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ROOT EXUDATE AND THE ABILITY OF CORN TO UTILIZE INSOLUBLE SOURCES OF IRON

KEY WORDS: Split medium technique, collecting root exudate, amino acids and organic acids in the exudate, corn plants under iron stress and utilization of insoluble iron sources.

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ABSTRACT

This work was to study the characteristics of root exudate from Fe-deficient and nondeficient corn plants as well as to study the ability of corn to utilize Fe-III from different sources.

A combined sand and water culture technique was designed in order to collect root exudate free of nutrients for direct analysis of organic acids and amino acids. Secondly it was to study the ability of the Fe-deficient plants to solubilize iron from $Fe(OH)_3$, $FePO_4$, alkali soil sample and acid soil sample.

Using the HPLC analysis, results indicate that various substances were released by the roots and there was a clear difference between normal and deficient plants. The amino acid analysis data showed a distinct difference in the amount and type of amino acids. Cystine, isoleucine, leucine and lysine were detected only in the exudate of Fe-deficient plants.

Results of Fe concentration in the above ground portion of corn plants reveraled different magnitude of increase and the highest increase was for $FePO_4$ and acid soil treatments.

INTRODUCTION

Most of iron in soil exist as Fe-III in a number of different forms. At high soil pH negligable concentrations of Fe^{3+} would exist in solution, Krauskopf (1972) & Olsen et al. (1982). One of the factors of solubilizing iron-III include :

a) Acidification as a decrease of pH of one unit will cause a theoretical increase of a thousand fold.
b) Chelation and/or complexation by organic acids, amino acids and humus substances.
c) Reduction of Fe-III to Fe-II and the solubility product constant for $Fe(OH)_2$ is far greater than for $Fe(OH)_3$, Olsen, et al., (1982).

On the other hand, different varities of plants exhibit varying abilities to mobilize iron from the soil and lower the pH and redox potential within the rhizosphere (Venkat Raju and Marschner, 1972, Clark and Brown, 1974, Hether, et al., 1984). Plants can also release reducing compounds into the medium Venkat Raju, et al., 1972, Clark and Brown, 1974. Lowering rhizosphere pH and releasing reducing compounds, were thought to be the two mechanism envolved by the Fe-stressed plants to solubilize and absorb iron by the roots, Azarabadi and Marschner, 1979, and Hether et al., 1984.

Monocotolydonous plants in general and Zea maize in particular are unable to lower the pH of the nutrient medium (with nitrate as a source of N) or produce riboflavin under the conditions of iron deficiency in the growth medium. However, corn is able to utilize iron-III hydroxide even at a high pH and high redox potential in the substrate; Azarabadi and Marschner (1979).

Accordingly, this work was conducted in order to study the characteristics of root exudates for Fe-deficient and non-deficient corn plants as well as to study the ability of corn to utilize Fe-III from different sources.

MATERIALS AND METHODS

Split Medium Technique;

A combined sand and water culture technique was designed, first to collect root exudate free of nutrients to be analysed directly for the organic acids and amino acids and secondly, to study the ability of Fe-deficient plants to solublize iron from different sources.

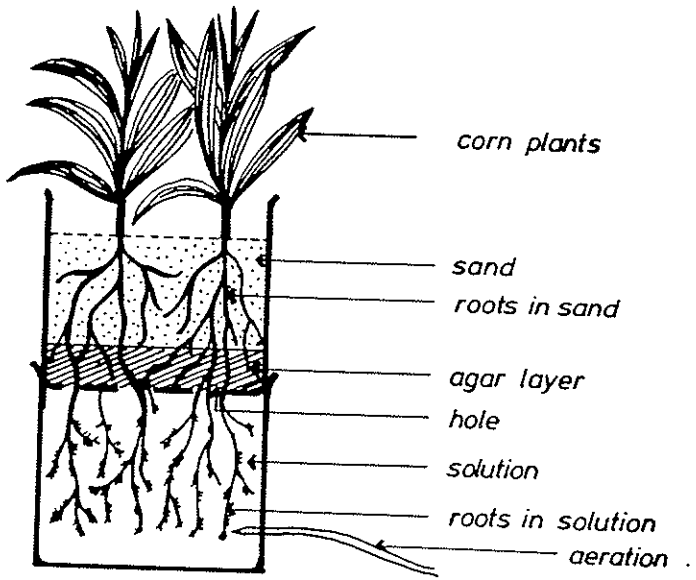

Fig. 1: split medium technique

In the split technique, two Neubauer plastic pots placed over each other were used. The upper pot was used as a sand culture but was modified to hold the sand and plants as well as permit the roots to penetrate to the lower pot. To maintain this, four-big holes were made in the bottom of the pot then a 1-cm layer of agar (4% in nutrient solution free of Fe with the Fungicide previcur 3.5x10-4%). The lower pot was fitted with a hole in the side to allow for aeration, Figure 1.

In this work corn plants were grown. Seeds of zea mays (variety Garba were first germinated between filter paper. Five seedlings were then transferd after 6 days to each of the upper Neubauer pots in which 450 g of acid washed sand were placed. The Seedlings were irrigated with complete nutrient solution. The complete nutrient solution had the following composition in M/L: $2.0x10^{-3}$ $Ca(NO_3)_2.4\ H_2O$, $0.75x10^{-3}$ K_2SO_4, $0.50x10^{-3}$ KH_2PO_4, $0.65x10^{-3}$ $MgSO_4.7\ H_2O$, $1.0x10^{-7}$ H_3BO_3, $1.0x10^{-6}$ $MnSO_4.H_2O$, $5.0x10^{-8}$ $(NH_4)_6\ Mo_7O_{24}.4\ H_2O$, $5.0x10^{-7}$ $Cu\ SO_4$, $5.0x10^{-7}$ $ZnSO_4.7\ H_2O$ and $2.0x10^{-4}$ MFe EDTA. The pH of the nutrient solution was 5.7.

The pots were placed in a growth chamber (24°C with 60% humidity for 10 hrs; and light intensity of 1300 foot candle for 14 hrs). After 5 days each pot with plants was placed over another pot.

A complete nutrient solution (CNS) or a nutrient solution-free of iron (NS-Fe) was placed in the lower pot. Plants were irrigated with CNS or NS-Fe according to the nutrient solution in the lower pot. The roots penetrated the agar layer and the root system in the lower pots was extensive. Visual symptoms of iron deficiency appeared on corn plants grown in the dificinet medium.

Collecting the Roots Exudate.

To collect root exudate free of interferring cations, a very diluted $CaCl_2$ solution ($5.0x10^{-4}M$) was used. The presence of Ca^{++} is thought to maintain root growth and middle lamella structure at normal conditions. In the lower pots, about 500 ml of the $CaCl_2$ solution pH 6.85 were placed and the free root system was washed with deionized water before it was immersed in the solution. The root exudates from Fe-deficient and non-deficient corn plants were collected every 24 hours period. About one liter sample was collected in two successive days for each pot of the deficient and non-deficient plants. The samples were directly frozen and kept for further amino and organic acid analysis.

Ability of Root System in Solubilizing Various Sources of Inorganic Iron.

To study the effect of the root in solubilizing relatively insoluble iron compounds, the following substance were added to the $CaCl_2$-solution in the lower pot of Fe-deficient plants.

a) A fresh precipitate of iron-III-hydroxide prepared from 1 g $FeCl_3$, 6 H_2O by, first dissolving the iron chloride in water then sodium hydroxide was added, the precipitatee $Fe(OH)_3$ was separated on a filter paper and the precipitate was washed with the diluted $CaCl_2$ solution used in the medium. The filter paper with the precipitate was placed in the lower pot of Fe-deficient plants.
b) 1g of $FePO_4$, very fine.
c) 5 g of an alkali soil, Tellain silty loam, pH 7.8, collected from El-Sharkia, Egypt. The organic matter and microorganisms were first destroyed in the soil by subjecting the soil to 650°C for 24 hrs in a muffle furance.

d) 5 g of an acid soil, Hohenbachern silty loam, pH 5.6, collected from Hohenbachern, Munich, West Germany.
The soil was also pretreated as mentioned above.

To each of these iron sources, 400 ml of the diluted $CaCl_2$-solution was added in the lower pot. A control treatment (only $CaCl_2$-solution) and a complete nutrient solution treatment were also made. Application of NS-Fe to the upper pot was made only at the beginning of the experiment. Aeration was adjusted and treatments were made in duplicate.
At the same time the above mentioned treatments were also conducted without plants in plastic bottles containing 400 ml of the $CaCl_2$ solution and fitted with aeration.

After 5 days corn plants were harvested in three parts:
1. Roots in solution (lower part)
2. Roots in sand (upper part)
3. Above-ground part (leaves + stems)

Surface contamination of sample was removed by washing first in diluted acid by dipping the sample in 0.001 N HCl, then with tap water and finally 2-times with bidistilled water.
The different plant parts were oven dried at 70°C and kept for analysis.

The solution in the lower pots as well as those in the plastic bottles (without plants) was filtered with Schleicher & Schüll, 185 mm folded filter paper, 512 1/2 No. 310 647. Soluble iron was determined in the filtrate.

Analytical Methods

a) Organic acids of the collected one liter sample of the exudate on the deficient and non-deficient plants was dried under vacuum at 40°C and the residue was dissolved in 5 ml deionized water. Organic acids were determined using the High Performance Liquid Chromatography (HPLC) model 302 Pumb-Gilson with 803 model monometric mudule and LKB 2151 variable wavelength manometer. 20 µ ml sample of the concentrated root exudate was injected in the HPLC Instrument. Detection was at 210 nm, 0.08 aufs and the chart speed was 5 mm, min^{-1}. The Column used Bio Rad HPX-87 H, 300x7.8 nm with a 2 cm micro-guard precolumn, filled with HPxB7 H. The columnelution was with 0.013 N H_2SO_4 and the flow rate was 0.4 $ml.min^{-1}$.

The HPLC retention time was evaluated for the unknown samples and compared with standard of certain organic acids.

b) Amino acids in the residue exudate samples was dissolved in 5 ml LiCl buffer. Amino acids were evaluated by the use of amino acid analyzer.

c) Iron content of the plant material was determined by the Atomic Absorption Spectrophotometer after wet ashing with 50 ml of a mixture of concentrated acids (600 ml NHO_3, 200 ml HClO, and 40 ml H_2SO_4).

d) Soluble iron in the solution containing different sources of iron was determined after drying the filtrate and the residue was desolved in 0.1 N HNO_3 and made to 50 ml.

e) pH of root exudate of the different iron sources in $CaCl_2$-solution in the presence and absence of plant-roots was measured.

RESULTS AND DISCUSSION

Organic Acids.

One of the major problem in evaluating organic acids in plant material or any extract as well as in investigating root exudate is to purify the material in quaestion from inorganic elements. Accordingly, the new split medium technique designed was a good tool not only to collect enough root exudate free from interfering ions but also could be useful in other research. Organic acids were determined in the root exudate of normal and chlorotic corn plants. Results, illustrated in Figure 2 indicate that various substances were released by the roots and there were a clear difference between normal and Fe-deficient plants with respect to the nature and amounts of the substances in the root exudate. It appears, that the iron-deficient roots exudate certain substances in relatively high amounts. Attempts were made to identify these substances and from the values obtained for the retention time; citric, pyruvic, glutaric, ∝-ketoglutaric, tartaric, glyoxylic and glyceric acids were released in the medium. The Fe-stressed plants exuded relatively high amounts, particulary, the glyceric acid which has adistinct absorption peak at 16.6 min. Riboflavin was not detected in the exudate samples. Also, there was a distinct peak after 23.2 min, only, in the exudate of the Fe-stressed plants. Work is going on to investigate these components in more detail. Previous work showed the accumulation of organic acids in iron deficient plants (Brown and Amber, 1970 and Venkat Raju, et al., 1972). Recently, Landsbeg (1981) indicated that during iron stress, symptoms of Fe chloroses are closely correlated

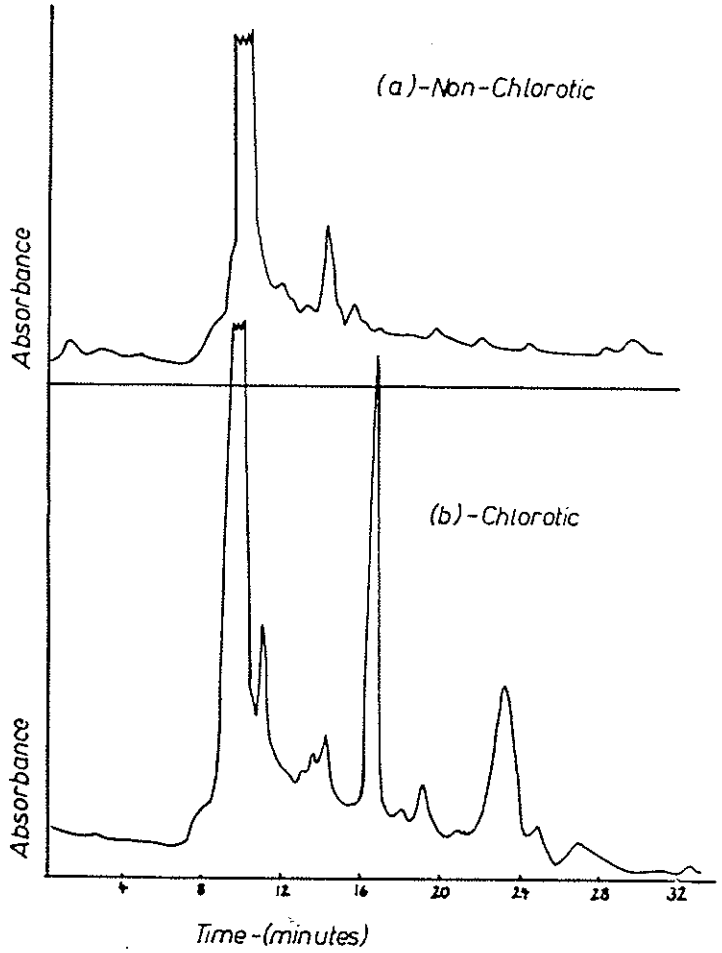

Fig. 2: HPLC Absorbing peakes and their retention time of root exudate of non-chlorotic(a) and chlorotic(b) corn plants

with the amount of organic acids particularly citrate and malate in the root tissues of monocots (barley, oats and millet) and dicots (sugar beet, peas, and beans) reaching to 330% over the control. Inspite of the accumulation of organic acids in the iron deficient plants there was no release of organic acids to the nutrient medium but there was a clear pH drop in the medium beside the release of reductants as riboflavin

TABLE 1

Amino acid content in the root exudate of non-chlorotic and chlorotic corn plants.

Amino Acids	Chlorotic plants nmol/l	non-chlorotic Plants nmol/l
Aspartic acid	-	7
Serin	-	18
Glycine	20	15
Alanine	8	8
Citrulline	-	42
Valine	-	8
Cystine	57	-
Isoleucine	5	-
Leucine	9	-
γ-Amino butaric acid	2	3
Ornithin	4	4
Lysin	13	-

caffeic acid, and chlorogenic acid (Venkat Raju et al., 1972 and Hether et al., 1984). the lowering of pH in the growth medium and effluex of reducing substance from the roots of plants under Fe-stress condition is known only for dicots and not for monocots as corn. Thus, the release of pronounced amounts of organic acids in the root exudate of chlorotic corn plants under investigation may explain the mechanism by which plants as corn overcome conditions of iron stress. Organic acids are known to form soluble and stable metal chelates with iron. Wallace, 1962, Chaberk and Martell, 1959.

Amino Acids.

Results reveal the presence of amino acids in the root exudate of both chlorotic and non chlorotic corn plants and there was a distinct difference in the amount and type of amino acids present, Table 1. 138 nmol/l of total amino acids compared to 105 nmol/l were found in the root exudate of the Fe-deficient and non deficient corn plants, respectively. As one liter was collected in two days i.e., half of this amount is released daily. It is worth to note, that 8 amino acids were detected in both the chlorotic and non chlorotic plants, but only four of them (glyceric, alanine, γ-amino butaric and ornithin) were present in about the same amount in the two exuadate samples.

The other four amino acids were, aspartic acid, serin, citrulline and valine in the exudate of Fe-sufficient plants and cystine, isoleucine, leucine and lysine in the exudate of Fe-deficient plants. Also, citrulline was the predominant amino acid in the exudate of normal plants while cystine was the predominant amino acid in the chlorotic exudate plants. Vancura (1964), identified 14 and 18 amino acids in the exudate of barley and wheat, respectively and indicated that cystine, glutamine, B-alanine, proline and γ-amino butaric acids were found in wheat exudate and not in barley exudate, on the other hand, barley contained cysteic acid and α-amino adipic acid. Thus, amino acids may also act as a chelator for iron, even small amounts is exuded (69 nmol/day) for the Fe-deficient plants, the accumulation effect of all complexing species may be appreciable. Wallace, 1962, Chaberack and Martell, 1959 reported that amino acids form stable chelates with microelements and the K-constant for Fe-cystine was 36 compared to 25 for Fe-EDTA.

Utilization of Insoluble-Fe-Sources by Corn.

It is worth to mention, that at the time of application of different iron sources, iron deficiency symptoms were observed on the corn plants grown under the NS-Fe and the grwoth was about the same for all pots. After 5 days from the application of Fe sources, chlorotic symptoms completly disappeared for plants of the $FePO_4$ and acid soil treatments, and slightly changed for the $Fe(OH)_3$ and alkali soil treatments.

Dry Weight.

Results in Figure 3 show that the highest total dry weight/pot were obained for corn plants grown when a complete nutreint solution was used. The lowest dry weight values were obtained for the control (-Fe) and when Fe-III-hydroxide or 5 g of the alkali soil were used as a source of Fe. On the other hand, the use of $FePO_4$ or 5 g of the acid soil caused a pronounced increase in total dry weight as the values were 5.2 g and 4.7 g, respectively compared to 3.8 for the control. The same trend was found for the growth of roots in solution and the above ground part (stem + leaves), but not for the roots in sand.

The disappearance of chlorosis and the increase in dry weight particularly from $FePO_4$ and acid soil treatments indicates that iron was utilized from these insoluble sources. Azarabadi and Narschner, (1979) found that chlorophyl content increased in young leaves

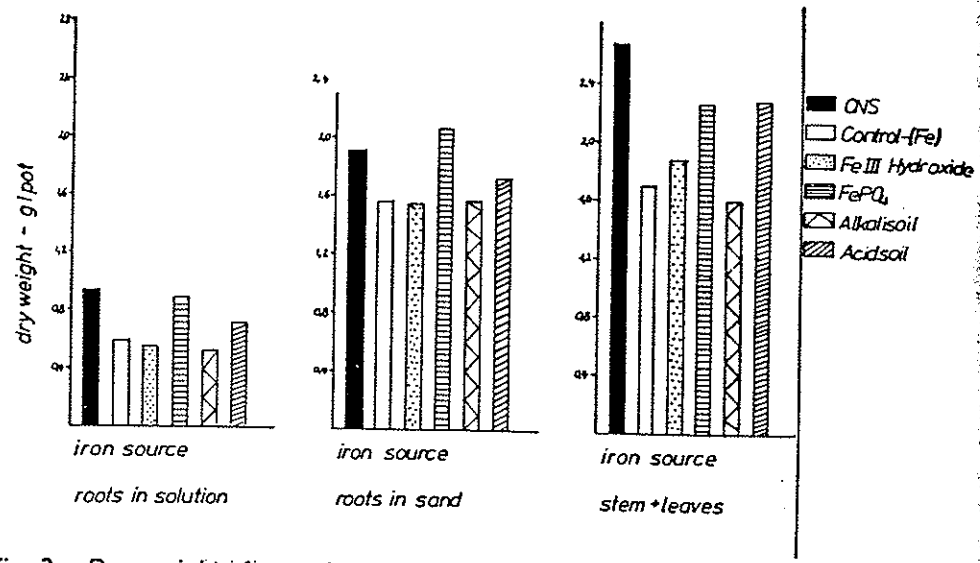

Fig. 3 : Dry weight of corn plants treated with different iron sources

FIG. 3: DRY WEIGHT OF CORN PLANTS TREATED WITH DIFFERENT IRON SOURCES

treated with fresh precipitated Fe-III-hydroxide. Thus, it is evident that the inorganic sources of Fe-III differ in thier regreening effect due to their difference in the anion associated with Fe.

Iron content in plant parts.

Inspite of the pre-washing of the roots in diluted acid and deionized water, relatively high concentrations of Fe were found in the roots grown in the solution (lower part) of all treatments including the CNS treatment as compared with the control Figure 4. The highest increase was for both the.$Fe(OH)_3$ and $Fe\ PO_4$. However, the increase found by the alkali soil treatment was about three times that of the control and the increase caused by the acid soil was 8-times that of the alkali soil. Such results suggests that in this active part of the root system, iron is partly absorbed and partly precipitated. The adsorption of iron on the surface could be a physical process due to the colloidal nature and high specific surface of $Fe(OH)_3$ and $FePO_4$. ashired et al., 1973 by studying the absorption and translocation of ^{59}Fe from various parts of corn roots (0-18 cm

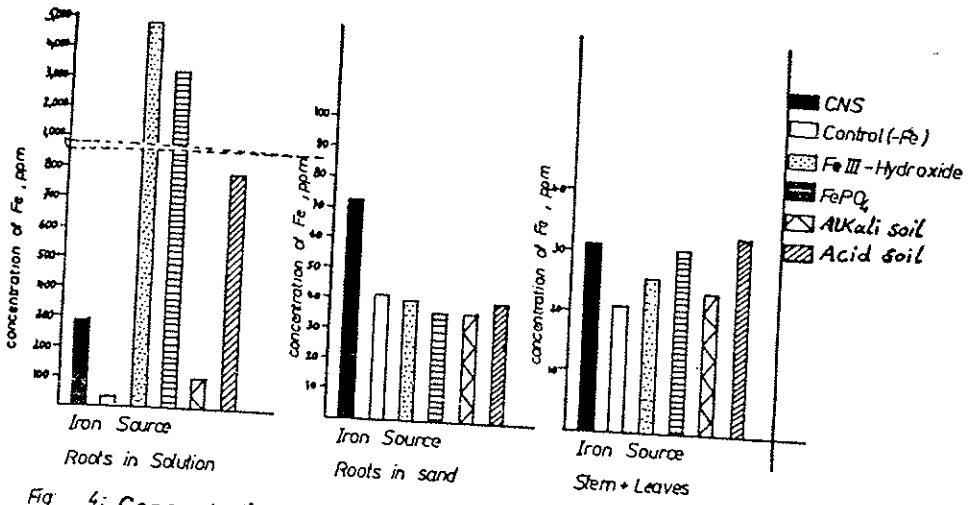

Fig. 4: Concentration of Fe in corn plants treatet with different Iron sources

FIG. 4: CONCENTRATION OF Fe IN CORN PLANTS TREATED WITH DIFFERENT IRON SOURCES

from the tip) found that accumulated ^{59}Fe in treated exised root zone was several times greater with $FeCl_3$ than with Fe-EDTA and suggested that precipitation of Fe from $FeCl_3$ is in the root surface or in the free space.

The uptake of Fe by the lower part of the roots follow the same trend as concentration and was more influenced by the concentration rather than by dry weight.

Except for the CNS treatment, the concentration of iron in roots grown in sand (upper part) were about the same as that of the control Figure 4. This indicates that the increase found in iron concentration in the lower part was not reflected on the iron content in the upper part due to the limited activity and growth in the later. In the CNS treatment iron concentration in the upper part was about doulbe that in the lower part. Such concentration could be considreed the actual absorbed concentration and any increase found for Fe content in the lower part is in the adsorbed or precipitated form.

TABLE 2

pH and soluble Fe in the $CaCl_2$-solution from different sources of iron.

Treatments	Without plants		With plants	
	pH	Fe ppm	pH	Fe ppm
CNS	5.7	3.0	6.6	2.6
Control (-Fe)	6.8	-	6.8	-
Fe-III-hydroxide	6.9	-	6.9	0.1
$FePO_4$	6.4	-	6.5	0.2
Alkali soil	6.7	-	6.9	-
Acid soil	6.6	-	6.9	-

The uptake of iron slightly increased in the upper part of roots of the $FePO_4$ treatment.

Results of Fe concentration in the above ground portion (stem + leaves) Figure 4 revealed a different magnitude of increase and the highest increase was for the $FePO_4$ and acid soil treatments as the concentrations of iron were about similar to that found when the CNS was used. There was a slight increase in the Fe content of stem + leaves caused by the Fe-III-hydroxide and alkaline soil treatments. Thus, the ability of the roots to utilize iron from relatively insoluble forms was evident from the plant recovery from chlorosis and the increase in iron content in root and consequently in leaves. Differences found amoung sources of iron could be related to their chemical nature and type of anion associated with Fe-III and consequently the ease by which organic acid, amino acids and possibly other chelators can react directly to form soluble and stable metal chelates. $Fe(OH)_3$ has a solutility product of 10^{-39} compared to 10^{-22} for $FePO_4$ and the critical concentration of Fe species in soils ranges from 10^{-9} to 10^{-8} molar. Olsen et al., (1982). Thus, iron may be present mainly as phosphate in the acid soil, while the carbonate, hydroxide and oxide, which are less soluble forms are probably dominant in the alkali soil.

Iron content in the $CaCl_2$-solution.

Results reveal that in the absence of plant roots, soluble iron was not detected in the $CaCl_2$ solution, Table 2. This is expected from iron hydroxide and iron

phosphate at this recorded pH vlaue (6.9 and 6.4) and aeration conditions prevailed. The absence of soluble Fe in both soil treatments could be due to the previous destruction of organic compounds which usually form soluble complexes with iron in soils, Elgala et al., (1976). Krauskopf (1972) stated that at pH >5 no iron can exist stable in oxidizing solution at concentration >0.01 ppm except in the form of organic complexes. In the presence of plant roots, data of the pH values in the $CaCl_2$ solution treatments indicates that corn plants did not cause a decrease in pH of the medium as previously found by Venkat Rajau and Marschner, 1972, Clark and Brown, 1974, Hether, et al., 1984). Small amounts of Fe were detected in the Fe-III-hydroxide and $FePO_4$ treatments as 0.1 ppm and 0.2 ppm were recorded, respectively. Such results confirm the previous speculation that exuded organic acids and amino acids solubilized Fe from the adsorbed or precipitated form at the root surface or in the root free space and this does mean that iron must be brought first in solution before it can be utilized by plants. Actually, the presence of chelated forms of organic and amino acids in soil solution will subject them to microbial decomposition or adsorption on clay minerals as no soluble iron was detected in the alkali and acid soil treatments even with an increase in the plant uptake of iron.

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