The Effect of Smut Gall Tumour Infection on Iron and Zinc Uptake and Distribution in Maize Seedlings

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SUMMARY

The amounts of Fe, and Zn were measured in maize seedlings infected by smut gall tumour (Ustilago maydis Dc. Cda.) and in healthy seedlings five days after infection. The amount of elements was also measured under different stress intensities. Due to the infection, as a biotic stress, the amount and distribution of examined elements have been changed. On the bases of the differences in the Fe distribution between the symptoms less and tumorial leaf parts, we have come to the conclusion that the infection also effects the mobilisation of Fe and Zn inside the plants. The Fe uptake was much higher in the infected plants and the tumour development also had an effect on the uptake and distribution of the examined elements. The experiments of infecting maize seedlings by monosporidial strain of crown gall tumour showed no tumour development. We found that the monosporidial strain also acts as a biotic stress and has an effect of iron and zinc distribution. We observed a slight difference in the iron and zinc contents in the roots of corn seedlings infected by different monosporidial sporidium concentrations, while the iron and zinc contents in the shoots were increased by the intensity of the infection. The roots do not form tumours. There is no difference between the roots of the infected and healthy corn seedlings. Since the Fe and Zn contents of the shoots of infected plants depend on the intensity of the infection, we have come to the conclusion that there must exist a „special" communication system regulating the transportation of the examined elements.

In the experiments with infected maize seedlings, it became necessary to get the iron chlorosis before the disease reaches the lethal phase. Although most of the iron reserves are located in the embryo, to accelerate the chlorosis, the endosperm was removed, and it was observed, that the iron chlorosis appears later in maize seedlings when the endosperm is removed. The relative chlorophyll content of the first and second leaves was measured in iron efficient and iron deficient maize seedlings at different times.

The higher IAA content of tumorial plant tissues is already known. The treatment with IAA decreases the iron concentration in the shoots and in the roots of +Fe precultured plants and increases at -Fe precultured ones. The TIBA retards the shoot-to-root transport of IAA. When the seedlings were treated simultaneously with IAA and TIBA, higher iron concentrations were observed in the shoots and in the roots of corn seedlings.

We found extremely high iron concentrations in the roots of infected seedlings and, in line with this, serious damage to the roots was observed that this can be caused by the high iron content generated free radicals. The results demonstrate that IAA has a role in the shoot to root communication.

Keywords: *IAA, TIBA, Iron and Zinc distribution, Chlorophyll content*

INTRODUCTION

The role of IAA is widely studied and known in the coordination of plant growth and development. It was also studied that plant hormones can play a role in the communication among different plant organs (Davies et al., 1987; Jeschke et al., 1997; Gomes-Cadenas et al., 1998; Ming Gong et al., 1997; Reed et al., 1998). In corn seedlings infected with smut gall tumour (*Ustilago maydis* Dc. Cda.), an increased iron uptake can be observed as a consequence of the higher iron demand of the symptom showing shoots, supposedly caused by a specific signal from shoot to root (Lévai and Kovács, 2000). The IAA production of tissues infected with smut gall tumour is higher, while the IAA-oxidase activity is low and the IAA is present mainly in a free form (Dvoreckaja, 1966; Archikovszkaja and Mihalevszkaja, 1967). The higher iron concentration in the infected corn seedlings is supposedly due to the increased IAA transport from the shoots to the roots. One of the aims of the present study was to determine the effect of IAA and TIBA treatments on the iron concentration in corn seedlings.

Fe deficiency (chlorosis) can also be observed on leaves which have sufficient iron contents. The decreased mobility and physiological activity of Fe is due to endogenous reasons (Römheld, 1997). The leaves or leaf parts also become chlorotic as a result of certain infections e.g. in maize seedlings infected by smut gall tumour. The leaf areas showing symptoms of infection are chlorotic and tumour development can be seen. Other leaf areas of the same leaf are green. The tumour development depends on the intensity of the infection. If the infection modifies the uptake and transport of different elements to the shoots, the question that should be answered is: does this modification effect depend on stress intensity and on tumour development? The effect of endosperm on chlorosis development was also studied. It was observed that the iron chlorosis appears later in maize seedlings when the endosperm is removed. The relative chlorophyll content of the first and of the second leaf was measured of iron efficient and iron deficient maize seedlings at different times. The Fe and Zn contents of the roots and shoots were measured in maize seedlings infected by different concentrations of compatible strains (tumour development) and monosporidial strain (no tumour development). The iron and zinc contents also were measured in symptom-less and symptom-showing leaf parts of infected corn seedlings. Grusak (1997) came to the conclusion that there should exist a communication system in plants that determines the uptake and distribution of iron. Any biotic stress has effects on the iron uptake and distribution in corn seedlings (Lévai and Kovács, 2001). Supposedly, the increased iron uptake and transfer to the infected shoots can be a result of a special communication system.

MATERIALS AND METHODS

Chlamidosporas of smut gall tumour (*Ustilago maydis* Dc. Cda.) were germinated on potato-glucose agar. The monosporidial strains were separated from the multisporidial mixture. The infection was induced with a 1:1 mixture of two compatible monosporidial strains. The inokuulum was diluted with distilled water corresponding to sporidium concentrations of 2,5, 5 and 7,5 x 10^3x mm^{-3}. The same concentrations were used for infiltration with a monosporidial strain. A single cross hybrid of corn (*Zea mays* L. cv. Norma) was used in the experiments. The seeds were surface sterilised in 18% hydrogen peroxid for 30 minutes and then washed five times in distillated water. The seeds were then transferred for 4 hours to the Erlenmeyer flask containing 10 mM $CaSO_4$ solution. The seeds were put on moistened filter paper in Petri dishes and germinated in the dark at 25°C for three days. The tips of the coleoptils of seedlings with 5-10 mm shoots were cut, then infected by vacuum infiltration method. The infected seedlings were then washed 5 times in distillated water to remove the sporidiums from the surface. The non-infected seedlings received the same treatments without sporidium. The seedlings were then transferred to a nutrient solution. The nutrient solution had the following composition: 2,0 mM $Ca(NO_3)_2$, 0,7 mM K_2SO_4, 0,5 mM $MgSO_4$, 0,1 mM KH_2PO_4, 0,1 mM KCl, 1 µM H_3BO_3, 1 µM $MnSO_4$, 0,25 µM $CuSO_4$, 0,01 µM $(NH_4)_6Mo_7O_{24}$. The Fe was added in the form of FeEDTA in concentration of 5 x 10^{-4} M. The nutrient solution was continuously aerated and changed every second day. There were two main plant groups: the plants of the first group were grown on an iron efficient nutrient solution, while the second group received no iron. There were maize seedlings with, and without endosperms in each main plant group. The endosperm was removed when the seedlings were transferred to a nutrient solution The relative chlorophyll content of the first and the second leaf was measured daily at the same time of day (14.00), between 8-12 days of age in both plant groups after the germination. The chlorophyll content was measured at three different places on the middle of the leaves. The number of repetitions was ten. The values are the mean of the 30 measurements. Plants were grown under controlled environment conditions with 70% humidity, a light intensity of 390 $\mu Em^{-2}s^{-1}$, a day-night temperature of 25-23°C and a light-dark period of 14-8 hours. The TIBA was mixed in Lanolin (Sigma) to 10^{-4}M final concentration applied to the mesocotyl when the seedlings were replaced to the nutrient solution. 5 µL of IAA at a concentration of 10^{-4}M was given to the leaf-tips at day 7 and 9. The treatments were repeated on the 12^{th} day after germination. TIBA was applied at 10^{-4}M as a drop to the leaf-bases, and ten hours later IAA was applied as leaf spray.

The samples were collected on the 5^{th} day after the infection. The roots of seedlings were washed in 0,1 N HCl to remove the apoplasmatic-bounded ions. The shoots and the roots were separated. The leafs of infected seedlings were then divided into symptom-showing and symptom-less parts. The samples were dried at 85°C. The content of elements was measured by OPTIMA 3300DV ICP-OA photometer (Perkin-Elmer). The relative chlorophyll content was measured by MINOLTA Chlorophyll Meter (Spad-502). After the last chlorophyll measurements the leaf areas also were measured by LI-COR (Model. LI-3000, Lambda Instruments Corp. USA).

RESULTS AND DISCUSSION

The effect of infection and its intensity on iron and zinc uptake and distribution

Infection, as a biotic stress, has a significant effect on the uptake and translocation of the measured elements. Differences were observed between the tumuorial and green leaf parts of infected corn seedlings in the Fe and Zn amounts. The Fe amount is higher in the shoots and roots of infected seedlings. The distribution of Fe between the different leaf parts shows that there is an intensive Fe accumulation in the green leaf parts. Its Fe content is 45% more than that of the control and 50% more than that of the tumorial leaf parts of the same leaf. The Fe amounts in the roots of infected seedling also are higher (13%). The total amount of Zn is lower in the shoots of infected seedlings in comparison to control, but there is a large difference of its content between the green and tumorial leaf parts. The Zn amount is higher by 54% in the green parts than in those of tumorial leaf parts. The results are shown in *Figure 1*.

Stress intensity also modifies the amounts and distribution of examined elements. Due to the infection with the smallest sporidium concentration, the Fe amounts increase in the shoots (12,4%) and in the roots (32,7%). Decrease of the Fe contents (-17%) in the shoots can be observed only at the highest sporidium concentration, while the Fe content of roots remains over the control level (8,5%). Similarities can be seen in the tendency of changing of Fe amounts in the shoots and roots under various stress strength. The Zn amounts also increase in the shoots parallel to stress intensity. The values are higher than the control.

The Zn content in the roots decreases as the stress intensity become higher and remain in all cases under the control. Results are shown in *Figure 2*.

Figure 1: **The amounts of Fe and Zn in the shoots and roots of corn seedlings infected with compatible strains of crown gall tumour (amounts in.μg x g^{-1} dry wt) Symbols: C: control, G: green leaf parts, T: tumorial leaf parts, A: the mean of G and T**

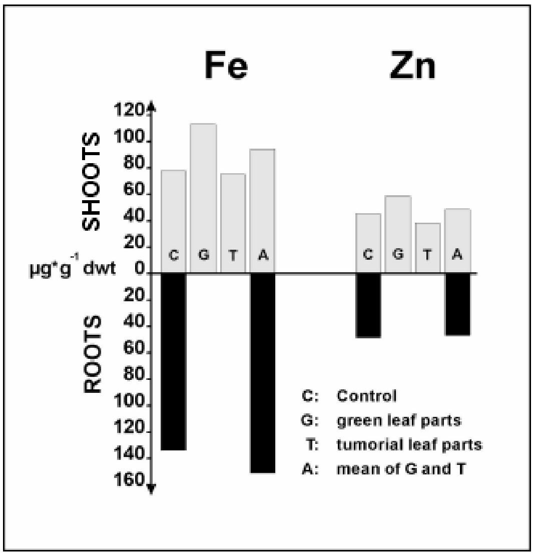
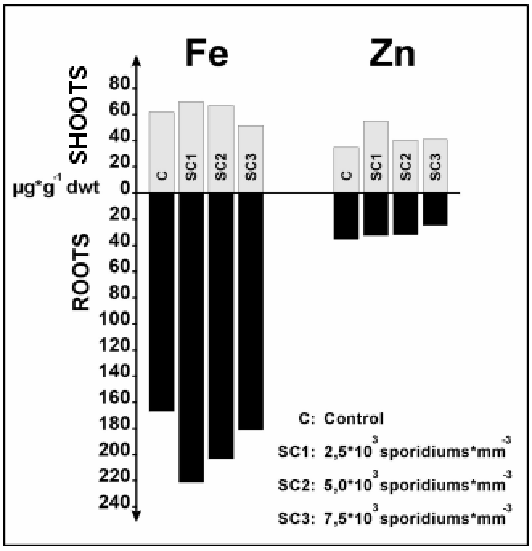
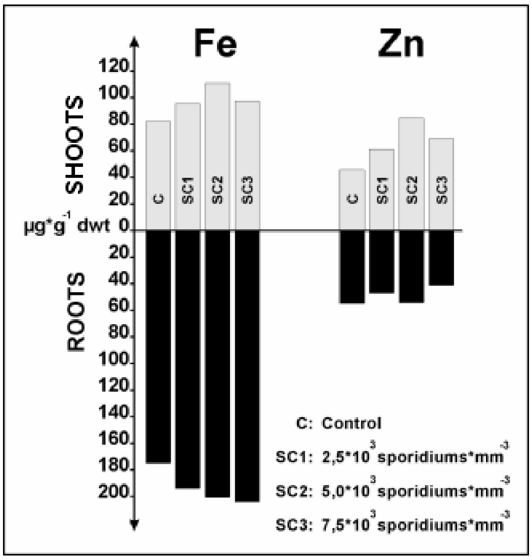


Figure 2: **The amounts of Fe and Zn in the shoots and roots of corn seedlings infected with different concentrations of compatible strains of crown gall tumour (amounts in.μg x g^{-1} dry wt) Symbols: C: control, SC1, SC2, SC3: sporidium concentrations of 2,5-5,0-7,5 x 10^3 sporidiums x mm^{-3}**



The monosporidial strain of crown gall tumour does no develop tumour but acts as a biotic stress. Due to the infiltration with a monosporidial strain, the Fe and Zn amounts increase in the shoots. Only the strongest stress caused a decrease in the examined amounts. The amounts of iron also increase in the roots according to stress strength. This tendency is in contradiction to that observed at infection with the compatible (tumour forming) strains of fungus (*Figure 3*).

Figure 3: **The amounts of Fe and Zn in the shoots and roots of corn seedlings infiltrated with different concentrations of monosporidial strain of crown gall tumour (amounts in.μg x g^{-1} dry wt) Symbols: C: control, SC1, SC2, SC3: sporidium concentrations of 2,5-5,0-7,5 x 10^3 sporidiums x mm^{-3}**



The effect of endosperm on chlorosis and leaf size of maize seedlings

To accelerate the appearance of the chlorois the role of endospermium on relative chlorofill content and leaf size were examined. There were no significant differences observed in the early phase of plant development in relative chlorophyll content of maize seedlings growing in an iron deficient nutrient solution. *Figure 4* shows that the presence of the endosperm and its missing have a moderate effect on the relative chlorophyll content of the first leaf of maize seedlings. The values are higher when the endosperms were removed. The differences are still higher in the second leaves (*Figure 5*).

Higher chlorophyll content was observed in the first leaves of endosperm-less seedlings, growing under Fe supply in comparison to ones growing without iron. The seedlings with endosperm show the same differences.

The chlorophyll contents in the first leaves of maize seedling with endosperm growing under iron supply are higher than the values of those which have no endosperm. This stands in contradiction with the observations represented in *Figure 1* and *2*. The results can be seen on *Figure 6* and *Figure 7*. In contrast to the results supposed at the beginning of the experiments, the results show that the removal of the endosperm affects on the iron distribution and on the transport-activity in the maize seedlings. The iron concentration was higher in the leaves of endosperm less plants (*Table 1*).

As *Figures 8-9* show, that the endospermium has a significant effect on leaf size. The leaves are smaller when the endospermium is removed, and the seedlings are grown on iron deficient nutrient solution.

Figure 4: **The relative chlorophyll content of the first leaves of maize seedling in different time after the germination**
(: Fe-deficient and endosperm-less,
: Fe-deficient with endosperm)

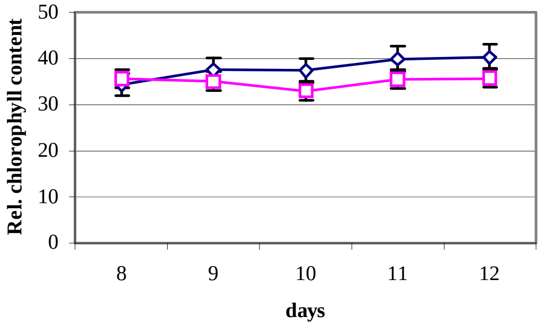


Figure 5: **The relative chlorophyll content of the second leaves of maize seedlings in different time after the germination**
(: Fe-deficient and endosperm-less,
: Fe-deficient with endosperm)

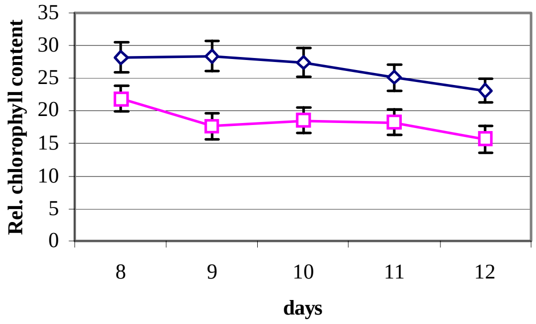


Figure 6: **The relative chlorophyll content of the first leaves of maize seedlings in different time after the germination**
(: Fe-efficient and endosperm-less,
: Fe-efficient with endosperm)

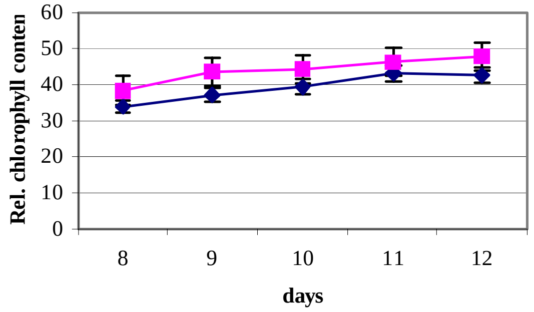


Figure 7: **The relative chlorophyll content of the second leaves of maize seedlings in different time after the germination**
(: Fe-efficient and endosperm-less,
: Fe-efficient with endosperm)

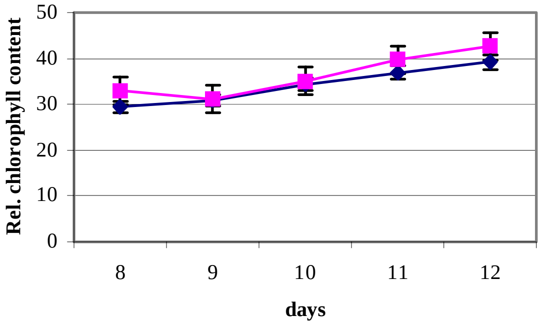


Table 1

Iron concentrations in the first and in the second leaves of maize seedlings with, and without endosperm growing under iron deficiency

Treatments	1. leaf Fe mg x kg^{-1} dw^{-1}	2. leaf Fe mg x kg^{-1} dw^{-1}
yes endosperm no Fe	25,6±2	21,3±5
no endosperm no Fe	57,3±6	36,9±5

Figure 8: **The leaf-areas of the first leaves of maize seedlings +/- endosperm and +/- Fe at the 12 days after the germination**

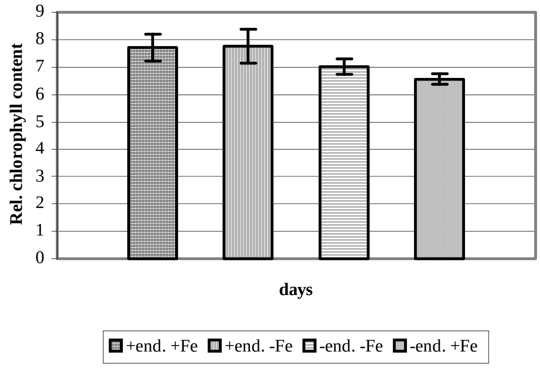
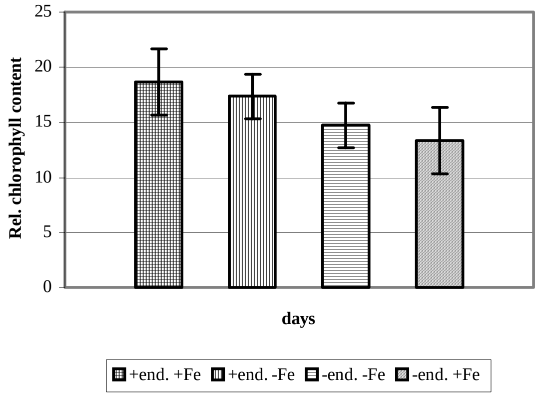


Figure 9: **The leaf-areas of the second leaves of maize seedlings +/-endosperm and +/- Fe at the 12 days after the germination**



The differences between endospermium less -Fe precultured and +Fe precultured seedlings with endospermium need further experiments.

The more intensive iron transport from the embryo has a delay-effect on iron chlorosis at endosperm-less maize seedlings growing under iron deficiency. The shortage in nutrients of seedlings improves the iron supply of the firstly developing leaves, that increases the chlorophyll content and consequently the production of organic matters.

The role of IAA and its retardant TIBA on iron uptake

The tumorial maize tissues have high IAA content. The effects of IAA treatment on the non-infected corn seedling are contradictionary. There are no significant differences between the control and IAA treated seedlings. The iron concentration is very slightly decreasing in the shoots and in the roots of +Fe precultured seedlings while an increasing effect can be observed at the -Fe precultured seedlings. Higher iron concentration can be observed in the shoots and in the roots when the seedlings were treated with IAA and TIBA. The results can be seen on *Figure 10* and *Figure 11*.

Figure 10: **Iron concentration of the shoots and of the roots of non infected, +Fe precultured corn seedlings (+C), treated with IAA (+CI) and with IAA and TIBA (+CIT) (mg/kg dwt.)**

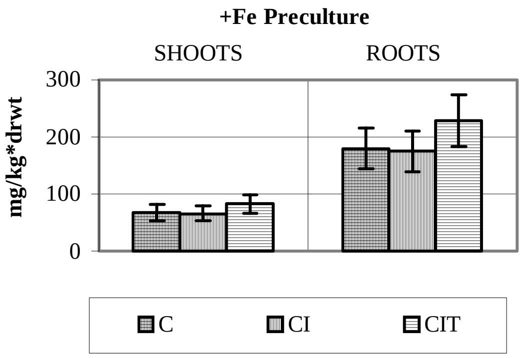
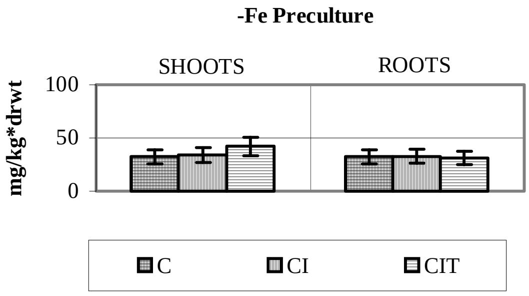
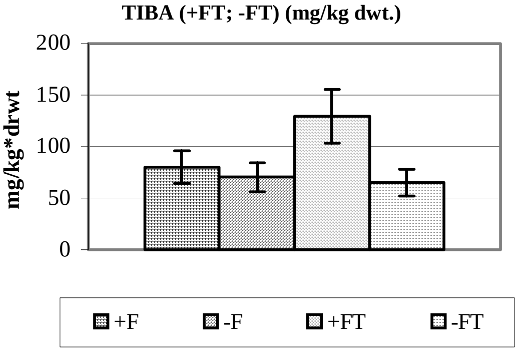


Figure 11: **Iron concentration of the shoots and of the roots of non infected, -Fe precultured corn seedlings (-C), treated with IAA (-CI) and with IAA and TIBA (-CIT) (mg/kg dwt.)**



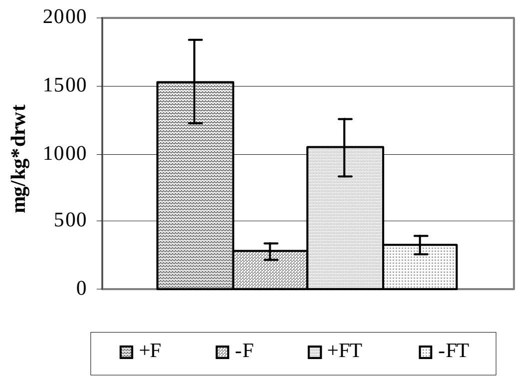
The infection acts as a biotic stress. The iron concentration is higher in the infected shoots in comparison to the non infected ones. The treatment with TIBA significantly increases the iron concentration in the infected shoots at +Fe precultured plants and decrease can be observed at -Fe precultured seedlings. The results are shown on *Figure 12*.

Figure 12: **Iron concentration of the shoots of infected, +Fe and -Fe precultured corn seedlings (+F; -F) and treated with TIBA (+FT; -FT) (mg/kg dwt.)**



Serious retardation of growth can be observed on the roots of infected seedlings, although the roots do not show the symptoms of infection (tumour development). The iron concentrations of the roots of infected seedlings are higher then it was observed at the non infected ones. There are extremely large differences between the iron concentrations of +Fe and -Fe precultured seedlings. The high iron concentration in the roots of +Fe precultured infected seedlings can be explained by an intensive iron uptake/accumulation of these roots. The results can be seen on *Figure 13*. The results demonstrate that IAA has a role in the shoot-to-root communication system. In contrast with our earlier suppose, to increase the level of IAA in the shoots the increased iron concentration cannot be observed. The TIBA treatment increased the iron concentration in the shoots and in the roots of corn seedling in all cases when +Fe preculture was applied. The TIBA treatment was especially effective at the infected-, and +Fe precultured seedlings. The effect of IAA is not in positive correlation with its concentration. An increased iron concentration of the shoots and roots could be observed when the TIBA treatment inhibited the transport of IAA from the shoots to the roots.

Figure 13: **Iron concentration of the roots of infected, +Fe and -Fe precultured corn seedlings (+F; -F) and treated with TIBA (+FT; -FT) (mg/kg dwt.)**



Conclusion, the regulation effect of IAA in iron uptake works at a very low IAA level. The high iron concentration in the roots of infected seedling is in contradiction with the high endogenous IAA level of corn seedling infected by smut gall tumour. The iron concentration of -Fe precultured infected seedlings is higher then the non-infected ones, and can be a consequence of a more intensive mobilization of reserved iron. To explain the extremely high iron concentration of the roots of infected and +Fe precultured corn seedlings, and to explain their damage, further experiments will be necessary.

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