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This is to certify that the

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EFFECTS OF PHOSPHORUS AND ZINC ON NORMAL AND ZINC SENSITIVE SOYBEAN CULTIVARS

By

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ABSTRACT

EFFECTS OF PHOSPHORUS AND ZINC ON NORMAL AND ZINC SENSITIVE SOYBEAN CULTIVARS

By

Hussein Mohammed Aly Ragheb

The effects of P level in the growth media on dry matter accumulation and Zn uptake in soybean plant parts varies between soybean cultivars, and depends upon the external Zn concentration. P had no effect on Zn uptake in tops and roots of the York and Beeson soybean cultivars grown at low and intermediate levels of Zn. But, P increased Zn uptake at high Zn Level. High P level induced Zn deficiency symptoms on the plants of both soybean cultivars; yet, Zn uptake and concentration in tops and roots was not reduced. Zn deficient plants had the highest P/Zn ratio which correlated with the intensity of Zn deficiency . These results support the metabolic model of the P-induced Zn deficiency.

The localization pattern of Zn in the leaves of both soybean cultivars agreed with its metabolic functions. The highest amount of Zn was localized in "soluble" form mainly in cytoplasm and vacuoles, and the next highest fraction of Zn was bound to cell wall and cell debris. In the "soluble" fraction three different forms of Zn-binding compounds were separated based upon their molecular size. P had no significant effect on both the localization pattern or the amount of Zn-binding compounds; however, high P level exhibited some effects upon the distribution of Zn between these forms.

High P level reduced the rate of total Zn uptake and translocation in both varieties, and reduced the rate of Zn accumulation in the roots of the Beeson variety. At the low Zn levl, P either decreased or had no significant effect on Vm values of either soybean variety. Any reduction was eliminated by increasing the external Zn concentration. However, only at the high Zn concentration range did P significantly increase the Km values of both soybean cultivars.

High P level had no effect on the protein content. Carbonic anhydrase was more sensitive to P than superoxide dismutase. The activities of both enzyme systems changed almost linearly with increasing the external Zn concentrations up to 3.06 uM Zn at all P levels, but carbonic anhydrase was more sensitive to external Zn concentrations.

بسم الله الرحمن الرحيم

In the name of Allah the most merciful and the most beneficient

To my wife

Her love and sacrifice made this work possible.

To my mother

Her prayers extend my ability to do this work.

To my brothers

their encouragements made me able to face the challenge.

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INTRODUCTION

INTRODUCTION

The increasing demands for food production today is a serious challenge that faces scientists worldwide. Therefore, the need for high yielding cultivars is obvious, and the use of higher rates of macronutrient fertilizers is a well known cultural practice to enhance yields. But, many other problems have arisen as a consequence of using higher rates of macronutrient fertilizers. One of problem is phosphorus (P) induced zinc (Zn) deficiency.

High levels of P fertilization have frequently been reported to induce symptoms which resemble those of Zn deficiency, or to aggravate Zn deficiency in crop plants (Olsen, 1972). Zn deficiency in crop plants has been extensively studied; however, the actual casual relationship and mechanisms involved are still unknown. Growth promotion and dilution effects; inhibition of Zn uptake; metabolic disorder due to P-Zn imbalances; and slow rates of Zn translocation are considered as possible explanations for P induced-Zn deficiency (Collins, 1981). The physiological nature of P induced-Zn deficiency has been emphasized based upon the observations that the induction of Zn deficiency is related to the P/Zn ratio rather than to the concentration of Zn "per se" (Boawn and Brown, 1968, and Sumner et al., 1982).

Studying the effects of heavy application of P on the metabolic functions of Zn on both molecular and subcellular levels could help to understand the physiological nature of P induced-Zn deficiency. Thus, this work was initiated to further explore the effects of high levels of P in the growth media on:

(1) The subcellular localization of Zn in two different soybean varieties,
(2) The distribution of Zn among the soluble Zn-binding compounds in soybean leaves,
(3) The kinetic characteristics of Zn uptake,
(4) and the activities of some Zn containing enzymes in soybean leaves.

CHAPTER I

CHAPTER I

LITERATURE REVIEW

Factors Inducing Zn Deficiency

There are many environmental factors which may induce Zn deficiency in plants. Many experiments conducted under different soil conditions, using different species have shown that P, pH, $CaCO_3$ and antagonism with other elements (Fe, Cu, Mn, S,...) are the most common factors that induce Zn deficiency symptoms.

Phosphorus-Zinc Interaction in Plants:

Phosphorus induced Zn deficiency has been extensively studied. There are a number of possible explanations for P induced Zn deficiency: growth promotion due to increased P causes Zn dilution in plants; inhibition of Zn uptake due to cation(s) added with P; metabolic disorder due to P-Zn imbalance; and slow rates of Zn translocation at increased P levels (Collins,1981).

Precipitation of Zn-phosphate inside plants (Biddulph, 1953; Boawn and Brown, 1968) or on the root surface (Kankoulakes, 1973; Malavolala and Govasliga, 1974) was the suspected cause of Zn-disorder in plants. But, evidence now shows that precipitation of Zn-phosphate does not fully explain the P-Zn interaction. Jurinak and Inouye (1962) showed that even at pH 8 the solubility of Zn-phosphate is sufficient to give a concentration of 15.7 uM soluble Zn, which would be more than adequate for plant needs. Precipitation of Zn-phosphate inside plants is not likely to induce Zn deficiency in plants (Boawn et al., 1970; Lindsay, 1972; and Reddy et al., 1973).

Phosphorus has many effects on Zn uptake and translocation. High P levels can either inhibit Zn uptake by roots (Safaya, 1976; Asif and Ajakaiye, 1974; and Takkar et al., 1976) or depress Zn mobility inside the plants (Warnock, 1970; Burleson et al., 1961; and Burleson and Page, 1967). Studies of P-Zn interaction in cereals (Brown et al., 1970; Sharma et al., 1968b; and Youngdahl et al., 1977), maize (Safaya, 1976 and Dwivedi et al., 1975), soybean (Wallace et al., 1978), navy bean (Pauli et al., 1968) and chickpea (Yadav and Shukla, 1982) showed that P affects either Zn absorption in roots or Zn translocation from roots with concomitant increase of Zn concentration in roots.

P-induced Zn deficiency may occur without reducing the Zn concentration in plants. High P level sometimes enhances or intensifies Zn deficiency without depressing or causing any change in Zn concentration in plant tops (Boawn and Brown, 1968; Millikan et al., 1968; and Marschner and Schropp, 1977). Moreover, Leece (1976 and 1978) reported that maize plants, grown in black earth soil, developed symptoms of Zn deficiency; yet, the expanded leaves had very high Zn concentration. The Zn deficiency was not attributed to P/Zn, Fe/Zn, Cu/Zn or Mn/Zn imbalance in the plant tops. He suggested that, under such conditions, Zn may be inactivated inside plants through a chelation process with unspecified, naturally occurring organic ligands.

Phosphorus may enhance plant growth and increase the P content and concentration in plant tissue. Thus, metabolic anomalies in plant tissues, which are caused from a lack of equilibrium between P and Zn will appear as Zn deficiency symptoms (Millikan, 1963; Millikan et al., 1968). Watanabe et al., (1965) reported that a dilution effect most likely occurs when the rate of plant growth exceeds the rate of uptake of a particular nutrient and, therefore, the concentration of that nutrient decreases. Sharma et al., (1968a) stated that total Zn uptake in plants generally increases with P application, but enhanced growth may sufficiently dilutes it to a deficient concentration. Dogar and Tang van Hai (1980) reported that the relation between growth rate and P-induced Zn deficiency suggests that the application of other nutrients that promote plant growth to Zn-deficient soils would also induce Zn deficiency. They found that increasing N levels in nutrient solution up to 2200 uM have a stimulatory effect on Zn absorption by rice roots. However most of absorbed Zn was accumulated in the roots and resulted in Zn deficiency in plants growing at a low Zn level (0.05 uM).

Boawn and Leggett (1964) and Watanabe et al., (1965) reported that the intensity of Zn deficiency symptoms and the P/Zn ratio were correlated and plant shoots containing a P/Zn ratio of about 400 indicated Zn deficiency. On the other hand, Stukenholtz et al., (1966) could not find any association between P/Zn ratio and loss of plant yield. The same conclusion was reported by Giordano and Mortvedt (1969).

Ghoneim and Bussler (1980) found that physiologically active Zn in leaves was decreased at a low Zn supply, in a more alkaline medium, and at high Fe levels in nutrient solution. Collins (1981) emphasized that this would seem to be a phenomenon different from Zn deficiency, and it may be due to the effects of high P concentration on Zn-dependent enzymes.

Loneragan et al., (1979) and Loneragan et al., (1982) showed that high P levels can induce P toxicity in *Trifolium subterroneum* and produce symptoms which are similar to those of P induced Zn deficiency, although in this case Zn levels are not reduced but rather P levels are increased. Symptoms can be alleviated by Zn application which promotes growth and dilutes the P in plant tissues. The results obtained by Singh et al., (1986) did not support this hypothesis; moreover, their results showed that P resulted in a significant decrease of Zn Levels in wheat tissue. The model of Zn inactivation invitro is still unsatisfactory, and generalizations from one species to another or from one soil or solution to another must be made cautiously (Tiffin, 1972).

Differences among cultivars are another basis that can be used to explain P-induced Zn deficiency. Differential genotypic responses of soybeans to Zn were observed under both culture solution conditions (Paulsen and Rotimi, 1968 and Wallace et al., 1973) and field conditions (Graves et al., 1980). Sumner et al., (1982) manipulated the original data obtained by Paulsen and Rotimi (1968) and Wallace et al., (1973) in terms of the model proposed by Sumner and Farina (1982) and showed that yield responses to added P and Zn were better explained by P/Zn ratio of the tissue. Morever, studying the differential genotypic sensitivity of soybeans to P-Zn-Cu imbalances they found that genotypes which appeared normal had normal P/Zn ratios while severe symptoms were associated with highly imbalanced P/Zn ratios illustrating the possibility of dividing the genotypes into two categories on the basis of sensitivity to P-Zn imbalances.

Calcium Carbonate and pH:

The pH of the growth medium is another factor which may induce Zn deficiency. Result obtained by Pauli et al., (1968) showed that P-Zn interaction is substantially influenced by $CaCO_3$ and/or high pH. The translocation of Zn from roots to leaves was decreased in the presence of $CaCO_3$ while that of P increased and induced Zn deficiency. Whether these effects were due to Ca or high pH was not clear. The results obtained by Chaudary and Loneragan (1972) and Hawf and Schmid (1967) showed that Ca and Mg depressed Zn uptake by wheat and bean plants. More recently, Adams et al., (1982) reported that the presence of Zn deficiency was clearly dependent on the interaction between pH and P levels in the growth medium. High P levels brought about Zn deficiency even though pH was as low as 5.2. The total concentration of Zn in leaves of Zn deficient plants was higher than that of normal plants. In alkaline soils, similar results have been documented (Adams, 1980; Khan and Soltanpour, 1978; Olsen, 1972). Wallace et al., (1978) reported that at high pH Zn concentration in leaves, stems and roots of soybean plants decreased with increasing P in nutrient solution. In contrast, increasing P at low pH resulted in high Zn concentration in leaves, stems and roots. White et al., (1979) reported that both Zn uptake and translocation were decreased by increasing the pH from 5.5 to 6.5 at all levels of Zn.

Zn Interaction with other Nutrients

Several investigators reported that other nutrient elements may have antagonistic effects upon Zn. The effects of Cu on Zn uptake, translocation and concentration in plants are not clear. Millikan (1953), Schmid et al., (1965), Hawf and Schmid (1967) and Bowen (1969) reported that Zn uptake was severely reduced by Cu addition, but the internal translocation was not altered. They suggested that Cu and Zn compete for the same carrier site. On the other hand, Sedberry et al., (1980) found that Cu application increased Zn content of rice leaves.

Iron also, to some extent, interacts with Zn. Nagarajah and Ulrich (1966), using sugar beets, found that by increasing the amount of Fe in nutrient solution, the Zn concentration was decreased. The results obtained by Watanabe et al., (1965) indicated that, at the highest Zn level used, increasing Fe concentration in the solution culture decreased Zn concentration in corn. For pinto beans, there was a trend toward increasing Zn concentration in the plant tissue when Fe concentration in the nutrient solution was increased. Aboulroos et al., (1983) reported that Zn uptake by barley was increased after adding Fe-EDTA.

There is very little information regarding S-Zn interaction on leguminous crops. Shukla and Prasad (1976) reported that S and Zn behave antagonistically with respect to the concentration and uptake of Zn in groundnut; however, others reported that S increased the concentration of Zn in plant tissues (Hassan and Olsen, 1966; Procopiou et al., 1976; Mukhi, 1979). Kumar and Singh (1979) found that S application at lower levels increased Zn concentration and, at higher levels, decreased it in leaves, pod husks, and grains of soybean plants.

Zn Status and Localization in PLants

Subcellular Localization of Zn in Plants:

The distribution between the essential mineral nutrients at subcellular levels in plant tissues can be considered as an image of their metabolic functions. The importance of subcellular localization of micronutrients relative to their functional role in cellular metabolic activities was accentuated as early as 1951 (Whatley et al., 1951). The changes in intracellular distribution patterns of micronutrients in concomitance with changes in growth and development of plant tissues were helpful in defining their roles (Rathore et al., 1972). Moreover, studying the differences in localization pattern of micronutrients among cultivars and varieties could be helpful in explaining the differential requirements and susceptibilities to micronutrients deficiencies.

Subcellular localization of Zn, among other essential metals, in various mammalian tissues and its relationship to the metabolic state has been thoroughly studied (Gyorkey et al., 1967; Edwards et al., 1961; Thiers and Vallee, 1957). In higher plants, however, Zn subcellular localization did not receive much attention and most of the work was confined to a few tissues at specific growth stages (Kositsyn and Igoshina, 1964; Turner, 1970; Rathore et al., 1972). Studying intracellular localization of Zn in tomato leaves, Kositsyn and Igoshina (1964) and Kositsyn (1965) found that approximately 80% of Zn was accumulated in the cell sap either in ionic form or in low molecular weight compounds; 10% was in the protoplasmic proteins; and roughly the same amount was in the mitochondria. In beans and sugar beet, however, Zn localization was different, only 34-35% and 60-68% of Zn, respectively, was detected in soluble form (Vlasyuk et al., 1963). Fujii (1954) reported that Zn is localized in nucleoli in a detectable amount. Rathore et al., (1972) reported that a much greater proportion of the absorbed Zn was localized in root mitochondria and nuclei of the Zn-sensitive Sanilac than in the Zn-tolerant Saginaw. In tropical legumes Johnson and Simons (1979) found that the highest Zn concentration existed in the cytoplasmic fraction of leaves. Recently, in a comprehensive review on Zn enzymes, Vallee (1983) reported that Zn is present in nucleus, nucleolus and chromosomes, and substantial amount of firmly bound Zn stabilize the structure of RNA, DNA and ribosomes.

The subcellular localization pattern of Zn in plant organs may concomitantly change with plant growth. The concentration of radioactive Zn was relatively high in the soluble fraction and in ribosomes isolated from young tissues (Polar, 1976). Aging resulted in a drop of overall Zn concentration followed by a drop in Zn concentration in soluble fraction and ribosomes; eventually a uniform Zn distribution pattern was observed. Zn-binding constituents in the soluble fraction and cell wall isolated from germinated corn tissues were further explored by Diez-Altares and Bornemisza (1967). Using different liquid extractant, they found that most of the radioactive Zn was recorded in the soluble protein fraction that has a high proportion of albumines and globulines. Moreover, Zn was predominant in the cell wall solubility groups of protopectins and hemicelluloses.

Zn Status in Plants:

In higher plants Zn mobility is not great and Zn became very immobile in older leaves (Rinne and Langston, 1960). The re-utilization of Zn and its rate of mobility to younger tissue is very low particularly in Zn deficient plants (Loneragan, 1975). Wittwer (1964) and Millikan and Hanger (1965), using ^{65}Zn either sprayed on or injected into leaves, observed that the movement of Zn is negligible and it is immobilized in the lamina of the treated leaves. These results suggested that Zn is strongly bound or occluded inside plant leaves.

In higher plants Zn is present in many forms. It is well known that Zn is closely associated with many enzyme systems either as a prothetic group or as a cofactor (Shkolnik, 1984). Mugwira (1970) reported that 87% of Zn in Sanilac beans plant tops was extracted in 0.2 M phosphate buffer at pH 7.0, and that the amount of extractable Zn in sodium salts of ligands increased with the Zn chelate stability constant. Thi results suggested that not all Zn in plants associated with proteins and Zn may be present in ionic form or bind to some other biological molecules. Using ryegrass as a test plant, Bremner and Knight (1970) observed that a large proportion of Zn was present as complexes of low molecular weight that are extractable in aqueous ethanol and water. These complexes were of limited stability and their behavior was pH dependent and exist in an anionic forms.

Zn and Enzymes Activities in Higher Plants

Zn-containing Enzyme Systems in Higher Plants:

It has been realized that Zn is an indispensable constituent of many enzyme systems and is most commonly found in metalloenzymes or metal-enzyme complexes. The functions of Zn in metal-containing enzyme systems are categorized into four categories: catalytic, structural, regulatory and noncatalytic (Galdes and Vallee, 1983). Riordan (1976) reported that Zn is found in 59 enzyme systems representing almost all the enzyme groups. The presence of Zn-containing enzyme systems in plants has been reviewed by Shkolnik (1984). Only carbonic anhydrase and superoxide dismutase will be consider here.

Carbonic Anhydrase:

Carbonic anhydrase was the first Zn-containing enzyme that has been detected and studyed in higher plants (Neish, 1939). Further experiments were undertaken and showed that carbonic anhydrase is present in parsley, spinach, peas, cotton, and soybean (Tobin, 1970; Randall and Bouma, 1973; Risiel and Graf, 1972; Ohki, 1976 and 1978).

Carbonic anhydrase catalyses the reversible decomposition of carbonic acid to carbon dioxide and water:

$$H_2CO_3 \longleftrightarrow CO_2 + H_2O.$$

It is present only in leaves and is involved to some extent in the process of photosynthetic CO_2 fixation (Burr, 1936).

In C3 plants carbonic anhydrase is located in the chloroplasts, while in C4 plants it exist in the cytoplasm (Everson and Slack, 1968; Waygood et al., 1969; Jacobson et al., 1975). In spinach leaves Poincelot (1972) reported that 63% of the total leaf carbonic anhydrase activity is located in chloroplasts in association with the stroma proteins, and this activity exhibits a distribution pattern similar to that of ribulose diphosphate carboxylase. These results indicate that CO_2 may be the substrate for the carbonic anhydrase, and consequently its function is to fix CO_2, which subsequently is liberated by glycol oxidase in the photorespiration. Jacobson et al., (1975) reported that carbonic anhydrase acts as a buffer and mediates short term transient pH effects. The enzyme is highly concentrated in the stroma, therefore, it is able to protect the stroma proteins from denaturation by local pH changes associated with H^+ pumps and incorporation of CO_2 into ribulos-1-5-biphosphate.

The activity of carbonic anhydrase is positively correlated with both Zn and protein-N contents in plants (Wood and Silby, 1952), and increased curvilinearly as the Zn status improved from deficiency to adequacy in cotton plants (Ohki,1976). Zn deficiency resulted in a severe reduction in carbonic anhydrase activity in the third and fifth trifoliate leaves in navy bean (*Phaseolus vulgaris* L.). The lowest Zn concentration in the fifth trifoliate was related to the lowest value of carbonic anhydrase activity (Edwards and Mohamed, 1973). The same relation was reported for soybeans (Ohki, 1978), and spinach (Randall and Bouma, 1973).

Superoxide Dismutase:

Superoxide dismutase (SOD) is another metal-containing enzyme system which contains Zn as well as other micronutrients (Cu, Mn, and Fe). SOD protects plant cells against the injurious effects of superoxide free radical (O_2^-)(produced in the biological oxidation) by catalyzing their breakdown to oxygen and hydrogen peroxide (Fridovich,1978).

In higher plants SOD was found to be composed of at least three isozymes. SODs isolated from wheatgerm and pea leaves contain three distinct isozymes (Beauchamp and Fridovich, 1973; Del Rio et al., 1978); whereas, greenpea SOD contains only two isozymes (Sawada et al., 1972). Recently, Sevilla et al., (1980a, 1980b) were able to characterize one of the SOD isozymes as a Mn-containing SOD, the other two isozymes were characterized as Cu-Zn-SODs. Reddy and Venkaiah (1984) reported that the Cu-Zn-SODs are localized in chloroplasts, while the Mn-SOD in associated with mitochondria.

Superoxide dismutases have been studied with respect to the interaction between Zn, Mn, and Cu, and the differential diagnosis of their deficiencies in plants (Del Rio et al., 1978). They suggested that SOD can be used as an indicator for Mn deficiency. The effects of Fe concentration in the growth media on the SOD system in peas was also investigated and proved to be useful in differentiating between Mn and Fe deficiencies (Garcia et al., 1981). In fronds of *Lemna gibba* L., Vaughan et al., (1982) found that SOD activity is correlated with Zn level in growth media; however, Cu level in the culture media causes little change in the enzyme activity.

Diagnoses of Zn Deficiency Using Enzyme Activities:

The activities of the enzyme systems that contain one or more of the micronutrients depends, in most cases, upon the level and/or the status of that micronutrient(s) in plant tissues. Brown and Hendrick (1952) postulated that the reduction in the activity of the metal-containing enzyme is a direct response to the metal stress in growth media; therefore, they proposed the use of enzyme activities as a physiological indicator for the diagnosis of micronutrient deficiencies in plants.

Total chemical analysis has been considered for a long time as a conventional way for diagnosis of Zn deficiency. However, the determination of carbonic anhydrase activity has been proven to be a better index for detecting Zn deficiency than total chemical analyses (Bar-Akiva and Lavon, 1969; Kessler, 1961). Under some conditions chemical analyses failed to associate with the appearance of Zn deficiency symptoms. Maize plants grown in alkaline black earth soils showed Zn deficiency, yet Zn content of leaves was sufficient to support healthy growth (Leece, 1976). The same results were observed under the P-induced Zn deficiency.

Zn deficiency in citrus trees often occurs along with Fe and Mn deficiencies. Because carbonic anhydrase is specifically sensitive to Zn content in leaves, Bar-Akiva and Lavon (1969) reported that the activity of the enzyme appears to be useful in detecting Zn deficiency when Fe and Mn deficiencies are occurring together with Zn deficiency.

Prediction of the hidden hunger of Zn in plants is another case in which using enzyme activities is more useful and helpful. A rapid test for detecting hidden hunger of Zn in different plant varieties was introduced by Dwivedi and Randhawa (1974). Using the test they found that at seedling stage, even with no Zn supplied, plants of all varieties did not show any Zn deficiency symptoms. Also, there was no difference in total Zn concentration and dry matter content compared with the higher Zn supply. However, there were distinct differences in carbonic anhydrase activities and in the amount of Zn bound to the enzyme protein in most of the plant varieties that were tested. At the later stages of growth, varieties that showed no deficiency symptoms were considerably different in enzyme activity and yield at no Zn compared to high Zn supply.

CHAPTER II

CHAPTER II

ZINC LOCALIZATION AND SOLUBLE ZN-BINDING COMPOUNDS IN LEAVES OF SOYBEAN PLANTS AS AFFECTED BY P AND ZN LEVELS IN THE GROWTH MEDIA

INTRODUCTION

Zn is required by plants in relatively small amounts compared to the other micronutrients; however, Zn participates in several widely distributed metabolic processes in plants. In most of these metabolic processes the functions of Zn as a mineral nutrient are a result of the strong tendency of Zn to form tetrahedral complexes with the naturally occurring metabolic compounds (Clarkson and Hanson, 1980). Therefore Zn in plants is either present as a part of an enzyme system or associated with compounds of low molecular weight through its coordination with amino, imidazole, and sulfhydryl groups (Passow and Clarkson, 1961).

Subcellular localization of Zn in plants has received little attention, and most of the research in this area has been restricted to a few tissues at specific growth stages (Turner, 1970 and Rathore et al., 1972). In tomato leaves 80% of Zn was accumulated in the cell sap either in ionic form or bound to low molecular weight compounds, 10% was in the protoplasmic proteins, and approximately 10% was in the mitochondria (Kositsyn and Igoshima, 1964). In beans and sugar beets, however, only 34-35% and 60-68% of Zn, respectively, was detected in soluble forms (Vlasyuk et al., 1963). On the other hand, a high Zn concentration was detected in the cytoplasmic fraction separated from leaves of some tropical legumes (Johnson and Simons, 1979). The subcellular localization pattern of Zn in plant tissues was found to be changed with the evolution of Zn-sensitivity and Zn-tolerance in plants (Rathore et al., 1972). A much greater proportion of the absorbed Zn was localized in root mitochondria and nuclei of the Zn-sensitive Sanilac than in the Zn-tolerant Saginaw.

P-induced Zn deficiency has been extensively studied (Wallace et al., 1978; Yadav and Shukla, 1982; Loneragan et al., 1979; Safaya, 1976; Boawn and Brown, 1968; Dogar and Tang van Hai, 1980; Singh et al., 1986, and Wagar et al., 1986). The physiological nature of the P-induced Zn deficiency was first reported by Boawn and Brown (1968). Their results showed that high P levels induced Zn deficiency symptoms on both beans and potatoes, yet the Zn content of tissue per se was not reduced by P addition. They concluded, therefore, that the high P levels interfered with normal Zn metabolism. Later results obtained by Collins (1981) and Leece (1976 and 1978) provide strong evidence that P-induced Zn deficiency is a type of metabolic malfunction, apparently unrelated to the Zn content in plant tissues.

This work was initiated to (1) investigate the differences in subcellular localization of Zn in leaves of Zn-sensitive and normal soybean cultivars, (2) characterize the changes, if any, in subcellular localization of Zn in leaves of soybean plants upon increasing the P level in the growth media, and (3) determine the effect of P on the Zn-binding ligands in leaves of soybean plants.

This information is essential for understanding the physiological nature of P-induced Zn deficiency.

MATERIALS AND METHODS

Plant Culture:

Two soybean cultivars, that have been classified as Zn-sensitive (York) and Zn-normal (Beeson) by White et al., (1979) based upon their response to Zn level in soil, were used as test plants.

Soybean seeds were germinated in acid-washed quartz sand in a growth chamber at 24 C for 6-7 days. Seedlings were irrigated daily using one-tenth Hoagland's solution minus P and micronutrients. Seedlings were removed by washing them out of the sand using distilled-deionized water, and homogeneous seedlings were selected and transplanted into 2.25 L polyethylene pots (2 seedlings per pot) that contained a continuously aerated one-tenth Hoagland's solution minus P and micronutrients. A week later plants were transferred into a one-half Hoagland's solution modified to contain 2 levels of P and 3 levels of Zn and containing the following basic constituents: 3 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 0.25 mM NaCl, 1 ppm Fe as Fe-Na-EDTA, 0.125 ppm Mn as $MnSO_4$, 0.01 ppm Cu as $CuSO_4$, 0.25 ppm B as H_3BO_3, and 0.005 ppm Mo as molybdic acid. Two levels of P (0.25 and 2.50 mM NaH_2PO_4) and three levels of Zn (0.076, 0.769, and 7.692 uM $ZnCl_2$) were applied. Zn was labeled in each pot by adding 1 uCi ^{65}Zn per 100 ug Zn.

The pH of the nutrient solution was adjusted to obtain pH 6.3 ± 0.2 using a solution of 0.1 N NaOH. Nutrient solutions were changed, at the beginning, every third day through two cycles and thereafter every second day. Plants were grown for 14 days, after transplanting into the treatment solutions, in growth chambers under a photoperiod regime of 16 hours light, at about 10 watts m^{-2} at the top of plants, and 8 hours dark. Five high-output fluorescent lamps (F 48 T 12/CW 1500) and six 25 W incandenscent light bulbs were used. The temperature was adjusted to 28 ± 1 C and 20 ± 1 C during the day and night respectively. Pots were arranged in the growth chambers in a randomized complete block, split-plot design, with cultivars as main plots and combinations of Zn*P levels as sub-plots. Each treatment was replicated 4 times.

Harvesting and Sample Preparation:

The experiment was terminated by removing plants from the nutrient solutions and placing them for two hours in a modified Hoagland's solution that contained no P or Zn. This was to assure that the adherent and precipitated Zn on the outer root surfaces would be dissociated. To desorb the reversibly accumulated Zn from the root free-spaces plants were placed in cold (8 C) 0.5 mM $CaCl_2$ for 2 hours. After that plants were removed from the $CaCl_2$ solution, the root systems thoroughly rinsed in distilled-deionized water 3 times for 10 minutes and blotted between filter papers. Plants were finally separated into roots, stems and petioles, and leaf blades. Fresh weights were recorded and the stems and petioles dried at 70 C for 24 hours. Leaf blades were prepared for fractionation by cutting them into small pieces of approximately 1 cm^2, thoroughly mixing the pieces, and dividing them into two parts. Five grams of leaves were removed for fractionation, and the remaining leaves were freeze-dried for 48 hours, weighed, ground to pass through a 40 mesh sieve, and stored for chemical analysis.

Homogenization and Subcellular Fractionation:

Five grams fresh leaves were homogenized in 25 ml of grinding solution for 3 intervals of 15 seconds each using a VirTis high speed homogenizer. The grinding solution was composed of 0.2 M ammonium acetate (NH_4OAc) and 0.33 M sucrose, adjusted to pH 6.9. The homogenization and the following fractionation steps were carried out at 4 C. The homogenate was filtered through a double layer of 20 um nylon cloth and the liquid pressed out of the residue. To assure complete recovery of soluble material and organelles the residue was washed twice using 10 ml of the grinding solution and the filtrates combined.

The filtrate was further fractionated into subcellular organelles using the differential centrifugation procedures as outlined by Polar (1976). A sorvall refrigerated ultracentrifuge (model RC5C) was used. First, the filtrate was centrifuged at 600 g for 15 minutes to separate the intact chloroplasts, nuclei, and cell debris. The pellets were washed with 5 ml of the grinding solution and recentrifuged at 600 g. The supernatants from the 600 g fraction were pooled and spun at 3000 g for 15 minutes. The 3000 g pellets which mainly contained chloroplast fragments were washed using 5 ml of grinding solution and centrifuged again at 3000 g. The supernatants from the 3000 g were pooled and spun at 18000 g for 15 minutes. The resultant pellets which contained the crude mitochondrial fraction were washed using 5 ml of grinding solution and recentrifuged at 18000 g. The supernatants from the 18000 g fraction were spun at 45000 g and the resultant pellets were referred to as 45000 g fraction. The non-sedimental fraction was referred to as the "soluble" fraction.

Each fractionated pellet was resuspended in 5 ml of the homogenizing solution and stored in a refrigerated state prior to analysis.

<u>Separation of Soluble Zn-binding Compounds:</u>

Gel filtration chromatography was used to fractionate the labile Zn-binding compounds from the "soluble" fraction. Sephadex G10-120 dextran gel (Pharmacia Fine Chemicals) was used. To avoid Zn binding on the residual surface charge of the gel particles, and to increase the recovery from the gel column, dextran gel was pretreated as described by Lonnerdal (1980).

Sephadex gel was first swollen in deionized-distilled water according to the instructions of the manufacturer. After swelling, 200 ml of the swollen gel was diluted to 500 ml with deionized-distilled water, then the pH of the gel suspension was adjusted to 10-11 using 0.1 N NaOH. One gram of sodium borohydride was added to the gel while stirring. Then the slurry was heated in a water bath at 80 C for 2 hours, allowed to cool and washed with deionized-distilled water. Gel was then equilibrated with 0.2 N NH_4OAc buffered at pH 6.9, and a column of 1.6 by 70 cm was packed.

Before running the samples, the gel column was calibrated with substances of known molecular weight (blue dextran and $^{65}ZnCl_2$). Samples with the appropriate volume were added through a sample applicator placed on the top of the gel surface. Samples were eluted using 0.2 N NH_4OAc buffered at pH 6.9 with the flow rate adjusted to give 12 ml h^{-1}. The outlet of the column was connected to a fraction collector and fractions (130) of 2 ml each were collected. Fractions were assayed for ^{65}Zn and P.

Total Chemical Analysis and ^{65}Zn Assay:

Dry, ground plant tissues were wet-ashed in a triple acid mixture (8:1:1 nitric:sulfuric:perchloric). Zn concentration was determined by atomic absorption spectrophotometry (Perken Elmer 303) and P concentration was determined using a flow injection autoanalyzer (Lachet). ^{65}Zn in fractionated pellets, "soluble" fraction and gel filtration fraction, was counted using a gamma-counter (Packard). To avoid the geometric effects on the counting efficiency, similar volumes (2 ml) were counted. At the same time a series of ^{65}Zn specific activities were prepared and counted.

RESULTS AND DISCUSSION

Zn Deficiency Symptoms:

The number of leaves that showed Zn deficiency symptoms was counted and a grading scale of five grades was established. One plus (+) was given to plants that showed no Zn deficiency symptoms, and 5 (+++++) were given to the plants when more than 80% of their leaves exhibited Zn deficiency. In between these two limits the other three grades were equally spaced. Table 1 shows the degree of Zn deficiency symptoms on soybean plants.

Zn deficiency appeared mostly on old leaves as an intervenial chlorosis and yellowish blotched spots. The recently developed leaves were irregularly shaped, waived, and curled around the middle vein. Leaves of soybean plants treated with high P level were generally pale green and the interveinal areas of the leaf blade were yellow. These

Table 1. Dry-matter, Zn, and P Uptake, and P/Zn Ratio in Shoot and Root Tissues of York and Beeson Soybean Cultivars as Affected by P and Zn Levels in Cultural Solutions.#

Treatments		Zn Deficiency	Dry-matter		Zn Uptake		P Uptake		P/Zn	
P	Zn		S	R	S	R	S	R	S	R$
mM	uM		g pot^{-1}		mg pot^{-1}		mg pot^{-1}			
			Zn-sensitive (York) variety							
0.25	0.076	++++	4.1	1.3	0.096	0.038	30.0	7.2	314	193
	0.769	+	7.2	1.4	0.332	0.056	23.9	7.6	71	137
	7.69	+	2.7	0.9	0.738	0.276	22.9	7.7	31	28
2.50	0.076	++++	4.6	1.3	0.124	0.036	49.4	29.1	400	808
	0.769	++	5.7	1.2	0.338	0.043	36.8	20.9	109	497
	7.69	+	3.2	0.9	0.918	0.534	31.2	18.0	33	34
			Zn-normal (Beeson) variety							
0.25	0.076	+++	6.2	2.1	0.143	0.064	36.7	5.8	258	91
	0.769	+	7.4	2.2	0.459	0.101	31.2	5.5	68	54
	7.69	+	5.4	1.9	1.370	0.924	27.2	5.8	20	6
2.50	0.076	++++	6.5	2.1	0.193	0.084	62.6	41.9	324	500
	0.769	++	7.4	2.2	0.439	0.108	54.7	33.2	127	313
	7.69	+	5.8	1.9	1.622	1.236	46.3	28.6	28	23
$LSD_{0.05}^{+}$			0.5	0.15	0.061	0.035	3.2	1.2	21	47
$LSD_{0.01}^{+}$			0.7	0.21	0.089	0.052	4.9	1.70	30	66

\# Each number is an average of 4 replicates.

\+ LSD was calculated according to Steel and Torrie(1980), and used to compare any two numbers within each column.

$ S - Shoots; R - Roots.

symptoms were similar to, but not identical with, the typical Zn deficiency symptoms described for navy beans.

Dry-matter Accumulation:

In both soybean cultivars at both P levels a consistent treatment effect was a significant increase in dry-matter accumulation in shoots with increasing Zn concentration in nutrient solution up to 0.769 uM (50 $ugZn \cdot L^{-1}$)(Table 1). However the magnitude of increasing dry-matter accumulation in the shoots of the Zn-sensitive York variety was higher than that of the Beeson variety (42.6%, 18% and 16.6%,11.9% in York and Beeson at low and high P level, respectively). Roots, in general, did not respond to the increasing levels of Zn up to 0.769 uM Zn. Further increases in Zn level up to 7.69 uM resulted in a significant decrease in dry-matter accumulation in tops and roots of both cultivars at both P levels. The Zn-sensitive York cultivar was much more affected by the high Zn treatment than the normal one (Beeson). The response of roots to a high level of Zn was similar to that of shoots. This reduction in dry-matter accumulation may be a direct effect of Zn toxicity at such a high level of Zn. Abd-Elgawad and Knezek (1981, unpublished data) found that the maximum dry-matter accumulation in the Beeson cultivar was obtained when 50 ug Zn per liter was used.

The data further indicate that the responses of the tested soybean cultivars to P treatments at different levels of Zn in the nutrient solutions were quite different. Dry-matter accumulation in shoots and roots of the Beeson variety did not show a significant response to P treatments at all levels of Zn. However, high P level in nutrient solution resulted in a decrease in dry-matter accumulation in the tops of the York variety at 0.769 uM Zn, but in increasing the dry-matter accumulation at 0.076 and 7.69 uM Zn. High P level in the growth media enhanced the growth of the Zn-sensitive variety York plants and, therefore, increased their ability to tolerate both high and low levels of Zn in the nutrient solution. Wallace et al., (1978) reported that increasing the P concentration in solution culture decreased leaf, stem and root dry-matter accumulation in three different soybean cultivars (Hawkeye, Haddso, and Wayen). Smilde et al., (1974) noted that P ameliorated Zn toxicity by increasing plant growth rather than by decreasing total Zn uptake.

Zn and P Uptake and Concentration:

Generally, both soybean cultivars responded similarly to Zn and P treatments, but the magnitude of this response was different (Tables 1 and 2). Zn uptake in tops and roots was markedly increased at both levels of P with increasing Zn concentration in nutrient solution. However, the amount of Zn uptake and translocation by the Beeson soybean variety was much higher than that by the York soybean variety. These results show the inherent variabilities between soybean cultivars and could be related to their differences in absorption and utilization of Zn.

The significant effect of P*Zn interaction on Zn uptake in soybean plant organs indicated that the effect of P on Zn uptake in soybean plant parts depends upon the Zn levels in the growth media. At levels of 0.076 and 0.769 uM Zn, high P did not significantly increase Zn uptake in shoots and roots. However, a Zn level of 7.69 uM P significantly increased Zn uptake in shoots and roots of both soybean cultivars. This increased Zn uptake, in general, was not

Table 2. Dry-matter, Zn , and P Concentration in Trifoliate Leaves, Stems, and Roots of York and Beeson Soybean Cultivars as Affected by P and Zn Levels in Cultural Solutions.#

Treatments		Zn Deficiency	Dry-matter		Zn Concentration		P Concentration	
P	Zn		S	R	S	R	S	R$
mM	uM		g pot^{-1}		mg kg^{-1}		g kg^{-1}	
			Zn-sensitive (York) variety					
0.25	0.076	++++	4.1	1.3	23.4	29.6	7.3	5.5
	0.769	+	7.2	1.4	46.3	39.5	3.3	5.3
	7.69	+	2.7	0.9	285.7	299.7	8.8	8.4
2.50	0.076	++++	4.6	1.3	26.9	29.3	10.8	23.3
	0.769	++	5.7	1.2	60.4	37.1	6.6	18.3
	7.69	+	3.2	0.9	285.1	573.3	9.7	19.4
			Zn-normal (Beeson) variety					
0.25	0.076	+++	6.2	2.1	23.3	30.8	6.0	2.8
	0.769	+	7.4	2.2	62.3	45.1	4.2	2.5
	7.69	+	5.4	1.9	253.9	487.6	5.0	3.0
2.50	0.076	++++	6.5	2.1	29.7	39.4	9.6	19.7
	0.769	++	7.4	2.2	59.8	50.1	7.4	15.5
	7.69	+	5.8	1.9	282.0	661.5	8.0	15.4
$LSD_{0.05}$+			0.5	0.2	26.2	24.1	1.1	2.3
$LSD_{0.01}$+			0.7	0.2	37.4	34.4	1.7	3.5

Each number is an average of 4 replicates.

+ LSD was calculated according to Steel and Torrie(1980),and used to compare any two numbers within each column.

$ R - Roots and S - Shoots.

associated with increasing growth and dry-matter accumulation and, therefore, resulted in a sharply increasing Zn concentration in plant parts (Table 2) with a maximum of 661.5 mg kg^{-1} in roots of the Beeson soybean variety. This high concentration of Zn could be toxic and may explain the reduction in dry-matter accumulation recorded at high Zn levels. Edwards and Kamprath (1974) found that P did not effect Zn uptake or translocation by corn plants. Millikan (1963), Watanabe et al., (1965), and Jackson et al., (1967) reported that P application either had no effect on Zn uptake or increased it.

P uptake in shoots and roots behaved similarly in both soybean cultivars. At all P levels P uptake in shoots decreased with increasing Zn level in nutrient solution. Zn deficient plants have the highest P uptake and concentration in the shoots. The increase of shoot growth with increasing Zn levels in nutrient solution up to some limit may account for the general drop in P uptake in shoots. Safaya (1976) reported that Zn deficient corn plants had the highest P concentration in their tissue, and they showed the highest P flux.

Data listed in Tables 1 and 2 show that, with the exception of the high Zn treatment, Zn uptake and Zn concentration did not explain the appearance of Zn deficiency on the plants treated with the high P concentration. In both cultivars, at the same level of Zn, Zn deficiency was always more severe on the plants which received the high P treatment, even though plant shoots contained almost the same amount of Zn. On the other hand P/Zn ratios were correlated well with Zn deficiency and always increased with increasing severity of deficiency symptoms. P significantly increased the P/Zn ratio only when 0.076 and 0.769 uM Zn were used. The increasing of P/Zn ratios occurred mainly as a result of increasing the P uptake by adding a high level of P. The association of high P/Zn ratio with Zn deficiency at a high level of P has been taken as strong evidence that under such conditions Zn deficiency is a reflection of a P/Zn imbalance in plant tissue. These results confirm what has been found by Sumner et al., (1982). Kahn and Soltanpour (1978) reported that the chlorotic leaves of dryland beans had higher mean levels of P and Zn, and lower Zn than the green leaves. However, in their work the increasing P content and decreasing Zn content in the chlorotic leaves gave rise to the high P/Zn ratios.

Subcellular Localization of Zn in Leaves:

Initially, the validity of the fractionation technique was tested to make sure that the observed localization patterns were not an artifact that occurred during the homogenization process. Soybean plants were grown under the same experimental conditions described above, but no radioactive ^{65}Zn was added into the nutrient solution. Leaves were taken and homogenized in the same grinding solution that contained three different known amounts of ^{65}Zn. Samples were fractionated using the same procedure described in the materials and methods section. Comparing the reconstituted ^{65}Zn activities in subcellular fraction with the amount of ^{65}Zn activities added at the beginning shows that the recovery ranged between 103.2 and 96.4% (Table 3). The results also show that the amounts of ^{65}Zn added to the homogenizing solution does not affect the percentage of ^{65}Zn found in each fraction, and that about 90% of the ^{65}Zn is recovered in the "soluble" fraction. The amount of ^{65}Zn bound to the cell wall and

Table 3. Percentage of ^{65}Zn Radioactive Recovery in Subcellular Organelles of Soybean Leaves Homogenized in Grinding Solution Containing different Amounts of ^{65}Zn.

		Subcellular Organelles						
Sample No.	^{65}Zn Added	600g Nu+Ch	3000g Br.Ch	18000g Mito	45000g Frac.	Soluble Frac.	C.W. C.De.	Recovery
	uCi	%						
1	0.080	0.9	1.2	1.2	1.1	91.6	4.1	103.2
2	0.161	0.9	1.3	1.3	1.1	90.8	4.6	99.0
3	0.241	1.1	1.2	1.2	1.1	90.5	4.9	96.4

Nu+Ch - Nucleus and Chloroplasts.
Br Ch - Broken Chloroplasts.
Mito - Mitochondria.
C.W. - Cell Wall.

cell debris is very small and likely results from the inadequacy of the washing procedure.

Tables 4 and 5 present the localization pattern of Zn in the leaves of soybean plants expressed as uCi pot^{-1} and percentage of the reconstituted total ^{65}Zn. The amount of ^{65}Zn localized in each subcellular organelle systematically increased as the overall Zn content increased with increasing concentration of Zn in the nutrient solutions. The percentage of ^{65}Zn localized in each subcellular organelle seems to be constant even when the Zn concentration in the nutrient solutions was increased from 0.076 to 0.769 uM. However, increasing the Zn concentration to 7.69 uM resulted in a significant increase in the percentage of ^{65}Zn localized in each subcellular fraction. The largest amount of ^{65}Zn in leaves was present in the "soluble" fraction. ^{65}Zn bound to the cell wall and cell debris were the next largest fraction. Assessment of this localization pattern leads to a general conclusion that the large storage of Zn in a "soluble" form (mainly in cytoplasm and vacuoles) establishes an equilibrium that controls, to some extent, the amount of Zn localized in each subcellular organelle.

Polar (1976) reported that the disadvantage of this fractionation technique comes from using a single type of grinding solution which may be suitable for a specific type of subcellular organelle but not for others. However the presence of ^{65}Zn activity in the large subcellular organelles such as nucleus, chloroplasts and mitochondria is convincing evidence against the possibility of having an artifact caused by a measurable leakage of ^{65}Zn out of these organelles during the homogenization and fractionation procedures.

Table 4 . Subcellular localization of ^{65}Zn (uCi/pot) in trifoliate leaves of York and Beeson Soybean cultivars as affected by P and Zn Levels in cultural solution.#

Treatments		Subcellular Organelles					
P	Zn	600 g Nu+Ch	3000 g Br Ch	18000 g Mito	45000 g Frac.	Soluble Frac.	C.W. C.Debri
mM	uM	uCi pot^{-1}					
		Zn-sensitive (York) variety					
0.25	0.076	0.017	0.015	0.016	0.012	0.298	0.168
	0.769	0.075	0.062	0.068	0.036	1.283	0.726
	7.69	0.358	0.383	0.256	0.062	2.814	1.742
2.25	0.076	0.033	0.022	0.037	0.017	0.483	0.233
	0.769	0.110	0.069	0.075	0.032	1.285	0.839
	7.69	0.658	0.354	0.283	0.102	3.153	2.238
		Zn-Normal (Beeson) variety					
0.25	0.076	0.017	0.019	0.035	0.022	0.540	0.155
	0.769	0.078	0.078	0.133	0.100	2.393	0.651
	7.692	0.654	0.339	0.428	0.254	6.776	2.256
2.25	0.076	0.052	0.032	0.045	0.028	0.750	0.212
	0.769	0.103	0.070	0.122	0.073	2.047	0.635
	7.69	1.623	0.383	0.693	0.282	7.256	3.201
$LSD_{0.05}$+		0.100	0.032	0.035	0.022	0.389	0.230

Each number is an average of 4 replicates.

+ LSD was calculated according to Steel and Torrie (1980), and used to compare any two numbers within each column.

Nu+Ch - Nucleus and Chloroplasts.

Br Ch - Broken Chloroplasts.

Mito - Mitochoneria.

C.W. - Cell Wall.

Table 5 . Subcellular localization of ^{65}Zn (percentage of reconstituted radioactivity) in trifoliate Leaves of York and Beeson soybeans cultivars as affected by P and Zn levels in cultural solutions.#

Treatments		Subcellular Organelles					
P	Zn	600 g Nu+Ch	3000 g Br Ch	18000 g Mito	45000 g Frac.	Soluble Frac.	C.W.+ C.Debri
mM	uM			%			
				Zn-sensitive (York) variety			
0.25	0.076	3.32	2.85	3.03	2.23	56.6	32.0
	0.769	3.25	2.77	3.01	1.62	57.1	32.3
	7.69	6.39	6.85	4.58	1.11	50.2	31.0
2.25	0.076	4.03	2.72	4.45	2.09	58.3	28.4
	0.769	4.55	2.91	3.11	1.32	53.3	34.8
	7.69	9.73	5.22	4.21	1.50	46.4	32.9
				Zn-Normal (Beeson) variety			
0.25	0.076	2.18	2.39	4.48	2.82	68.5	19.6
	0.769	2.26	2.27	3.88	2.90	69.9	18.9
	7.692	6.13	3.18	3.99	2.38	63.3	21.1
2.25	0.076	4.66	2.82	4.05	2.50	67.0	19.0
	0.769	3.20	2.33	3.95	2.41	66.7	21.4
	7.69	12.02	2.85	5.16	2.12	53.9	24.2
$LSD_{0.05}$+		0.95	0.52	0.52	3.92	4.9	3.3

Each number is an average of 4 replicates.

+ LSD was calculated according to Steel and Torrie (1980), and used to copmare any two numbers within each column.

Nu+Ch.- Nucleus and Chloroplasts.
Br Ch - Broken Chloroplasts.
Mito - Mitochondia.
C.W. - Cell Wall.

The presence of Zn in chloroplasts, nucleus, and mitochondria is in agreement with its functions. Zn is known to be an essential part of carbonic anhydrase enzyme, which is mainly a chloroplast enzyme (Poincelot, 1972; Randel and Bouma, 1973; and Jacobson et al., 1976). Zn is also somehow related to DNA and the protein moiety in these organelles (Riami and Heyde, 1970; Slater et al., 1971; and Zimmer, 1973).

A high P level in the nutrient solution significantly ($p < 0.05$) increased the quantity of ^{65}Zn localized in most subcellular organelles at the high Zn concentration in the nutrient solution. This follows the significant increase of the overall Zn content in leaves. However, P did not effect the relative amount of ^{65}Zn localized in all separated organelles, except for chloroplasts and nucleus (600 g fraction).

Considering the amounts of ^{65}Zn presented in the "soluble" fraction and bound at cell wall and cell debris, soybean cultivars exhibits some differences in their relative amounts. The percentage of ^{65}Zn activity bound by the cell wall and cell debris in the leaves of Zn-sensitive soybean (York) was higher than that in the leaves of the normal soybean (Beeson). The reverse is true for the "soluble" form. Polar (1976) reported that cell wall fraction separated from leaves of broad beans bind Zn through the uronide-carboxy groups of their polysaccharides.

Zn-binding Compounds:

The data presented in Table 4 show that the largest proportion of ^{65}Zn in soybeans is accumulated in the "soluble" fraction. In this fraction Zn is associated with some of the cytoplasmic enzymes, coordinated with amino, imidazolyl and sulfhydryl groups (Passow and Clarkson, 1961), and may be present in ionic form. To separate each type of these compounds and measure its proportion in the "soluble" fraction, a gel permeation technique was used. Using the most suitable gel type (Sephadex G10-120), long gel beds (70 cm) and collecting a small fraction volume (2 ml), one can separate molecules which differ to some extent in size and properties.

Data presented in Figures 1 and 2, and in Table 6 show that three different fractions that contained ^{65}Zn were eluted out of the gel column. These three fraction are of three different molecular weights. The first fraction, fraction "A", which eluted at the void volume of the column contains compounds of molecular weight more than 700. This fraction mainly constitutes the Zn-containing proteins. Fraction "C", which eluted at the elution volume of $^{65}ZnCl_2$, may contain the ionic form of Zn. In between these two fractions fraction "B" was eluted. This fraction should contain those compounds with a molecular weight lower than 700 and greater than that of $^{65}ZnCl_2$. Separation by gel chromatography depends upon the molecular size, and does not show whether the separated fraction contains one compound or more. Therefore, the results obtained using this technique should be carefully interpreted.

Data listed in Table 6 present the amounts of soluble Zn-binding compounds and their relative percentage in the "soluble" fraction extracted from leaves of soybean cultivars. The amount of ^{65}Zn activity recovered in fraction "A" and fraction "B" are, in general, significantly increased by increasing Zn supply up to 0.769 uM at all levels of P. ^{65}Zn recovered in fraction "C", however,

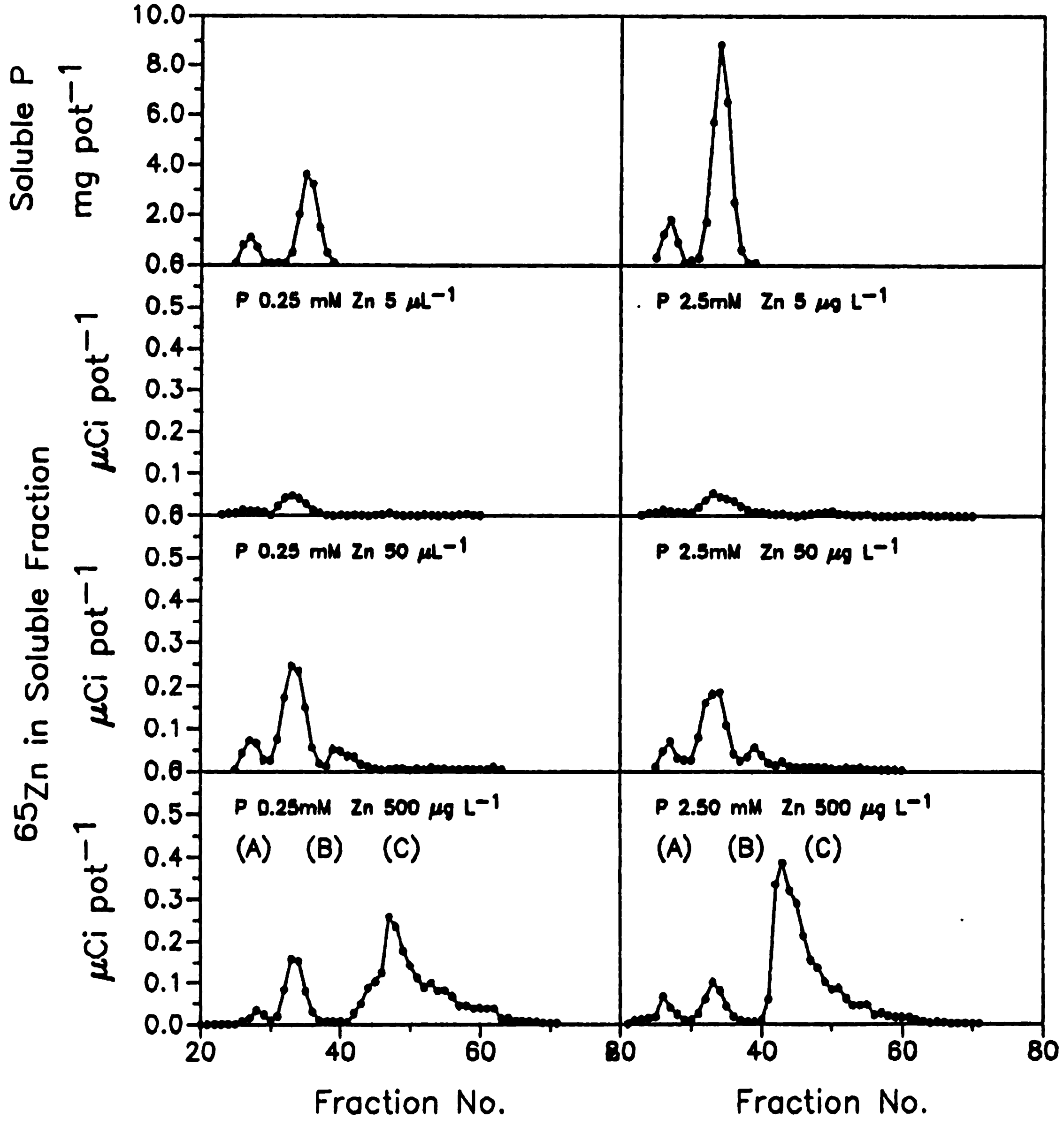
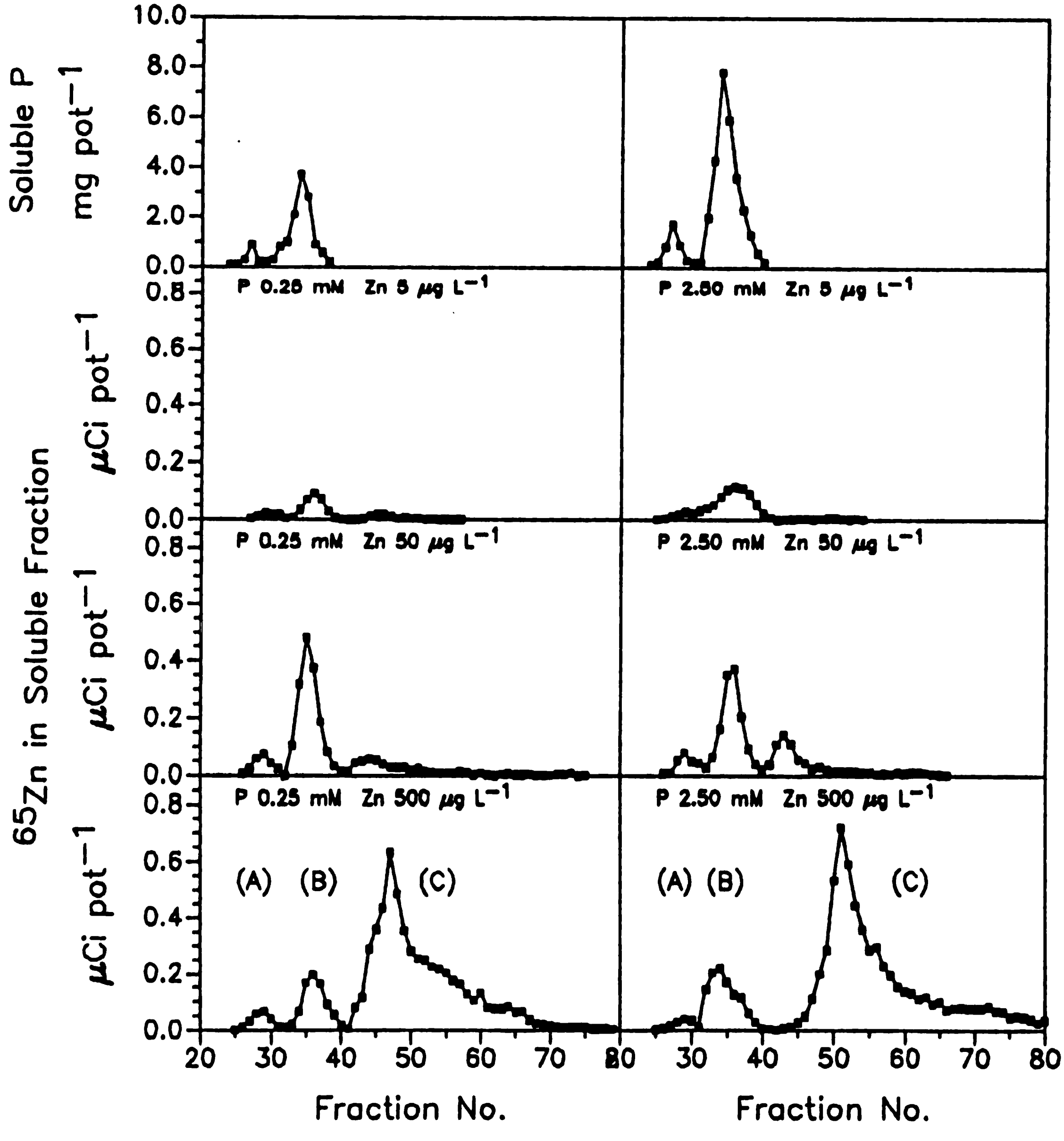


Fig. 1. Chromatography of NH_4OAc–Soluble Zn–Binding Compounds Extracted From Leaf Blades of York Soybean and Separated on Sephadex G10–120.



Fif. 2 . Chromatography of NH_4OAc–Soluble Zn–Binding compounds Extracted From Leaf Blades of Beeson Soybean and Separated on Sephadex G10–120.

Table 6 . NH$_4$OAc-soluble Zn-binding compounds extracted from trifoliate leaves of York Beeson soybean cultivars as affected by P and Zn levels in cultural solution.#

Treatment		M.W. of Zn-binding Compounds@					
P	Zn	>700	<700	Ionic	>700	<700	Ionic
mM	uM	uCi pot^{-1}			% of Total Soluble Zn		
		Zn-Sensetive (Yorke) variety					
0.25	0.076	0.055	0.169	0.075	18.7	56.4	24.9
	0.769	0.197	0.893	0.252	14.7	66.0	19.4
	7.69	0.104	0.434	2.426	3.5	14.8	81.7
2.50	0.076	0.064	0.288	0.140	13.2	58.3	28.5
	0.769	0.158	0.680	0.457	12.2	51.4	36.5
	7.69	0.101	0.310	2.674	4.6	9.9	85.5
		Zn-normal (Beeson) variety					
0.25	0.076	0.082	0.338	0.109	15.4	63.9	20.6
	0.769	0.363	1.551	0.371	15.9	67.9	16.2
	7.69	0.186	1.290	5.222	2.7	19.1	78.2
2.50	0.076	0.108	0.618	0.073	13.8	76.8	9.4
	0.769	0.326	1.684	0.169	15.0	77.2	7.8
	7.69	0.191	1.272	5.625	2.2	18.0	79.8
LSD$_{0.05}$+		0.089	1.429	0.258	2.5	42.6	41.8

@ The molecular weights are inducted from the execlution limit of the Sephadex Gel G10-120.

Each number is an average of two replicates.

+ LSD was calculated according to Steel and Torrie(1980), and used to copmare any two numbers within each column.

increased with increasing Zn supply up to 7.69 uM. It appears that the low-molecular weight Zn-binding compounds function as an intermediate between the ionic form and Zn-containing proteins, and thus, help to regulate the amounts of Zn containing proteins in leaves. Consequently, the relative amount of ^{65}Zn present in each fraction was kept constant regardless of increasing Zn concentration in the nutrient solution up to 0.769 uM Zn. Increasing the Zn concentration in the nutrient solution to 7.69 uM resulted in increasing the amount of "soluble" Zn in leaves as shown in Table 4. However, by increasing Zn supply to 7.69 uM a greater proportion of this "soluble" Zn is present in the ionic form and reaches a maximum at the high P level. At that level of external Zn supply the ionic form could be toxic and has a direct impact on the formation of Zn containing enzymes, and also may have some effect upon the activities of the Zn-containing proteins present in fraction "A".

Even though P did not show significant effects upon the amounts of Zn bound to any of these fractions, a high P level exhibits some effects upon the distribution of Zn between these fraction. These effects do not vary in the same way in the two different soybean cultivars. In York, high P level increases the amount of Zn bound to proteins and to the small molecular weight compounds in the leaves of the plants grown at 0.076 uM external Zn supply by 16.4 and 70.4%, respectively, but decreases the amount of Zn bound to these fractions by 19.8 and 23.9 % in plants grown at 0.769 uM Zn, and by 2.9 and 28.6% in plants grown at 7.69 uM external Zn supply, respectively. Also, high P level increases the ionic form recovered from the leaves of York variety grown at 0.076, 0.769, and 7.69 uM external Zn supply by 86.7, 81.3, and 10.2%, respectively. It has been shown above (Table 4) that high P level did not affect the amount of "soluble" Zn in York variety grown at 0.769 uM Zn and increased it by 62.1% in the leaves of the York variety grown at 0.076 uM external Zn supply. On the other hand the Beeson variety of soybean behaves alike at 0.076 uM external Zn supply with respect to fraction "A" and fraction "B", but the amount of Zn present in the ionic form decreased at high P level with increasing the external Zn supply up to 0.769 uM. These results revealed that high P level in general did not affect the total "soluble" Zn in the leaves of soybean plants, but changed to some extent the distribution of Zn between the different forms. By increasing the external P supply, Zn tended to accumulate in an ionic form and somehow did not participate in the formation of Zn-containing enzymes (proteins) and Zn-containing intermediates. This affect is quite clear in York variety and could explain its response to the external P and Zn supply.

The gel filtration chromatograms presented in Figures 1 and 2 show the P-containing fractions overlapped with the Zn-containing fractions "A" and fraction "B". P could be a part of Zn-containing proteins. However, these data cannot tell whether or not both P and Zn are bound to the same small molecular weight compounds.

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CHAPTER III

CHAPTER III

EFFECT OF P LEVEL IN NUTRIENT SOLUTION ON THE KINETICS OF ZN UPTAKE, TRANSLOCATION, AND ACCUMULATION BY TWO SOYBEAN CULTIVARS.

INTRODUCTION

Although P-Zn interaction has been extensively studied, considerable disagreement exists in the literature concerning the influence of P upon Zn uptake (Olsen, 1972). In some cases P decreased the total uptake of Zn (Loneragan, 1951; Langin et al., 1962; Asif and Ajakaiye, 1974; Takkar et al., 1976; Malavolta and Gorostiage, 1974; and Safaya, 1976); however, other investigators found that the application of P reduced Zn concentration in plant tissues but had no effect or increased total uptake and translocation of Zn (Millikan, 1963; Boawn and Leggett, 1964; Henning, 1971; Adriano, 1970; and Wallace et al., 1[illegible]. However, there is a general agreement that P and Zn interact i[illegible]de plants, and that roots are, most likely, the site of this interaction (Burleson et al., 1961; Loneragan and Asher, 1967; Sharma et al., 1968a; and Sharma et al., 1968b). This view was strongly supported by the results obtained by Dwivedi et al., (1975) showing that high P levels in the growth media rendered the applied Zn unavailable to leaves by immobilizing nearly 40% of the total absorbed Zn in the roots.

Olsen (1972) reported that the effects of applied P on the total uptake of Zn depends upon the balance between two factors: the yield response and the concentration of Zn in the tops of the plants. Therefore, a wide variation in the effects of P on total uptake of Zn has been reported, and it can not be predicted whether applied P will increase, decrease or have no effect on total uptake of Zn by plants. However, most of the investigations conducted on the P-Zn interaction in plants considered the total amount of Zn taken up over a period of time as a measure of the effects of P on uptake or absorption of Zn without examining the effect of the time and treatment on the growth of the roots, which usually mask the true interaction. Thus in studying the P-Zn interaction, it is necessary to distinguish between growth mediated interaction effects on total uptake of Zn, and the interaction effects on the amount of Zn taken up per unit weight of roots per unit time (Safaya, 1976).

A thorough examination of the literature revealed that Zn uptake has been mainly studied using a specific plant tissue (Bowen, 1969 and Rathore et al., 1970), excised plant roots (Hassan and Tang van Hai, 1976; Veltrup, 1978; Bowen, 1973), and intact plants (Chandel and Saxena, 1980; and Chaudhry and Loneragan, 1972). Most of these workers examined the kinetic character of Zn uptake under normal growth conditions. However, little work has been done to investigate the effects of increasing the P level in the growth media upon the rate of Zn uptake and its kinetic character. Safaya (1976) found that P reduced Zn flux through corn roots. Dogar and Tang van Hai (1980), using rice as a test plant, found that low P levels had no or slightly increasing effect on the rate of Zn uptake. But, high P levels suppressed Zn uptake.

In view of the fact that the effects of heavy application of P on the uptake of Zn by soybean plants varies between cultivars, and that there is a great need to know the effect of P on the rate of Zn uptake, it was important to investigate the impact of increasing P level in the growth media upon the rates of Zn uptake, translocation, and accumulation by soybean cultivars, and upon the kinetic characters of these processes.

MATERIALS AND METHODS

Short time Zn uptake studies were conducted using two soybean cultivars grown in nutrient solution labeled with ^{65}Zn. Soybean seeds were planted and cultured hydroponically as described in Chapter II, except that seedlings were grown in the greenhouse supplemented with 8 sodium-metal high intensity lights placed approximately at 85 cm above the tops of the plants. The same nutrient solution was used except for P and Zn which were kept at minimum concentrations (0.05 mM and 1.52 uM, respectively) to prevent the appearance of any Zn deficiency symptoms.

By the end of a three-week growth period soybean plants were transferred into nutrient solution that have the same components and 4 different levels of P (0.25, 0.50, 1.00, and 2.50 mM NaH_2PO_4). The pH of the nutrient solution was adjusted to pH 6.0 ± 0.2 by using a different amount of solid phase $CaCO_3$, the pH monitored daily and the nutrient solutions changed when the pH changed by more than 0.5 pH units. Plants were grown in these solution for 48 hours to reach a steady state and raise the magnitude of the phosphate pool inside the plants. After 48 hours soybean plants were transferred into the nutrient solution with treatments developed to give 14 different levels of Zn as $ZnCl_2$. Zn concentrations used ranged between 0.076 and 76.5 uM Zn. The nutrient solutions were labeled using $^{65}ZnCl_2$ so that a final specific activity of 1 uCi per 25 ug Zn was maintained. All the treatment combinations were arranged in a randomized complete block design and replicated three times. Plants were grown in the uptake solutions for 24 hours, after which the experiment was terminated by taking the plants out of the uptake solution, washing the roots in deionized water three times each of 1 minute, and then rinsing them in cold (4-8 C) 0.5 mM $CaCl_2$ three times (1 minute each washing), and finally washing the roots in deionized water again.

After blotting the roots between filter papers, plants were separated into roots, stems and petioles, and leave-blades, and then dried at 70 C for 24 hours. Plant materials were ground to pass through a 40 mesh sieve. ^{65}Zn in the dry, ground plant tissues was counted using a gamma counter (Packard). The amount of Zn taken up by each plant tissue, expressed as uM Zn per gram dry matter, was calculated from the amount of ^{65}Zn detected in each plant tissue and the specific activity of ^{65}Zn in the uptake solutions. The rates of Zn uptake, translocation, and accumulation were calculated for each Zn level at each P level.

To calculate the kinetic characters of Zn uptake, translocation, and accumulation of soybean plants the Lineweaver and

Burke double reciprocal plots, as described by Hofstee (1952) were established. The rates of Zn uptake, translocation, and accumulation at an external Zn concentration of 0.076 uM were unusually high. The low Zn contents inside the plants at the beginning of the uptake period may account for that. To avoid any significant effects on the calculation of the kinetic characters at the low external Zn concentration, the first Zn level (0.076 uM Zn) was excluded.

RESULTS AND DISCUSSION

Effect of P Levels on the Rate of Total Zn Uptake:

Figure 1 shows the effect of increasing P concentration in the growth media upon the rate of total Zn uptake of the intact soybean plants as a function of the external Zn concentration. The rate of Zn uptake by both soybean cultivars increased rapidly with increasing the external Zn concentration upto 15.3 uM Zn. Thereafter, the rate of Zn uptake remained almost constant with further increases in the external Zn concentration up to 76.5 uM Zn. The rate of total Zn taken up by the York soybeans was higher than that of Beeson soybeans at low external Zn concentrations (up to 3.82 uM Zn). However, at higher external Zn concentrations the rate of total Zn uptake of both cultivars was similar.

The effect of P levels in the nutrient solution on the rate of Zn uptake appeared to vary with cultivars and external Zn concentration. At low Zn concentrations (upto 3.82 uM Zn) P showed no significant effect ($p<0.05$) upon the rate of total Zn uptake in either cultivar. Increasing P concentration to 0.5 mM P had no effect on the

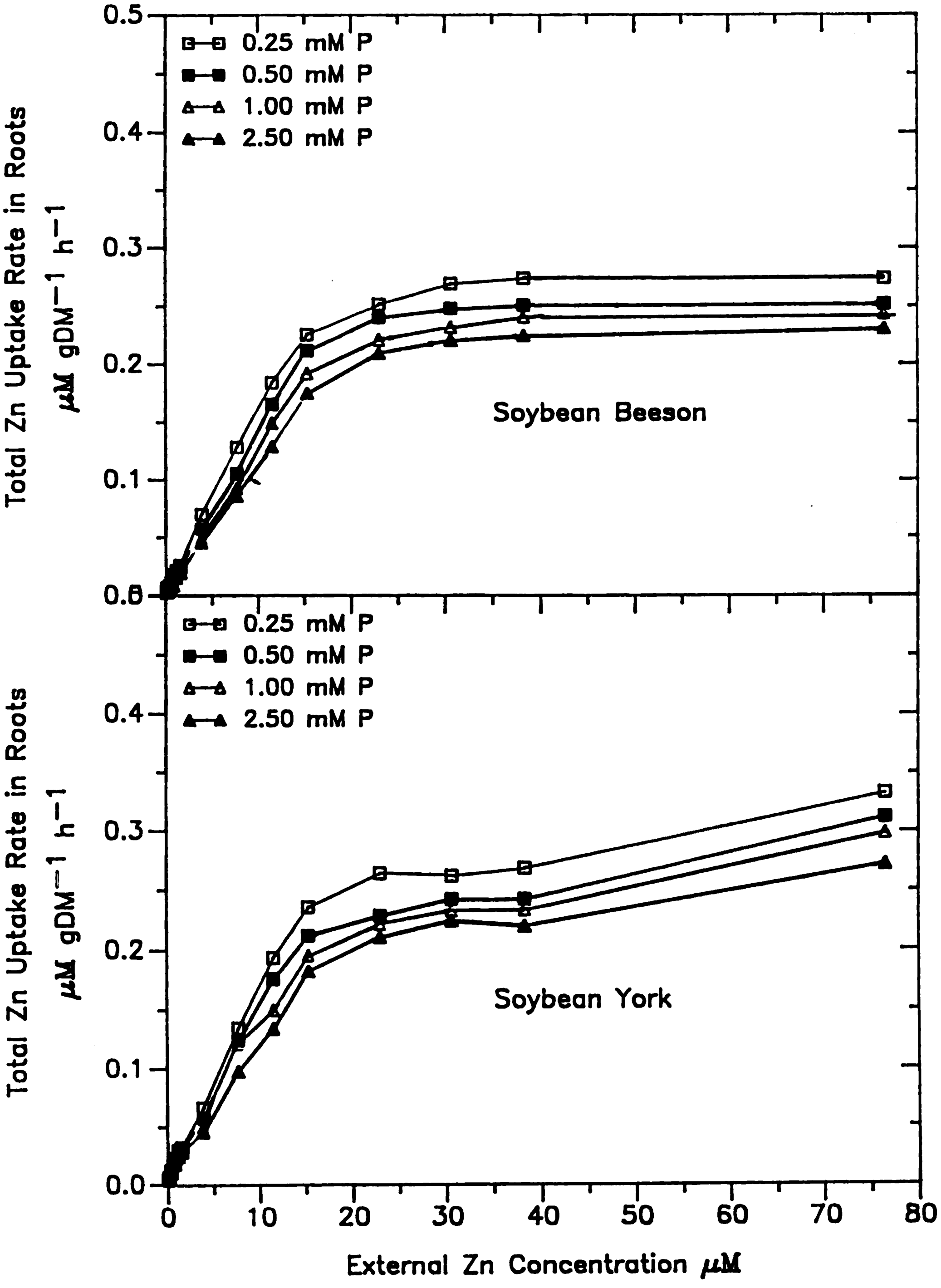

Fig. 1 . Effect of P levels in nutrient solution on the rate of total Zn uptake by soybean plants.

rate of total Zn uptake of Beeson soybeans; however, P significantly decreased the rate of the total Zn taken up by the plants of York soybeans. The rate of total Zn uptake at external Zn concentrations higher than 3.82 uM Zn was suppressed significantly ($p<0.05$) by further increments of P application beyond 0.5 mM. Within the short uptake period of 24 hours the effect of P on the growth can be neglected, thus the reduction in the Zn uptake rate by high levels of P is not mediated by the effect of P on the growth rate, and P may have a direct effect on the uptake systems of the soybean plant roots. These results further demonstrate that, considering the total Zn uptake rates, soybean York is more susceptible to P-Zn interaction. Safaya (1976) and Dogar and Tang van Hai (1980) reported that application of high P levels reduced the rate of Zn uptake by corn and rice plants, respectively.

Effect of P Level on the Rate of Zn Translocation into Shoots and Accumulation in Roots of Soybean Plants:

The results presented in Figures 2 and 3 show the influence of P level upon the rate of Zn translocation into shoots and accumulation in roots of the soybean plants. The effect of high levels of P on Zn translocation into shoots and accumulation in roots was different for the two cultivars. At low levels of Zn (up to 3.82 uM) application of P had no impact on either Zn translocation or accumulation in either soybean cultivar. But, at external Zn concentration higher than 3.84 uM, applications equal to or greater than 0.5 mM P significantly reduced the rate of Zn translocation, and increased the rate of Zn accumulation in the roots of York

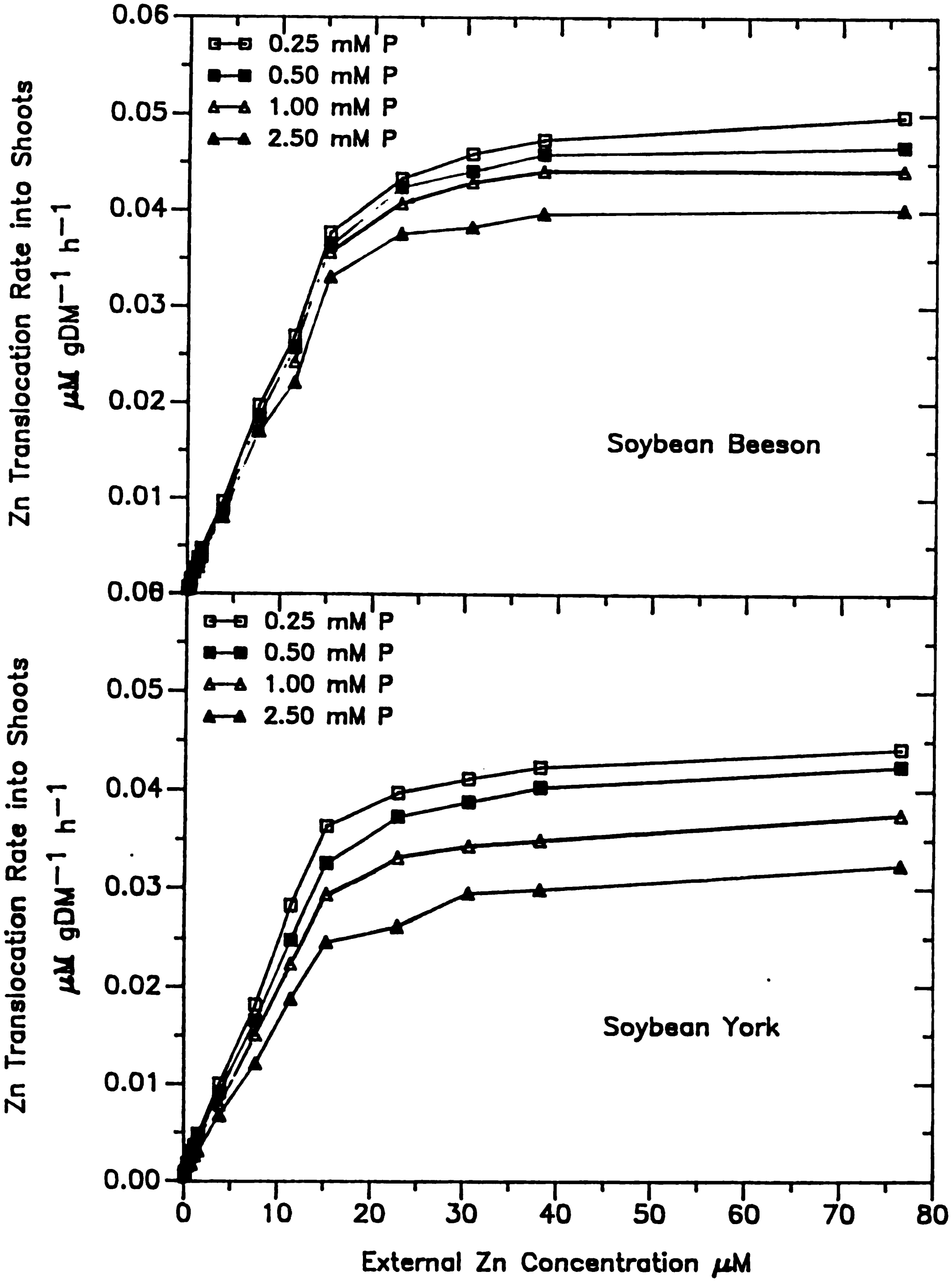

Fig. 2 . Effect of P levels in nutrient solution on the rate of Zn translocation into the shoots of soybean plants.

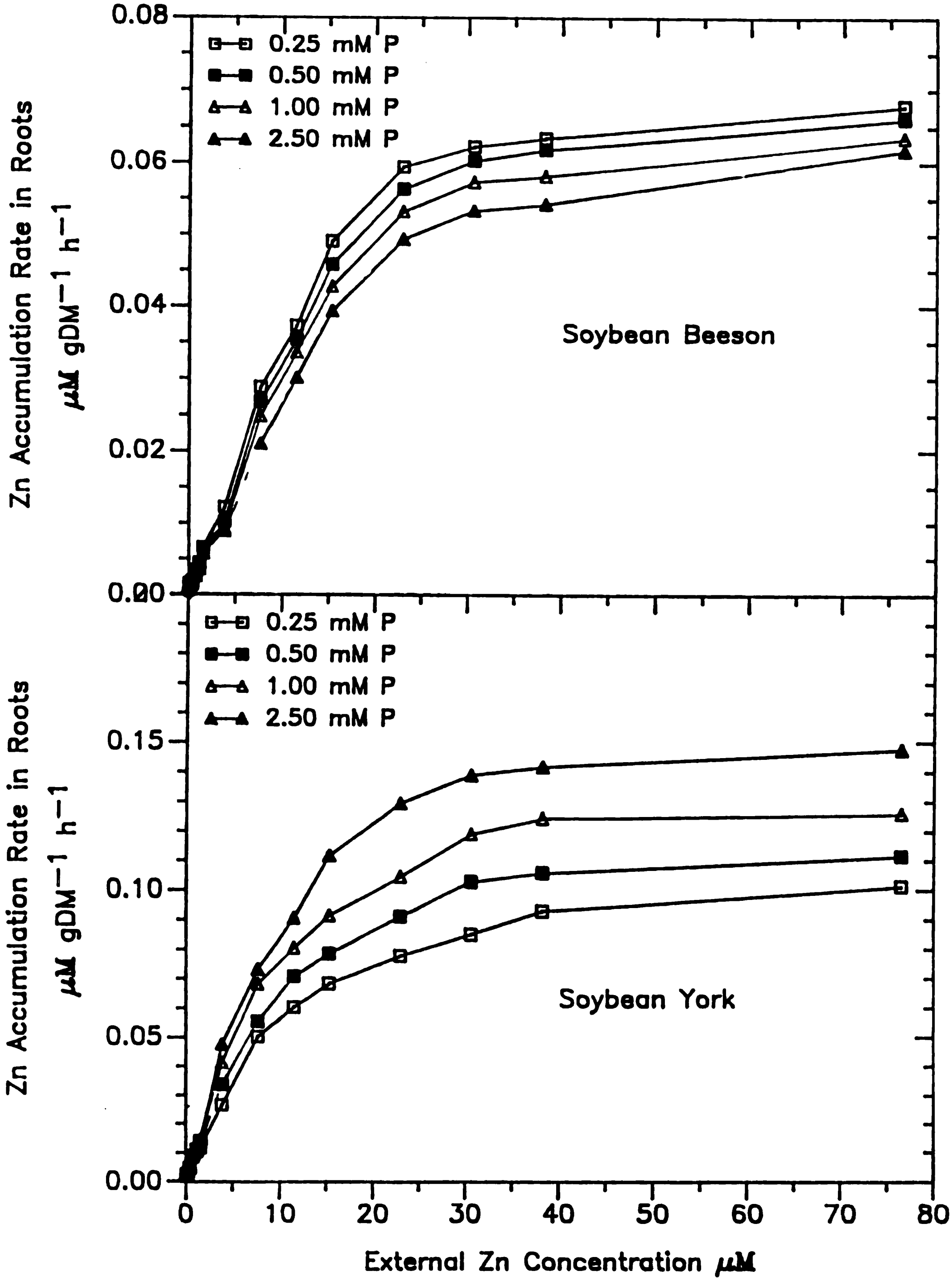

Fig. 3 . Effect of P levels in nutrient solution on the rate of Zn accumulation in the roots of soybean plants.

soybeans. These results are in agreement with those reported by Stukenholtz et al., (1966) and Sharma et al., (1968). With Beeson soybeans, however, only the application of 2.5 mM P accounted for the decline of the rate of both Zn translocation and Zn accumulation. The high levels of P in the nutrient solution most probably inhibit Zn accumulation in the tops of soybean plants, first by reducing the rate of its absorption through the epidermal or the external surface cell layer of the roots, and secondly by suppressing its release through the endodermis cells into the root xylem. The suppression of the rate of Zn uptake and Zn translocation occurred, in most cases, at the high external concentration range of Zn, thus the effect of P may be expressed on the second phase of the Zn uptake system, which was characterized by a low affinity for Zn.

Effect of P Level on the Kinetic Characters of Zn Uptake, Translocation, and Accumulation by Soybean Plants:

The kinetic characters, Vm and Km, of the ions absorption and translocation system(s) are used to measure, respectively, the capacity and the affinity of the system(s) to the ions under consideration (Epstine and Hagen, 1952; and Epstine, 1972). If Km is a small number, the affinity between the absorption and translocation systems and the ion under consideration is high. The reverse is true as the Km approaches unity. Vm is related to the amount of the carrier(s) present and the rate at which it turns over.

Data presented in Table 1 shows the effect of increasing P level in nutrient solution on the kinetic characters of Zn uptake, translocation, and accumulation by plants of the soybean cultivars

Table 1. Effect of P Level on the kinetics characters of total uptake, translocation, and accumulation of Zn by two soybean cultivares.#

	Soybean York				Soybean Beeson			
	Low Zn Range		High Zn Range		Low Zn Range		High Zn Range	
P	0.153-1.53 uM		3.82-76.48 uM		0.153-1.53 uM		3.82-76.48 uM	
	Vm	Km	Vm	Km	Vm	Km	Vm	Km
mM	uMg^{-1}h^{-1}	uM	uMg^{-1}h^{-1}	uM	uMg^{-1}h^{-1}	uM	uMg^{-1}h^{-1}	uM
				Total Zn Uptake				
0.25	0.079	1.83	0.484	20.8	0.064	2.37	0.478	21.7
0.50	0.067	1.67	0.493	26.1	0.065	2.68	0.519	30.1
1.00	0.049	1.47	0.691	35.1	0.064	2.96	0.562	38.9
2.50	0.045	1.65	0.546	41.9	0.029	1.45	0.540	40.9
F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	31.5**
LSD$^{@}$								5.5
				Zn Translocation				
0.25	0.023	5.74	0.081	26.2	0.015	3.0	0.108	37.9
0.50	0.017	4.54	0.077	27.6	0.012	3.0	0.112	42.7
1.00	0.013	3.83	0.073	30.7	0.014	4.0	0.108	43.3
2.50	0.008	2.37	0.057	27.6	0.013	3.9	0.091	37.7
F	7.49*	n.s.	10.6**	n.s.	n.s.	n.s.	n.s.	n.s.
LSD$^{@}$	0.008		0.011					
				Zn Accumulation				
0.25	0.042	3.43	0.132	14.7	0.009	1.4	0.191	54.9
0.50	0.030	1.94	0.139	11.8	0.012	2.2	0.224	71.1
1.00	0.025	1.50	0.153	10.2	0.013	2.6	0.289	98.6
2.50	0.025	1.33	0.181	10.8	0.014	3.3	0.256	95.4
F	n.s.	n.s.	46.1**	7.2*	n.s.	n.s.	n.s.	n.s.
LSD$^{@}$			0.011	2.5				

\# Each number is an average of three replications.

** Significant at $p<0.01$

* Significant at $p<0.05$

@ LSD at $p<0.05$

York and Beeson. When the external Zn concentration increased, both Vm and Km increased. The data convincingly demonstrates the changes in the state of the carrier system in response to the high P level in the nutrient solution. Nissen (1974) reported that the state of the carrier system(s) may be changed as a result of changing many factors such as the total electrolyte concentration, and electrical potential difference across the plasma membrane.

At the external low Zn concentration range (0.076-1.53 uM Zn) P either decreased or had no significant effects on Vm of both soybean cultivars. Since P and Zn are not competitive for the same carrier or the same absorption site, this reduction in Vm could be a result of changing the conformation of the carrier or the uptake site. High P concentrations may also reduced the rate at which the carrier turns over. The competitive inhibition of Zn absorption by Ca^{2+} ions, which are present in the nutrient solution at relatively high level associated with high P levels could be another explanation for the reduction of Vm. Chaudary and Loneragan (1972) showed that the inhibitory effects of Ca^{2+} increased as its concentration increased from 250 uM to 10 mM. The reduction of Vm by increasing P concentration in the nutrient solution is eliminated by increasing the external Zn concentration range to 3.82-76.5 uM Zn.

The affinity of the absorption system(s) for Zn ions, as manifested by the magnitude of Km value, is affected by increasing P concentration in the growth media. Only at the high Zn concentration range did P significantly increase the Km values of the absorption system in the plants of both soybean cultivars. High P concentration in the nutrient solution may reduce the affinity of the absorption system(s) to Zn ions, and thus ultimately reduced the Zn uptake rates at high Zn concentration ranges as shown in Figure 1. The translocation system(s) seems to be unaffected by the external P concentration as indicated by its relatively unchanged Km values (Table 1). Thus the changes in the translocation rates, reported above in Figures 1,2 and 3, as the P level increased could be a direct effect of the decreasing or increasing the accumulation rate of Zn in roots.

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CHAPTER IV

CHAPTER IV

CARBONIC ANHYDRASE AND SUPEROXIDE DISMUTASE ACTIVITIES IN SOYBEAN LEAVES AS AFFECTED BY HIGH P LEVELS IN THE GROWTH MEDIA AND THEIR USE IN DIAGNOSING ZN DEFICIENCY.

INTRODUCTION

Total concentration of Zn in plant parts, as measured by the conventional method of plant analysis, usually enables reliable diagnosis of Zn deficiency in plants. However, Ghoneim et al., (1978) and Dwivedi and Takkar (1974) reported that diagnosis of Zn deficiency based upon the content of Zn in leaves is not assured. Moreover, under some conditions the total Zn concentration in plant parts does not reflect the true nutritional status of Zn in plants. Leece (1976a and 1978) reported that leaves of 30-day old maize plants grown in alkaline black earth soil amended with P showed Zn deficiency symptoms even though the concentration of Zn was sufficient to support healthy growth; therefore, the author concluded that decreasing the active Zn associated with of P/Zn imbalance, rather than the total Zn concentration was the main reason for pronounced Zn deficiency. The results presented in Chapter II showed that when P supply was increased to 2.5 mM P, leaves of soybean plants of two cultivars expressed symptoms very much like those of Zn deficiency even though the concentration of Zn in shoots and roots was high.

Like many other micronutrients, Zn is an indispensable component of many enzyme systems in higher plants. Thus enzymes activities have been proposed and employed as physiological indicators for the diagnosis of micronutrient deficiencies in different species of higher plants (Brown and Hendricks, 1952; Leece, 1976a; and Smith, 1983). Carbonic anhydrase, aldolase, ribonuclease and superoxide dismutase activities have been employed in diagnosing of Zn deficiency; however, carbonic anhydrase was the enzyme system most sensitive to changes in the concentration of Zn in higher plants (Bar-Akiva and Lavon, 1969; Bar-Akiva et al., 1971; Randall and Bouma, 1973; Ohki, 1976 and 1978; Gibson and Leece, 1981; and Shrotri et al., 1983). Also the interaction between Mn, Cu, and Zn in plant cells was evaluated by studying the metalloenzyme system superoxide dismutase (Del-Rio et al., 1978). Moreover, Dwivedi and Takker (1974) found that the biological assay is helpful in predicting the deficiency of Zn at early stages of plant growth and the hidden hunger of Zn in plants.

To be reliable in diagnosing Zn deficiency and assessing the status of Zn in plants, the behavior of Zn-containing enzymes under well defined nutritional conditions have to be known. Very little is known about the effects of raising the external concentration of P on the activities of Zn-containing enzymes in soybean plants. Therefore, as a first step in attempting to use the activities of Zn-containing enzymes as a bioassay for Zn deficiency, the effect of raising the phosphate pools on these enzyme systems must be studied. The present experiment was initiated to study the effects of raising the external P concentration on the activities of carbonic anhydrase and superoxide dismutase in the leaves of two soybean cultivars. Also the relations between the activities of these two enzyme systems, the concentration of Zn, and dry-matter accumulation in the leaves of soybean plants are addressed.

MATERIALS AND METHODS

Plant Culture:

Soybean (*Glycine max L.*) cv York and cv Beeson seeds were sown in acid-washed white quartz sand in the greenhouse for 7-8 days and watered every day with deionized water. Seedlings in the early unifoliate leaf stage were chosen for homogeneity, washed out of sand and transplanted into polyethylene pots whichc contained 2.25 L of continuously aerated one-tenth strength Hoagland solution minus P and micronutrients. Seedlings were grown in this solution for one week, then transferred into treatment solutions that had the initial composition as follows: 3 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM NH_4NO_3, 1 ppm Fe as Fe-Na-EDTA, 0.15 ppm Mn as $MnSO_4$, 0.25 ppm B as HBO_3, 0.1 ppm Cu as $CuSO_4$, and 0.05 ppm Mo as molybdic acid. All possible combinations of three levels of P (0.1, 0.5, and 2.5 mM NaH_2PO_4) and five levels of Zn (0.077, 0.382, 0.675, 3.059, and 7.648 uM Zn as $ZnCl_2$) were applied. The treatment combinations along with the soybean varieties were arranged in a split randomized complete block design in which varieties were distributed over the main plots, and the P-Zn treatment combinations over the sub-plots. All treatments were replicated five times. The pH of the nutrient solutions was monitored daily and ranged between 5.5 and 5.8. The nutrient solutions were changed when the pH dropped to pH 5.3. Plants were grown in the greenhouse under supplemental light, using sodium metal halide lamps, for 16-hour photoperiods.

Sampling and Sample Preparation:

Two weeks after starting the treatments the experiment was terminated by cutting the plants just above the fibrous roots. Roots were washed three times in deionized water, three times in 0.5 mM $CaCl_2$, then rinsed in deionized water again, and blotted between filter papers. After that roots were weighed and freeze-dried for 48 hours.

Leaf blades were separated from stems. After taking the fresh weight, leaves were cut into small pieces, mixed thoroughly, and 2 five-gram samples were taken for superoxide dismutase and water-soluble proteins extraction. The other portion was freeze-dried for 48 hours and, after taking the dry weights, samples were stored desiccated in the refrigerator.

Stems and petioles were dried at 70 C for 48 hours. To correct the freeze-dried weight to the 70 C dry weight, samples were taking from both freeze-dried roots and leaves and weighed before and after drying at 70 C. The weights before and after drying at 70 C were similar. All dried plant samples were ground to pass through a 40 mesh sieve, and stored for chemical analyses.

Enzymes Extraction and Assay:

A:Carbonic Anhydrase:

One half gram of finely ground leaf blades was homogenized with a chilled mortar and pestle with 10 ml cold 0.1 M Tris-HCl buffered at pH 8.0 and containing 1 mM EDTA and 5 mM dithiothreitol as described by Ohki (1976). The homogenate was passed through four layers of nylon cloth, the crude homogenate collected in a plastic vial embedded in an ice bath, and immediately assayed for carbonic anhydrase activity.

Carbonic anhydrase activity was assayed by the CO_2-veronal buffer procedures described by Wilbur and Anderson (1948) and modified by Chen et al., (1970) to include a digital pH meter to monitor the pH changes. One ml of the crude homogenate was mixed with 3 ml of 0.02 M veronal buffered at pH 8.0, then 2 ml of cold CO_2-saturated deionized water was added and the time (seconds) required for pH to drop to 6.3 was recorded. Each extract was assayed 4 times, and the extract diluted whenever the time was 10 second or lower. The enzyme units in the homogenate were calculated as: EU = 10(To-T)/T where To is the time required for pH to drop to pH 6.3 for the uncatalyzed reaction mixture.

B: Superoxide Dismutase (SOD):

Superoxide dismutase was extracted by grinding fresh leaf blade tissue in a grinding solution containing 50 mM Tris-HCl buffered at pH 7.5 and 0.1 mM EDTA. All operations were carried out at 4 C in an ice bath. Leaf blade samples were blended in the grinding solution (1:6 w/v) using a Vir Tis homogenizer set at maximum speed for 1 minute. The homogenate was then filtrated by pressing through four layers of nylon cloth, and the filtrate centrifuged at 15000 g for 15 minutes in a Sorvall RC5C refrigerated ultra centrifuge. The resultant crude supernatant was immediately used for the enzyme assay.

SOD activity in the crude extraction was assayed photochemically as described by Beauchamp and Fridovich (1971) and modified by Giannopolitis and Ries (1977). Being independent of other enzymes and proteins, the photochemical procedure is more reliable when using the crude extraction for the enzyme assay (McCord and Fridovich, 1969). In this procedure the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by the superoxide-free radicals generated by the action of light on riboflavin was measured. One unit of SOD was defined as that amount of the enzyme that caused 50% inhibition of the reduction of NBT at 25 C.

The reaction mixture was composed of 1.3 uM riboflavin, 13.0 mM methionine, 63.0 uM NBT and 0.5 M sodium carbonate (pH 10.2). The reaction mixture was first prepared, then volumes of three ml each were transferred into glass test tubes selected for uniform thickness and color. An appropriate volume of the crude extract (10 ul) was added and the test tubes were held in a rotating holder and immersed in a water bath at 25 C. A circular fluorescent lamp was attached on the outside wall of the water bath, and the entire assembly fitted in a box lined with aluminum foil. The reaction was initiated and terminated by turning the light on and off. The photochemical reaction was run for 5 minutes, at the end of which the absorbance at 560 nm was recorded. SOD was assayed four times for each extract.

Total Chemical Analysis:

Finely ground plant materials were digested in sulphuric-peroxide digestion mixture as described by Parkinson and Allen (1975). Total P and N were determined using a flow injection autoanalyzer

(Lachet). Zn, Fe, and Mn were determined using the atomic absorption spectophotometry (Perken Elmer 303).

Water-soluble and Total Protein:

Water-soluble protein in a water extract of fresh leaves (1:6 w:v) was measured by the method of Potty (1969). Ovalbumin (Sephadex Fine Chemical) was used as a standard. Total protein was calculated by multiplying the percent of total N-NH_4 by 6.25.

RESULTS AND DISCUSSION

Zn Deficiency, Plant Growth, and Nutrient Composition:

The appearance of Zn deficiency on the leaves of soybean plants at harvest time was recorded using the same scale described in Chapter II. Zn deficiency symptoms developed in both Zn deficient and induced Zn deficiency treatments (Tables 1 and 2). Addition of Zn in the nutrient solution corrected the Zn deficiency; whereas, P intensified the Zn deficiency. The induction of Zn deficiency by heavy application of P was more pronounced in the York variety. At high Zn levels plants developed chlorosis which was most likely toxicity rather than deficiency symptoms.

The effects of P and Zn supply on the growth and nutrient composition of soybean tops and roots were in agreement with the results reported in Chapter II showing that soybean varieties differed with respect to P and Zn interaction. The York variety seems to be more susceptible to the induction of Zn deficiency by heavy

Table 1. Dry-matter and P, Zn, Fe, and Mn uptake in shoots and roots of soybean York as affected by P and Zn concentrations in the growth media.[#]

P	Zn	Zn Deficiency	Dry-matter S	R	P Uptake S	R	Zn Uptake S	R	Fe Uptake S	R	Mn Uptake S	R[@]
mM	uM		g pot^{-1}		mg pot^{-1}		ug pot^{-1}		mg pot^{-1}		ug pot^{-1}	
0.1	0.077	+++	5.2	1.3	25.4	7.2	154	54	0.8	0.7	358	152
	0.382	++	5.7	1.4	23.9	7.4	353	89	0.7	1.1	311	104
	0.765	+	6.3	1.6	23.5	7.5	340	84	0.7	0.9	312	88
	3.059	+	7.1	1.8	26.0	8.9	467	132	0.7	1.2	333	95
	7.648	+	4.6	1.5	17.0	7.1	720	291	0.5	1.1	339	73
0.5	0.077	++++	5.6	1.5	55.6	37.6	168	60	0.9	1.6	506	162
	0.382	+++	5.6	1.4	50.2	32.2	348	86	0.7	1.9	371	145
	0.765	++	5.6	1.3	46.7	26.7	314	70	0.7	1.6	390	158
	3.059	++	5.8	1.4	51.5	30.3	497	110	0.6	1.9	391	139
	7.648	+	5.6	1.4	46.5	31.4	973	265	0.6	1.9	330	102
2.5	0.077	++++	5.7	1.3	55.2	34.2	170	56	0.8	1.5	515	197
	0.382	+++	5.9	1.5	57.0	35.1	381	86	0.7	1.9	395	165
	0.765	+++	5.5	1.1	44.6	19.1	301	62	0.6	1.4	348	144
	3.059	++	5.1	1.3	52.0	32.6	471	114	0.6	1.9	426	158
	7.648	+	5.5	1.2	42.1	29.5	945	437	0.6	1.5	424	135
LSD$_{0.05}$[+]			0.7	0.2	5.5	3.7	161	26	0.3	0.4	096	87

[#] Each number is an avearage of 5 replications.

[+] LSD is calculated according to Steel and Torris (1980), and used to compare any two numbers within each column of both Table 1 and 2.

[@] S - Shoots and R - Roots.

Table 2. Dry-matter and P, Zn, Fe, and Mn uptake in shoots and roots of Beeson soybean as affected by P and Zn concentration in the growth media.#

P	Zn	Zn Defi-ciency	Dry-matter S	R	P Uptake S	R	Zn Uptake S	R	Fe Uptake S	R	Mn Uptake S	R@
mM	uM		g pot^{-1}		mg pot^{-1}		ug pot^{-1}		mg pot^{-1}		ug pot^{-1}	
0.1	0.077	+++	6.7	2.3	26.1	5.8	196	82	1.6	0.7	436	923
	0.382	++	7.5	2.2	22.9	6.8	373	104	1.3	0.8	480	262
	0.765	+	8.1	2.2	20.6	5.5	448	119	1.3	0.8	435	168
	3.059	+	7.6	2.2	20.0	5.0	642	133	1.3	0.9	405	94
	7.648	+	7.1	2.1	21.1	5.3	924	319	1.1	1.3	347	113
0.5	0.077	++++	6.6	1.8	87.5	43.9	240	69	1.6	1.7	593	202
	0.382	++	7.9	1.5	79.9	34.0	340	97	1.3	1.7	526	183
	0.765	++	8.6	2.3	69.8	40.1	500	109	1.4	2.3	586	258
	3.059	+	8.1	2.1	67.3	38.5	746	138	1.3	2.4	618	166
	7.648	+	7.5	2.1	70.7	41.0	1094	323	1.6	2.7	560	122
2.5	0.077	++++	6.7	1.7	99.8	57.8	198	67	1.7	1.9	689	216
	0.382	++	7.8	1.9	79.1	48.0	382	85	1.4	2.2	586	158
	0.765	++	8.2	1.7	77.8	37.8	487	80	1.4	1.9	603	171
	3.059	+	6.8	1.7	53.0	32.1	616	141	1.2	2.3	516	130
	7.648	+	7.6	1.8	47.5	34.0	1018	485	1.5	2.5	543	105
LSD$_{0.05}^{+}$			0.7	0.2	5.5	3.7	161	26	0.3	0.4	96	87

\# Each number is an average of 5 replicates.

\+ LSD is calculated according to Steel and Torrie(1980), and used to compare any two numbers within each columne of both Table 1 and Table 2.

@ S - Shoots and R - Roots

application of P than is the Beeson variety. Only at the low P level did increasing the external Zn concentration up to 3.06 uM Zn accounts for a gradual and significant increase in dry-matter accumulation in tops and roots of the York variety. In Beeson, however, dry-matter accumulation in tops and roots was significantly increased over the lowest level of Zn with each level of Zn up to 3.06 uM Zn at P level of 0.1 mM P, and upto 0.765 uM Zn at 0.5 and 2.5 mM P.

Zn uptake in tops and roots of both soybean varieties were regularly and significantly increased with raising the external Zn supply at all P levels. But the most striking effect was the insignificant effect of raising the P levels on Zn uptake in tops and roots of both soybean varieties at external Zn concentrations up to 3.06 uM Zn. But P shows a general effect of decreasing the Zn uptake in the roots of the Beeson soybean variety. The increasing of Fe uptake in the roots of these plants may account for this decrease of Zn in the roots. On the other hand, heavy application of P significantly increased Zn uptake in tops and roots of both soybean varieties at a Zn concentration of 7.65 uM Zn. This increase in Zn uptake could be a direct impact of P toxicity and increasing the permeability of cell membranes in roots. Wallace et al., (1978) reported that at low pH, a high P level resulted in increasing the Zn contents of leaves, stems, and roots of soybean plants. Results reported by Safaya (1976) showed that when 10 ppm Zn was added to a soil culture, high P increased Zn uptake in tops and roots of 27-days old corn plants.

The induction of Zn deficiency in soybean plants by heavy application of P is not associated with increasing the dry-matter accumulation in tops and roots, but rather is associated with increasing the P uptake in tops and roots while not changing Zn uptake. Figures 1 and 2 show that the induction of Zn deficiency by heavy application of P is concomitant with increasing the P/Zn ratio. Thus the physiological P/Zn imbalance rather than the dilution of Zn in tops might be an acceptable reason for developing Zn deficiency symptoms in response to heavy application of P. Boawn and Leggett (1964) and Watanabe et al., (1965) reported that the intensity of Zn deficiency symptoms and the P/Zn ratio were correlated. Recently Sumner et al., (1982) found that soybean genotypes which appeared normal had normal P/Zn ratios while severe symptoms were associated with highly imbalanced P/Zn ratios. P toxicity is another explanation for inducing Zn deficiency by adding high P level to the growth media. Loneragan et al., (1982) has shown that when high P and low Zn were supplied, P was absorbed and translocated in such excess that it would be toxic.

Considering the uptake of Fe and Mn in the tops and roots of soybean plants, data presented in Tables 1 and 2 show that Zn has a considerable antagonistic effect upon the uptake of Fe in the tops and Mn in the roots of York variety at low P level and increasing the P supply reduced these antagonistic effects. Soybean Beeson expressed the same behavior with the exception that Zn increased the Fe uptake in roots at all P levels. However the uptake of Fe and Mn at all Zn levels did not correlate with the appearance of Zn deficiency

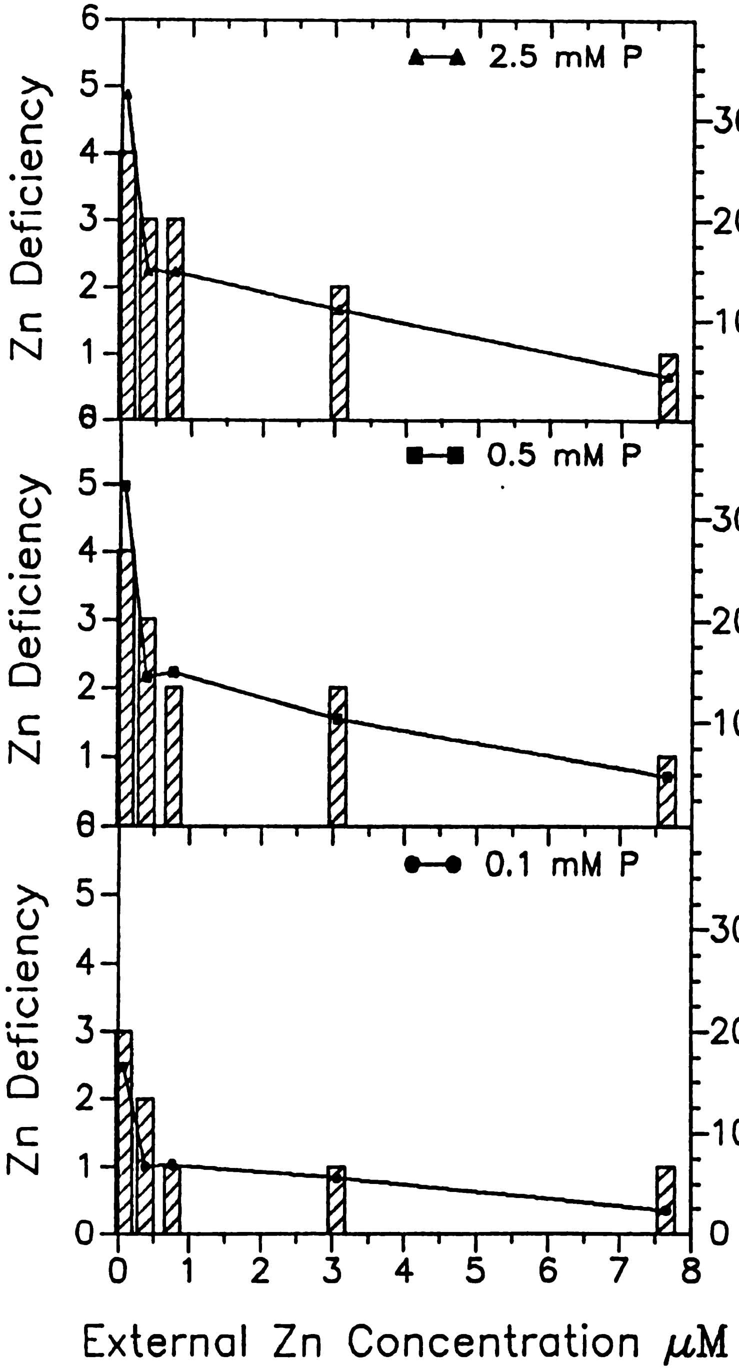

Fig.1 . The Relationship between Zn Deficiency and P/Zn Ratio in Shoots of York Soybean at Three P Levels.

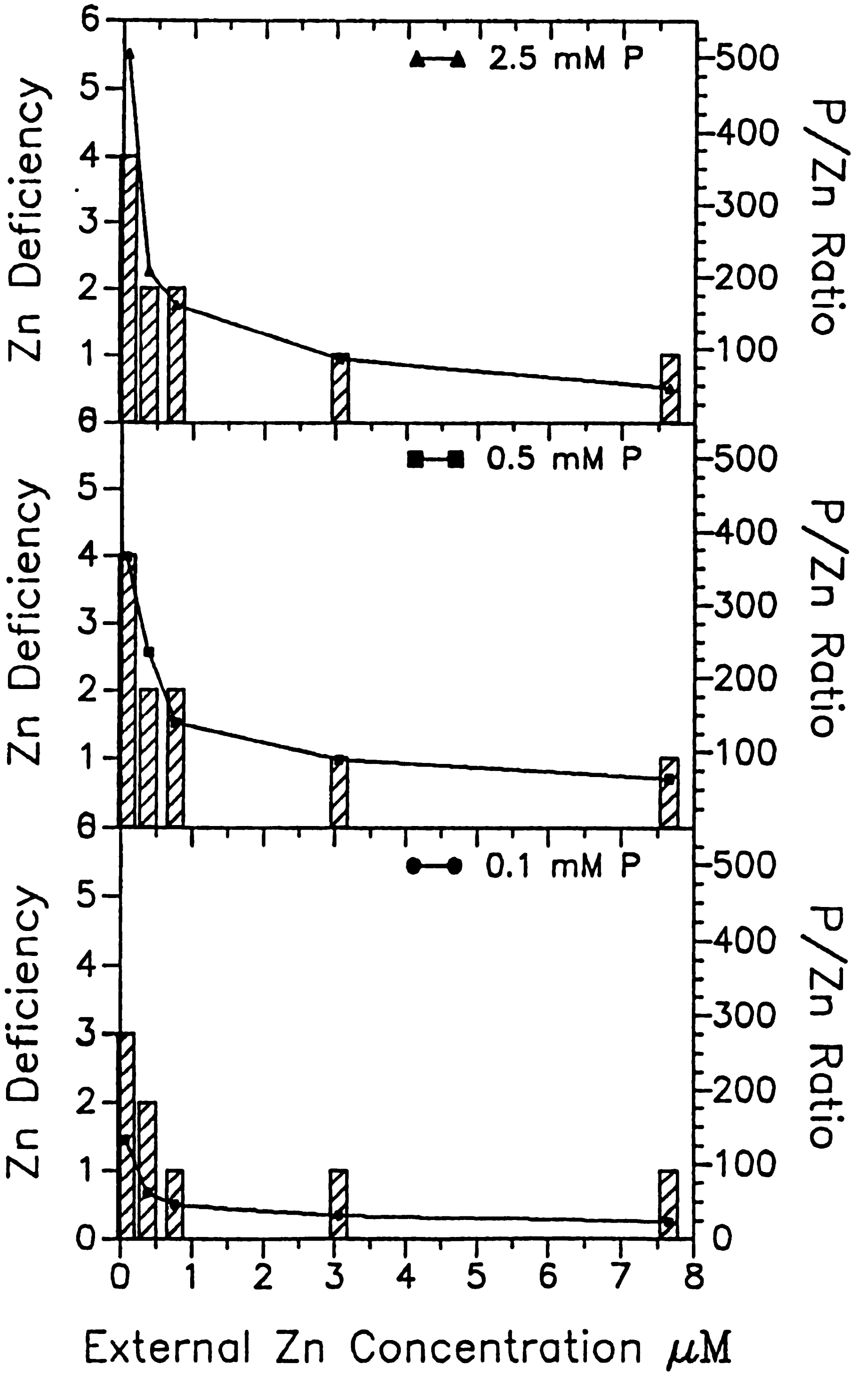

Fig. 2 . The Relationship between Zn Deficiency and P/Zn Ratio in Shoots of Beeson Soybean at Three P Levels.

especially when high P levels were added and thus both Fe and Mn appear to have no effect on P and Zn interaction.

Water-soluble, Total Proteins, and Carbonic Anhydrase:

The effects of Zn and P applications on water-soluble, total proteins, and carbonic anhydrase activity in soybean leaves are listed in Tables 3 and 4. Water soluble and total proteins in leaves of both soybean varieties increased continuously with increasing the external Zn concentration at all P levels but only the increases in soluble proteins were significant. The reduction in water-soluble and total proteins always occurred in the leaves of those plants which were Zn deficient. However P application has no influence on the protein content of the leaves of either soybean variety. These results further emphasize the fact that Zn is essential for protein synthesis. Inactivation of RNA polymerase (Falchuk et al., 1977), disintegration of ribosomes (Prask and Plocke, 1971), and increasing the activity of RNase which enhanced the rate of RNA degradation (Sharma et al., 1982) are three distinct explanations for the adverse effect of Zn deficiency on protein synthesis and protein contents in plants.

The activities of carbonic anhydrase in the leaves of soybean plants of both varieties was increased significantly by raising the external Zn concentrations at all P levels. Zn has been reported to be an indispensable component of and essential for the activity of carbonic anhydrase in plants (Tobin, 1970; and Ohki, 1976). The concomitant increases of carbonic anhydrase activity in leaves and dry-matter accumulation in the tops of soybean plants with increasing external Zn supply is strong evidence for the importance of carbonic anhydrase in photosynthesis and CO_2 fixation as indicated by Randall and Bouma (1973), Ohki(1978), and Shrotri et al., (1983).

The reduction in carbonic anhydrase activity, on the other hand, is associated with the occurrence of Zn deficiency, which is not the case with respect to uptake of Zn in tops of soybean plants (Table 1 and 2). Although the concentration of Zn in the leaves of soybean plants exhibits little change with increasing the external concentration of Zn up to 3.059 uM Zn (Figures 3 and 4), an almost linear increase in carbonic anhydrase activity occurs with increasing Zn supply up to 3.06 uM Zn. Thus carbonic anhydrase is sensitive to the changes in the external Zn supply and quickly responds to the occurrence of Zn deficiency even before the appearance of deficiency symptoms. Therefore, carbonic anhydrase has been used as an index for detecting Zn deficiency in higher plants (Bar-Akiva and Lavon, 1969; Bar-Akiva et al., 1971; Dwivedi and Takkar, 1974; and Ohki, 1978). Moreover, Gibson and Leece (1981) reported that carbonic anhydrase differentiated between active and inactive Zn in corn leaves and thus is considered as a suitable biochemical index for what was called "physiologically active Zn".

The carbonic anhydrase enzyme system is also sensitive to the P levels in the growth media. Increases in the activities of carbonic anhydrase, which are sometimes significant, are recorded when P application increased to 0.5 mM P. However, further increases in P supply accounted for a significant reduction in carbonic anhydrase activity. These reductions in the carbonic anhydrase activities are associated to a great extent with the appearances of Zn deficiency in

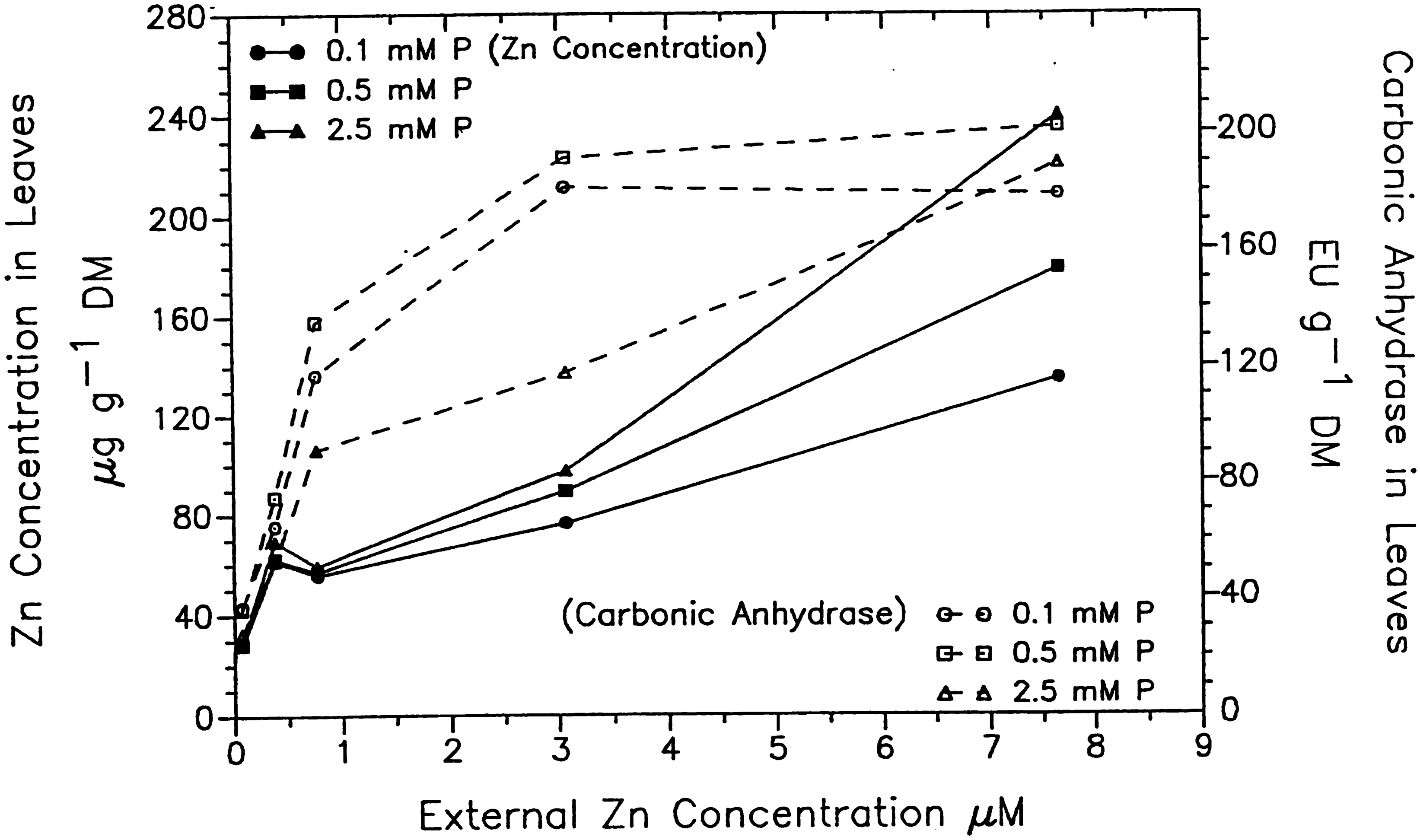

Fig. 3 . Effect of External Zn Concentration on Zn Concentration and Carbonic Anhydrase Activity in Leaves of York Soybean at Three P Levels.

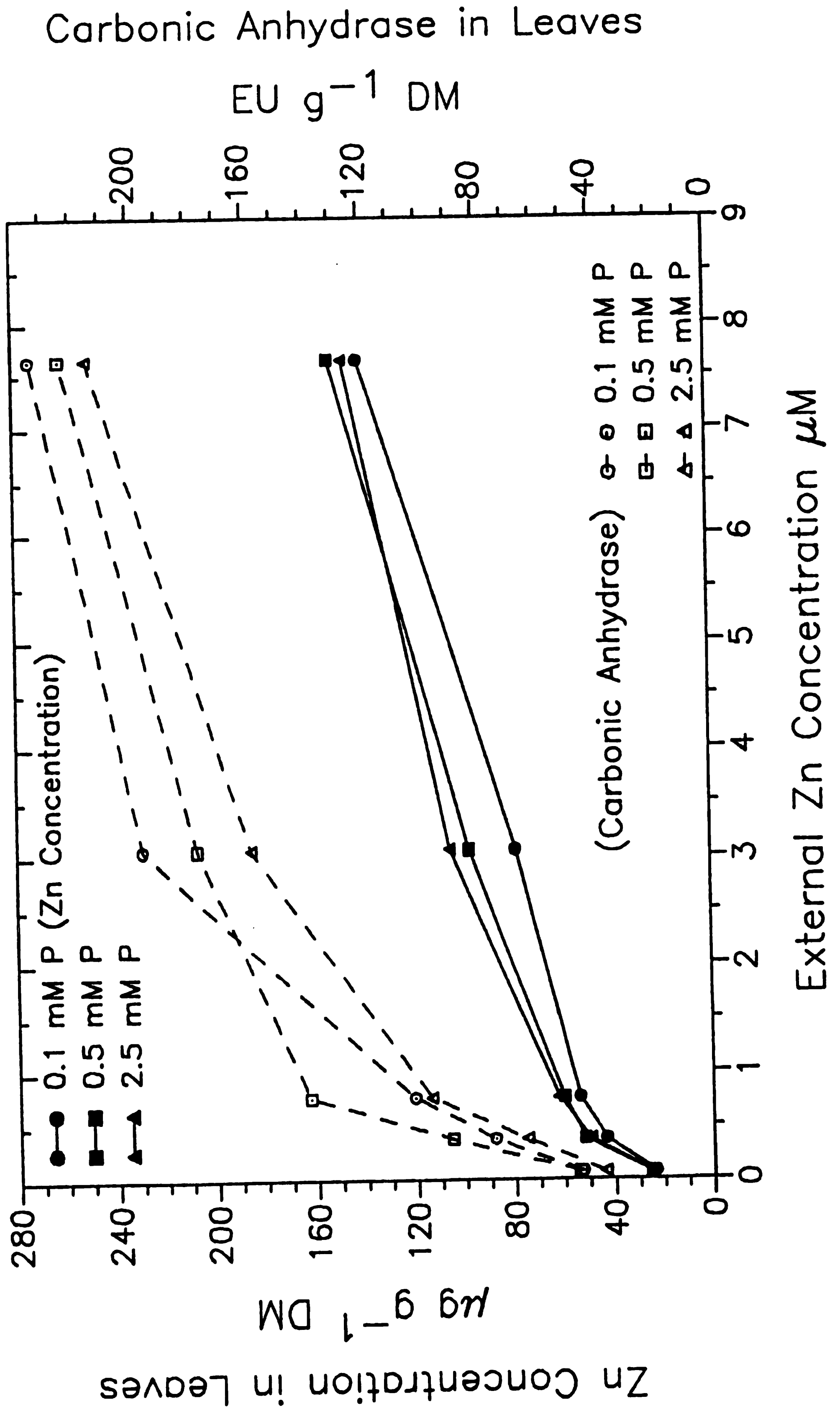

Fig. 4 . Effect of External Zn Concentration on Zn Concentration and Carbonic Anhydrase Activity in Leaves of Beeson Soybean at three P Levels.

the plants of the York variety grown at 2.5 mM external P concentration. Two possible reasons might explain this inverse effect of high P level. Raising the P pools inside the leaf tissues may inhibit the enzyme activity or somehow reduce the amount of Zn which is available for forming the enzyme from the apoenzyme. Further studies are required to understand these results.

Superoxide Dismutases:

The effects of Zn and P supply on the activities of superoxide dismutase in leaves of soybean plants are listed in Table 3 and 4. Superoxide dismutase is another Zn-containing enzyme system which contains Mn, Cu, and perhaps Fe as well. The activity of the enzyme, thus, increased with increasing the external Zn concentration at all P levels. The increase in enzyme activity was almost linear only up to 0.765 uM Zn. The external P supply showed no distinct effect on the enzyme activities at external Zn concentration up to 0.765, but raising the P level reduced the enzyme activity at higher Zn levels.

The relation between the activity of superoxide dismutase and Zn concentration in soybean leaves is presented in Figures 5 and 6. It is quite clear that the enzyme is only sensitive to the changes in Zn concentration in leaves at low Zn levels and up to 0.765 uM Zn. However, the activity of the enzyme shows small changes, which are not significant, with the corresponding changes in the concentration of Zn in soybean leaves. The superoxide dismutase enzyme system contained Cu, Mn, and Fe beside the Zn, therefor, Zn in not the only limiting factor that controls the activity of this enzyme system and

Table 3. Total and water-soluble protein and carbonic anhydrase and superoxide dismutase activities in the leaves of York soybean as affected by the concentrations of P and Zn in the growth media.#

P	Zn	Proteins W.S	Proteins Total	Carboninc anhydrase		Superoxide dismutase	
mM	uM	mg g^{-1}DM		EU g^{-1}DM	EU g^{-1}Pro	EU g^{-1}DM	EU g^{-1}Pro
0.1	0.077	47	327	37.0	115.8	2319	7140
	0.382	51	329	64.6	200.1	2803	8541
	0.765	55	374	117.0	309.8	3318	8872
	3.059	59	386	181.3	469.7	3535	9158
	7.648	82	393	178.9	455.2	4675	11896
0.5	0.077	42	317	36.2	113.8	2386	7514
	0.382	55	345	74.7	215.4	2674	7751
	0.765	55	348	135.3	389.6	3418	9827
	3.059	55	350	191.3	546.6	3351	9574
	7.648	80	365	201.8	552.9	4079	11175
2.5	0.077	45	317	28.1	89.8	2248	7113
	0.382	46	340	53.0	158.0	2526	7448
	0.765	47	325	91.0	276.4	3502	10773
	3.059	59	338	117.7	353.6	3192	9444
	7.648.	83	347	189.4	545.8	3893	11219
$LSD_{0.05}^{+}$		13	56	18.5	75.5	509	1146

Each number is an average of three replications.

+ LSD was caculated according to Steel and Torrie(1980), and used to compare any two numbers within each column of both Table 1 and 2.

Table 4. Total and Water-soluble protein, and carbonic anhydrase and superoxide dismutase activities in the leaves of Beeson soybean as affected by the concentrations of P and Zn in the growth media.

P	Zn	Proteins[#] W.S	Total	Carbonic[$] anhydrase		Superoxide[#] dismutase	
mM	uM	mg g^{-1}DM		EU g^{-1}DM	Eu g^{-1}Pro	EU g^{-1}DM	EU g^{-1}Pro
0.1	0.077	67	337	45.6	135.3	2051	6086
	0.382	69	347	75.7	218.2	2704	7793
	0.765	79	357	123.5	345.9	3336	9345
	3.059	81	384	196.6	512.0	3452	8990
	7.648	84	374	234.1	625.2	3779	10104
0.5	0.077	63	338	46.3	113.6	2081	6157
	0.382	73	348	90.6	260.3	2692	7736
	0.765	76	366	139.4	380.9	3313	9052
	3.059	78	389	207.6	533.7	3543	9108
	7.648	82	384	263.8	687.0	3653	9513
2.5	0.077	64	341	37.8	110.9	2497	7323
	0.382	60	352	64.4	183.0	3080	9033
	0.765	63	366	97.7	266.9	3482	9514
	3.059	72	353	158.9	450.1	3382	9581
	7.648	81	354	244.6	691.0	3241	9154
LSD$_{0.05}$[+]		13	56	18.5	75.5	509	1464

Each number is an average of 4 replications.

$ Each number is an average of 3 replications.

+ LSD is calculated according to Steel and Torrie(1980), and used to compar any two numbers within each column of both Table 1 and Table 2.

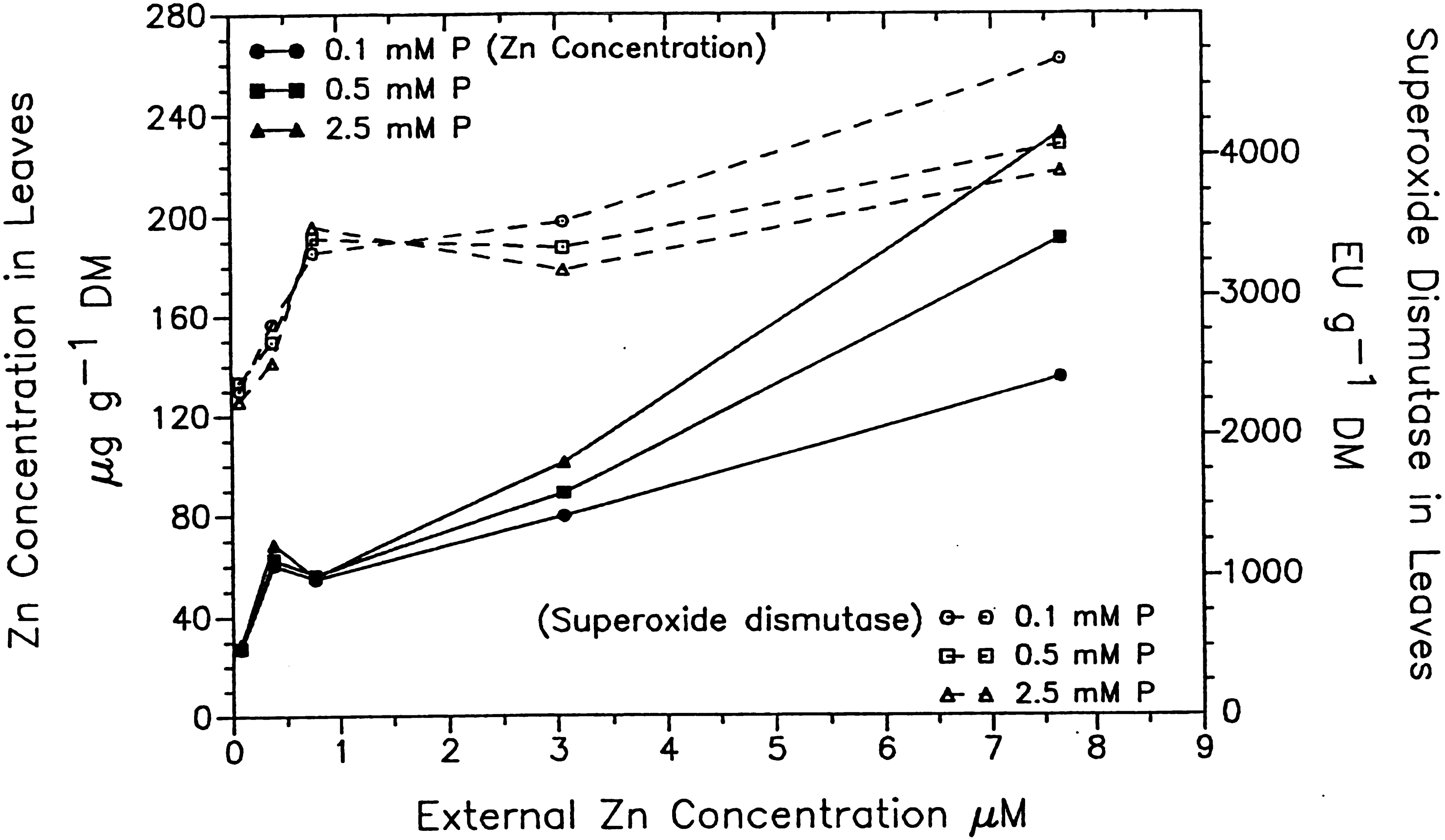

Fig. 5 . Effect of External Zn Concentration on Zn Concentration and Superoxide Dismutase Activity in Leaves of York Soybean at Three P Levels.

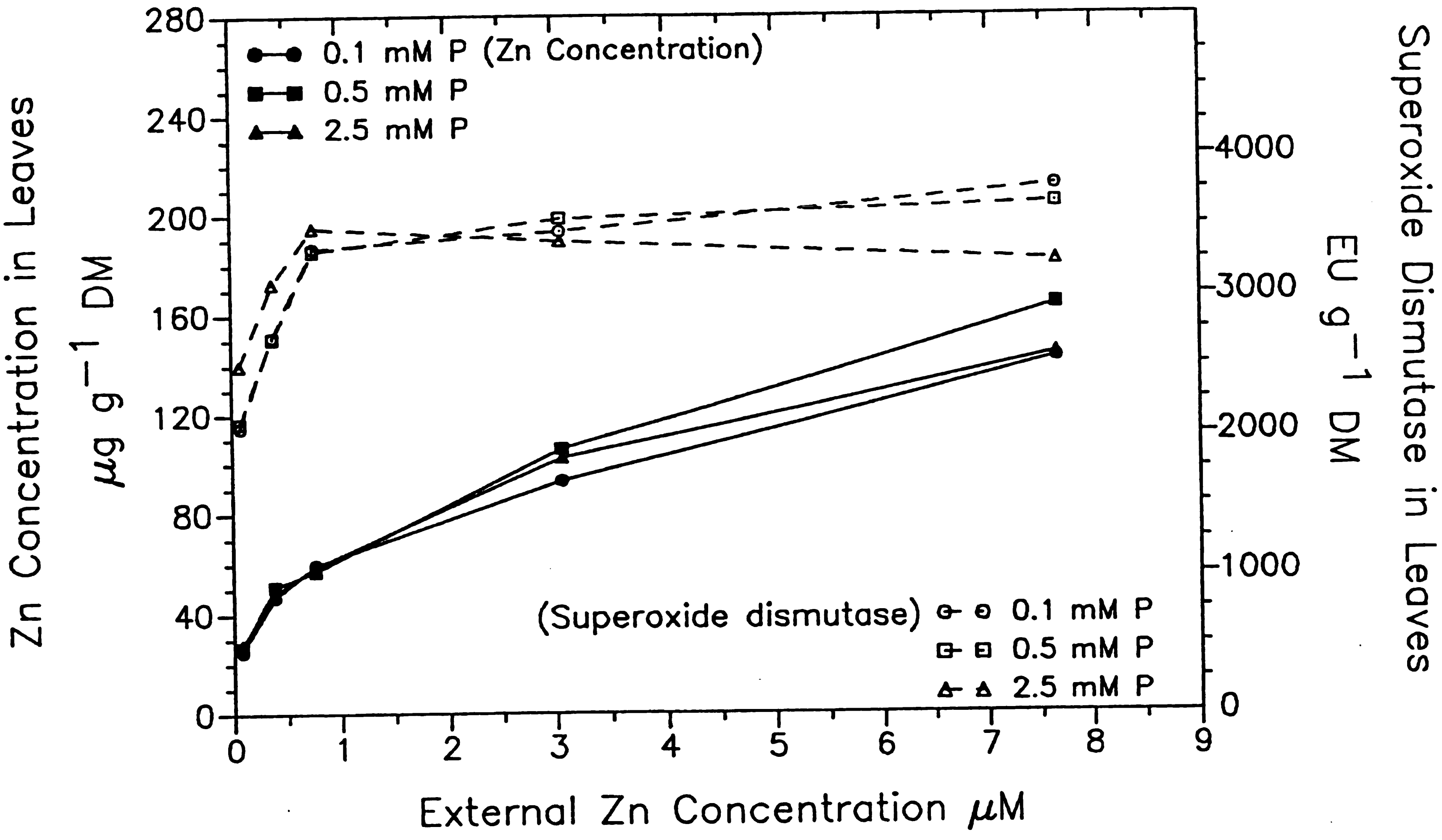

Fig. 6 . Effect of External Zn Concentration on Zn Concentration and Superoxide Dismutase Activity in Leaves of Beeson Soybean at Three P Levels.

its activity can not exceed that limit by increasing the Zn concentration unless the other limiting components are increased.

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SUMMARY AND CONCLUSIONS

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A solution culture technique was used to study the effect of high P in the growth media on the uptake and localization of Zn, the kinetic characteristics of Zn absorption system(s), and the activities of some Zn-containing enzymes in two soybean cultivars. A Zn-sensitive soybean (variety York) and a Zn normal soybean (variety Beeson) were used. Radioactive Zn was used to monitor the localization of Zn in plant cells, and to detect the soluble compounds that naturally bind Zn inside the plant cells.

Soybean cultivars responded similarly to Zn treatments but not to P treatments. In both cultivars dry- matter accumulation in plant tops was increased with increasing Zn concentration in the nutrient solution up to 0.769 uM Zn. Higher Zn levels resulted in a significant reduction in dry matter accumulation in tops and roots of both cultivars. P significantly increased the dry matter accumulation in tops of the York variety at low and high Zn levels, but decreased it at the intermediate Zn level. In the Beeson soybean variety, P did not significantly affect the dry matter accumulation in tops.

At low and intermediate Zn levels increasing the external P concentration had no significant effects on Zn uptake in tops and roots of either cultivar. However, heavy application of P significantly increased Zn uptake in top and roots of those soybean plants grown at a high Zn level. This increase in Zn uptake could be a direct effect of P and/or Zn toxicities.

High P level in the growth media induced Zn deficiency symptoms on the plants of both soybean cultivars, yet the uptake and the concentration of Zn in the tops and roots changed little. The appearance of Zn deficiency was not associated with either Zn uptake or Zn concentration in the tops, but was well correlated with P/Zn ratios-- Zn deficient plants always have a higher P/Zn ratio. The York variety was more susceptible to P-induced Zn deficiency than the Beeson variety.

Zn localization pattern in the leaves of soybean plants showed that the largest amount of Zn in soybean leaves was present in the "soluble" fraction. Zn bound to cell wall and cell debris were the next largest fraction. The percentage of Zn activity bound at the cell wall and cell debris in the leaves of the Zn-sensitive soybean variety (York) was higher than that in the leaves of the normal soybean variety (Beeson). Further fractionation of the Zn "soluble" fraction using the gel permeation technique revealed that in the "soluble" form, mainly in cytoplasm and vacuoles, Zn is bound to at least three different types of compounds: proteins (enzymes), ionic compounds, and intermediates. P had no singnificant effect on either localization pattern or the amount of Zn-binding compounds; however, P level exhibits some effect upon the distribution of Zn among these compounds.

The effect of P level in the growth media on the kinetic characteristics of Zn uptake, translocation, and accumulation in the intact soybean plants were investigated. Soybean plants were exposed to 14 different Zn concentrations, ranging between 0.076 and 76.48 uM Zn, and 4 different P levels (0.25, 0.5, 1.0, and 2.5 mM P) for 24 hours absorption periods. Radioactive ^{65}Zn was used to monitor the amount of Zn taken up by soybean plants. In both soybean varieties high P levels reduced the rate of total Zn uptake and translocation. The rate of accumulation of Zn by roots of the Beeson variety were decreased with increasing P level, but in the York variety, P increased the accumulation rate of Zn in roots.

At a low range of Zn concentration P either decreased or had no effects on Vm values of the absorption systems in both soybean varieties. Most probably this reduction is a result of changing the conformation of the absorption site(s) or reducing the rate at which the carrier turns over. The reduction of Vm by increasing P concentration in the nutrient solution is eliminated by increasing the external Zn concentration range to 3.82-76.48 uM Zn. P significantly increased the Km values of the absorption system(s) of both soybean cultivars at the high Zn concentration range.

Water-soluble and total proteins in leaves of both soybean cultivars increased with increasing the external Zn concentration at all P levels. However, P application has no influence on the protein contents of leaves of either soybean cultivar. Zn is known to be an essential part of RNA polymerase and ribosomal RNA, and therefore the immediate impact of its deficiency is on the reduction of protein contents in the leaves. Also, at all P levels increasing the external Zn concentrations increased the activities of carbonic anhydrase and superoxide dismutases. However, these enzyme systems respond differently to P application. Carbonic anhydrase activity was increased by adding the first increment of P, but further P additions resulted in a significant reduction of the enzyme activity. The effect of P on SOD activity depends upon the external Zn concentration. At Zn concentrations up to 3.06 uM Zn, P had no influence on SOD activity; however, P significantly reduced the activity of SOD when the external Zn concentration increased to 7.65 uM Zn.

The activities of both carbonic anhydrase and SOD changed almost linearly with increasing external Zn concentration up to 3.059 uM Zn at all P levels, but carbonic anhydrase was more sensitive and more susceptible to the P concentration in the growth media.

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