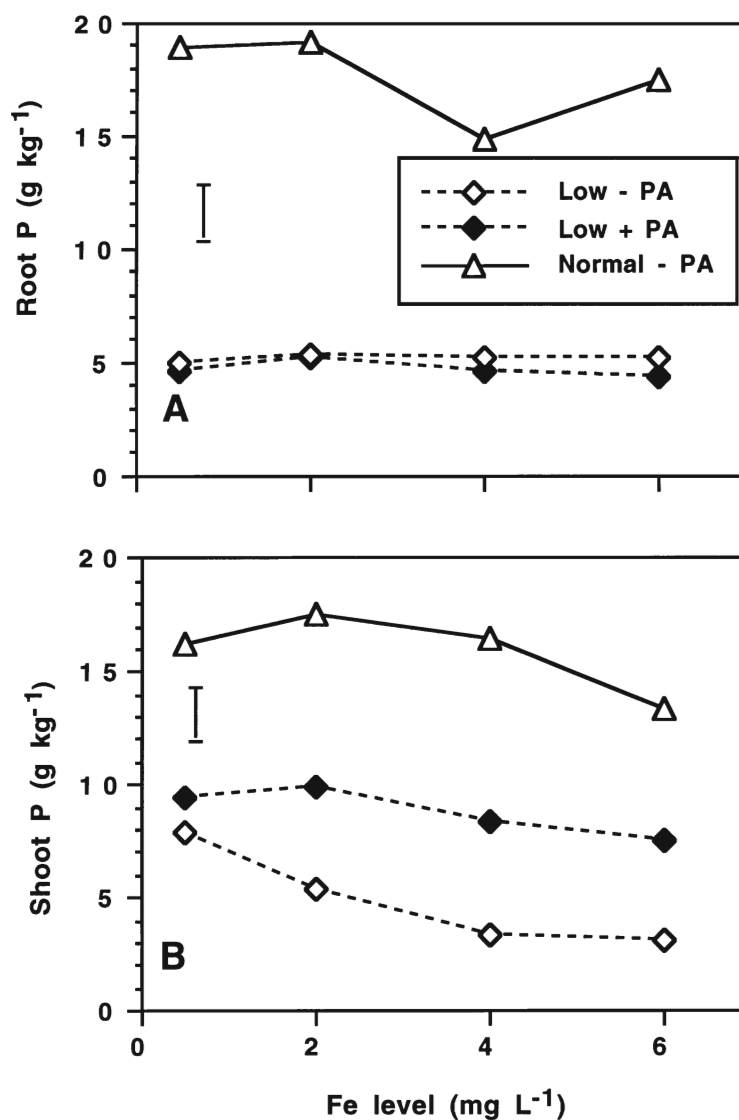


Fig. 3. Effect of Fe level and PA addition on the P concentration in the roots (A) and shoots (B) of wheat plants grown hydroponically for 16 days with a low level of macronutrients (0.1x). Plants grown with a normal level of macronutrients (1x) without PA serve as the control. Bars depict the LSD (0.05) which can be used to compare different treatment means within the same Fe level or Fe levels within the same nutrient treatment.

Better growth when plants were fertilized with chelated iron compared to FeSO_4 has been reported previously (Mordvedt, 1982). However, the growth of both plant species supplied with Fe^{3+} was better than we expected, since earlier work with maize showed much less growth when plants received Fe^{3+} compared to Fe-chelate (Hageman et al., 1961). We also could not find any current work in the literature where Fe^{3+} was used as the sole source of iron, which led us to believe that Fe^{3+} would not be as effective as Fe^{2+} or Fe-chelate. We speculate that the reasonably good growth with Fe^{3+} could be related to the young age of the plants, and the likelihood that they are still acquiring some of their nutrients from seed reserves.

In the absence of PA, growth of wheat plants was affected by nutrient level only when Fe^{2+} was the iron source, while the growth of soybean plants was affected by nutrient level when iron was supplied as either Fe^{2+} or Fe^{3+} (Table 2). Similarly, addition of PA resulted in significant increases in growth of wheat when the iron source was Fe^{2+} (only with the low nutrient supply), and of soybean (at both nutrient levels) when the iron source was Fe^{2+} or Fe^{3+} . A similar enhancement in plant growth from PA addition was noted in our previous work when wheat and tomato plants were grown with Fe^{2+} as the iron source (Wang et al., 1996).

For both species, the effect of PA addition was exhibited as an increase in the length of roots and primary root branches when the iron source was Fe^{2+} or Fe^{3+} , and a higher number of primary and secondary root branches (for soybean only) (Tables 3 and 4). When PA was present, roots of both species grown with low nutrients were also characterized by longer and/or more root hairs (data not shown). These findings are similar to previously described work with wheat (Zeko et al., 1996), and support the hypothesis that PA-induced enhancement of plant growth is related to changes in root morphology. Root morphological parameters such as length and number of lateral roots and root hairs are known to increase the absorption area of the root system (Peterson and Peterson, 1986; Peterson, 1992), which should lead to greater availability of mineral nutrients to the plant. Longer and thinner roots have also been shown to be more efficient in nutrient uptake by increasing the root surface area, and as a result the

potential availability of nutrients (Jungk and Barber, 1974; Robinson and Rorison, 1983; Barber, 1984). The nutrient data (Tables 5 and 6) showed that PA addition resulted in increased accumulation of macro- and micronutrients by the shoot. Soybean plants receiving PA generally accumulated more cations under both Fe^{2+} - or Fe^{3+} - nutrition, while wheat plants accumulated more cations as well as more P (especially when grown with Fe^{2+}). The lack of increase in shoot P for Fe^{2+} -grown soybean plants receiving the low-nutrient supply could be due to the small amounts of P present in the solution (Table 1). Plants of both species grown with Fe-chelate had longer roots, and more root branches, than those grown with Fe^{2+} or Fe^{3+} , and accumulated more nutrients in their shoots; which likely resulted in their better plant growth. For both species, PA addition to Fe^{2+} -, and in some cases, to Fe^{3+} -plants, resulted in root morphological parameters, plant dry weights, and nutrient contents that were similar to those of plants grown with Fe-chelate (Tables 2 to 6).

Findings from the plant growth, root morphology and nutrient uptake data indicate that PA is acting as a nutrient absorption enhancer by inducing changes in root morphology that result in increased surface area, more efficient nutrient uptake, and ultimately better plant growth. Similarly, the lack of nutrient and PA effects on plant growth and root morphology of both species when provided with Fe-chelate, is evidence that Fe-chelate may also act as a nutrient absorption enhancer. Other evidence is provided by the nutrient data which showed a higher content of nutrients in the shoots of Fe-chelate-grown plants, than those grown with either Fe^{2+} or Fe^{3+} without PA (Tables 5 and 6).

We speculate that the similar responses on plant growth and root morphology for the chelate and the PA may be due to common functional groups in their chemical structure, which is characterized by the presence of multiple carboxylic groups and a high negative charge. The number of these charged carboxyl groups varies from five for the Fe-chelate to up to forty for PA. While Fe-chelates are widely used to provide Fe to plants, they can also induce micronutrient deficiencies when present in excess amounts (Clark, 1982; Wallace and Wallace, 1992). This effect is presumably due to competitive binding of the cation micronutrients to the

carboxyl groups of the chelate (Lindsay, 1984). Large-chain carboxyl polymers (like PA and polyacrylates) also function in water-treatment applications by binding Ca^{2+} , thereby preventing its precipitation with PO_4^{3-} (Kemmer, 1988; Koskan et al., 1992). Therefore, we speculate that Fe-chelate and PA might temporarily sequester mineral nutrients, (especially Ca and P) away from the roots by creating a double radius of positively and negatively charged layers. In this regard, cations such as Ca^{2+} are attracted by the negatively charged PA or Fe-chelate, creating a “cloud” of positive charges which in turn attracts anions such as P.

Throughout the literature, there are examples of changes in root morphology for plants grown under Ca or P deficiencies. Longer and/or finer roots, and the presence of root hairs are known to increase P uptake, and often result when the P supply is limiting (Jungk and Barber, 1974; Föehse et al., 1991; Blair, 1993). The addition of PA or Fe-chelate to the nutrient solution resulted in a finer root system with more root hairs (data not shown), which could be a response to a temporary lack of nutrients. This sequestering of nutrients by PA or Fe-chelate does not appear to result in a nutrient deficiency, but rather causes a temporary shortage of nutrients, as plants did not show any decrease in growth, or any visible deficiency symptoms. When such a temporary nutrient deficit occurs due to binding with PA or with Fe-chelate, we believe that the plants respond by changing their root morphology; and the resultant increase in root surface area facilitates the increased uptake of nutrients. Our nutrient data in this study is in agreement with earlier work with wheat and tomato which showed that PA addition to plants grown with a low or very low level of macronutrients increased the plant accumulation of all the nutrient elements, especially P and Ca (Wang et al., 1996).

For both species, when the iron source was Fe^{2+} , PA addition had the largest impact on plant growth, nutrient uptake, and root morphology. Because Fe^{2+} at high concentrations has been shown to cause decreases in plant growth (Hageman et al., 1961; Fageria, 1990), we varied the Fe^{2+} level available to wheat plants from low to high in order to investigate whether the PA effects on root morphology and plant growth were related to the amount of available iron. When the Fe^{2+} level was increased from 0.5 to 6 mg L^{-1} , our data showed a sequential

decrease in growth of low-nutrient plants (both roots and shoots), which did not receive PA (Fig. 1). This decrease was accompanied by the presence of a visible coating of reddish-brown color on the roots (data not shown). Similar root coatings, known as iron plaque, are common for wetland species such as *Typha latifolia* L., rice (*Oriza sativa*), as well as upland species grown under waterlogged conditions (Green and Etherington, 1977; Boone et al., 1983; Taylor et al., 1984). The plaque formation occurs under reducing conditions that bring about the oxidation of Fe^{2+} to the less soluble Fe^{3+} , and increases with Fe concentration and higher pH (Taylor et al., 1984). The presence of this plaque is thought to have a detrimental effect on the capacity of roots to absorb nutrients, because when observed under the electron microscope the cell walls were damaged and there were no root hairs (Taylor et al., 1984). The addition of PA prevented the formation of iron plaque (data not shown), and overcame the negative effects of excess Fe^{2+} on plant growth (Fig. 1). Our data indicates that this effect is due to PA-induced changes in the root morphology, as at every Fe^{2+} level PA addition increased the length of roots, and the number of primary root branches (Fig. 2).

In the absence of PA, an increase in Fe^{2+} level resulted in a decrease in the concentration of P of the shoots (Fig. 3B), which indicates that less P was available for transport to the shoot. It is well known that P and Fe nutrition frequently interact such that high levels of Fe can decrease P uptake, while high levels of P can induce Fe deficiency (Brown and Jones, 1977; Bassiri et al., 1979). Thus, in our study increasing the Fe^{2+} level may have made P less available, possibly because of precipitation with iron. As evidenced by the lack of iron plaque on PA-treated roots and the higher accumulation and concentration of P in the shoots (Fig. 3), PA seemed to ameliorate the precipitation of P by excess iron. However, from our data we cannot determine with certainty whether the additional shoot P in PA-treated plants is the result of less P precipitation, or of the PA-induced changes in root morphology. Additional research is needed to address this question.

In summary, the effect of PA on plant growth, root morphology and nutrient accumulation is dependent on the source of iron, while the mechanism (e.g. Strategy I and II)

by which different plant species acquire iron does not seem to influence the plant response to PA. Both wheat and soybean were able to use all three iron sources (Fe^{2+} , Fe^{3+} , and Fe-chelate), but both grew better with Fe-chelate. The inclusion of PA increased plant growth and shoot nutrient content, and changed root morphology when the iron source was Fe^{2+} or Fe^{3+} , and resulted in values that were similar to plants receiving Fe-chelate. For both species, there was no PA effect on either plant growth, nutrient accumulation, or root morphology when plants were supplied with Fe-chelate. PA also appears to ameliorate the negative effects of high iron concentration (on plant growth, root morphology, and shoot P status), presumably by preventing the precipitation of P. Based on these data we conclude that both PA and Fe-chelate act as nutrient absorption enhancers by temporarily sequestering nutrients, which induces changes in root morphology that results in a subsequent increase in nutrient uptake, and in better plant growth.

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