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Ph.D. Theses

BIOLOGICAL EFFECTS OF CYCLIC HYDROXAMIC ACIDS,

THEIR ROLES IN MICROELEMENT UPTAKE

AND MICRONUTRIENT TOLERANCE

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1. INTRODUCTION, OBJECTIVES

Cyclic hydroxamic acids are produced as secondary metabolites primarily of the economically important species of the *Poaceae* family, and excreted through their roots. Due to the manifold biological roles of this group of organic compounds, their properties have been extensively investigated since their discovery.

However, following the review article of NIEMEYER appeared in 1988, no studies summarizing the properties of cyclic hydroxamic acids have been published. As no review paper on the various biological roles of such compounds has been compiled in Hungarian, one of the major objectives of the present Theses was to summarize recent knowledge in this field. For this reason almost 200 communications, appeared in 60 Hungarian and international scientific journals, as well as book-chapters and another materials were used. The chemical properties of the cyclic hydroxamic acids, their occurrence in plants, and their biological roles together with explanation thereof were surveyed. Special emphasis was made on the phytosiderophore function, isolation and structural determination of cyclic hydroxamic acids. For the sake of completeness and comparison, the characteristics of the mugineic acid-type compounds and of the siderophores of microbial origin were briefly discussed. Collection and elaboration of the literature data for the preparation of the thesis and the dissertation were closed in February 2001.

The other goal of the present Theses was to confirm and clarify the role of cyclic hydroxamic acids in the microelement uptake. The hydroxamic acids released by the plants, form complexes with iron in the soil solution thereby facilitating the uptake of iron. Uptake of the iron complexes of the added hydroxamic acids was also recognized in plants, which did not produce cyclic hydroxamic acids. The role of cyclic hydroxamic acids in the iron uptake is also substantiated by the fact that many of the bacterial and fungal substances responsible for the iron uptake and complexation are hydroxamate-type compounds, and some of these derivatives carry the hydroxamate group in cyclic form, similarly to the cyclic hydroxamic acids produced by the plants. The mugineic acid-type compounds, possessing complex-forming properties and excreted by the species of the *Poaceae* family through their roots, show similar properties to those of the hydroxamic acids of microbial origin. It was established that besides the iron uptake, this group of compounds also plays role in the uptake of copper, zinc and manganese. Thus, the plants of the *Poaceae* family may produce various types of complex-forming compounds. From preliminary studies it is concluded that the release of mugineic acid and cyclic hydroxamic acid by the various species is rather different. When $FeCl_3$ is added, the species releasing high amounts of mugineic acid produce small amounts of cyclic hydroxamic acid or do not produce such compounds at all. On the contrary, species with low mugineic acid production show up with high cyclic hydroxamic acid excretion. It was proved earlier that even the daily cadence of excretion of these two types of substances are different.

Besides iron, cyclic hydroxamic acids form complexes also with another metal ions, therefore I anticipated that the uptake of such metals can also be facilitated this way. Keeping this in mind, I investigated the role of cyclic hydroxamic acids in the uptake of iron, manganese, copper, zinc and nickel. In parallel studies I investigated the role of the cyclic hydroxamic acids in the microelement tolerance, as well as the excretion of cyclic hydroxamic acids by the different plant species upon addition of Fe(III)-EDTA.

Clarification of the role of cyclic hydroxamic acids in the microelement uptake is considered a new biological function of related compounds. Therefore, investigation of these effects is important from both theoretical and practical points of view, and such studies permit to turn to another questions and to the appropriate explanations. Among the plant species producing these compounds there are several cultivated plants, such as *Triticum aestivum*, *Secale cereale* and *Zea mays*, as well as certain dangerous, frequently occurring weeds, such as *Agropyron repens* or *Echinochloa crus-galli*. The cultivated species are used as foodstuffs, raw materials for the food industry, or as fodder-plants. The microelement uptake of the cultivated plants determines their microelement content, which has an indirect influence on their quality and nutriment value. Either a decreased or enhanced microelement content may be disadvantageous, as even the essential microelements could be potentially toxic in concentrations higher than the optimum value. In the case of the cultivated plants, which serve the basis of the nourishment-chain, the lack or excess of microelements finally appears at the human level, that is at the end of the chain. The higher the cyclic hydroxamic acid excretion of a plant species is, the higher its ability for microelement exploration is. This is most particularly important because during the harvest of voluminous crops, significant amounts of microelements are removed whose recirculation has not been elaborated. The weed species producing cyclic hydroxamic acids are competitors of the cultivated plants. For the explanation of the details of this competition, and for the evaluation of the competitiveness of the weed species the knowledge of a more efficient microelement uptake is essential. Naturally, the competitiveness of the weed species is also enhanced by the allelopathic effect of the cyclic hydroxamic acids.

Abbreviations:

DIMBOA-glucoside = GDIMBOA: 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one

DIBOA-glucoside = GDIBOA: 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one

cHx = cyclic hydroxamic acid

The structure of the cyclic hydroxamic acids and their glucosides

The cyclic hydroxamic acids are present in form of glucosides in the differentiated plant tissues. In case of tissue damage specific glucosidase enzymes release the aglucones.

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2. METHODS AND MATERIALS

The role of cyclic hydroxamic acids in microelement uptake was investigated in nutrient solutions. I used the following plants for the experiments: plants not producing cyclic hyroxamic acids: cucumber (*Cucumis sativus* cv. Joker F1; cv. Budai Korai) and bxbx maize mutant (*Zea mays* cv. bxbx); a maize cultivar that produces cyclic hydroxamic acids (*Zea mays* cv. Norma). In the examination of cyclic hydroxamic acid secretion I chose cultivated wheat (*Triticum aestivum* cv. Mv16) and rye (*Sevale cereale* cv.Kisvárdai) as well.

The plants were cultivated in nutrient solutions in a climatic chamber with illumination for 16 hrs at 240W/m^2, thermoperiod: 25/22^{o}C, humidity: 65-70%. The nutrient solutions for cucumber and the maize plants were prepared as described by CSEH *et al*. (1982) and TREEBY *et al*. (1989), respectively. In the experiments aimed at investigating the microelement tolerance, the microelement content of the above nutrient solutions was enhanced, and the effect of the cyclic hydroxamic acids was studied under such conditions. From both species 20 plants were cultivated in 3 L of the nutrient solution, but maize species were also raised in 1-L volume of the solution by using 10 plants in such cases. For comparison of the release of hydroxamic acid, 15 maize and 25-25 wheat or rye plants were cultivated in one-liter nutrient solution. The solutions were continuously aerated and exchanged in each 3rd day. The mode and extent of the addition of iron were different for the individual experiments.

For the experiments the cyclic hydroxamic acids were used in form of the readily soluble glucosides, since in such a form their phytotoxic effects appear only at higher concentrations, they are more stable, nevertheless their complexation ability is very similar to that of the aglycones. These glucosides are not available in the market; therefore I isolated these substances at our Department from young, etiolated rye and maize shoots. In the experiments with the maize species mainly GDIMBOA was used, as it is the predominant hydroxamic acid in this plant.

The application of the hydroxamic acid glucosides carried out in two ways. In the first case the plants cultivated in solutions deficient in the element(s) to be investigated were incubated in a solution of the complex formed from the element and the glucoside for 1-6 hrs, and then the plants were put back into the respective, metal-deficient solutions (treatment for a determined period). In the other cases the glucosides were added to the nutrient solutions on the third, or on the third and the sixth days before taking a sample (continuous treatment) so that the complex was in contact with the plant roots for 3-6 days. For control treatments either the cyclic hydroxamic acid or the element(s) were discarded. Following 1-3 days of the administration of the cyclic hydroxamic acid, the shoots of the experimental plants were harvested and dried, and the microelement content was determined by using the ICP technique, or with the aid of an atom absorption spectrometer. The developed and observed differences are significant at a P=1% level in most cases, the less frequently observed P=5% level and the non-significant differences are indicated in the Tables with the marks * and n.s., respectively, beside the measured values, or above the columns in the Figures.

As samples a part of the shoot above the node of the maize choleoptyl and a part of the shoot above the cucumber cotyledons were taken. In the charts and figures the data obtained with these shoots parts are shown.

In accord with the daily cycle of excretion, the release of hydroxamic acid of the different species was measured in the morning hours. The quantity of the released hydroxamic acid, and the cyclic hydroxamic acid content of the plant samples were determined by means of the HPLC technique and standard curves following a multi-step purification procedure. Excretion of the mugineic acid-type phytosiderophore was measured according to the method of ZHANG *et al*. (1989).

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3. RESULTS

Considering the limited volume of the Thesis I present the results of some representative set of trials.

3.1. Microelement uptake

3.1.1. The effect of the treatment with the cyclic hydroxamic acid glucoside for a determined period on the microelement uptake of cucumber at various pH values:

The cucumber plants, pre-cultivated in a medium free from microelements, were placed for 2 hours onto the microelement solution, which corresponded in concentration to the basic solution (CSEH *et al*. 1982), and supplemented with $5x10^{-5}$ mole/liter of $FeCl_3$, or 10^{-7} mole/liter of $NiSO_4$, and then they were put back onto the solution free from the microelements. The pH of the microelement solution was adjusted to 4.0, 5.5 and 7.0, and a group of the plants at the various pH's was treated with 10^{-4} mole/liter of GDIBOA. The microelement content and the dry weight of the shoots was determined in samples taken after 3 days, and the results are summarized in Table 1 and in Figure 1 and 2.

Table 1.

The effect of GDIBOA and the pH on the microelement content and the dry weight (mg/kg dry weight; mg/piece of shoot) of the 19 days old cucumber shoots

	Treatment					
Element	4,0	4,0 GD	5,5	5,5 GD	7,0	7,0 GD
Cu	16,5	15,6	13,4	14,5	14,9	15,8
Fe	90,0	65,0	101,0	81,0	74,0	111,0
Mn	124,0	131,0*	113,0	123,0*	126,0	122,0 n.s.
Zn	185,0	233,0	177,0	184,0 n.s.	232,0	228,0 n.s.
Ni	17,3	13,4	16,2	15,0	16,0	13,8
Dry weight	21,6	21,6	20,4	19,7	20,2	18,3

Note: The values and the abbreviation GD in the second row relate to the pH and the addition of GDIBOA, respectively.

The addition of GDIBOA resulted in the increase of the Cu, Fe, Mn and Zn content at pH=5.5 and 7.0, at pH=7.0, at pH=4.0 and 5.5, and at pH=4.0, respectively. The Cu and Fe content decreased upon the addition of the hydroxamate at pH=4.0, and pH=4.0 and 5.5, respectively. The Ni content was lowered at each pH upon treatment with the hydroxamate.

The addition of GDIBOA resulted in the decrease of dry weight of shoots at pH=5,5 and 7,0 with 3,9 and

9,4%. The dry weight of shoots was not effected by the addition of GDIBOA at pH=4,0. As increasing the pH value the inhibitory effect also increased.

In another cases GDIMBOA was applied instead of GDIBOA for the treatment of the cucumber plants pre-cultivated in the absence of the microelements. In such cases the treatment carried out for 4 hrs at pH=4.0 or pH=6.0, and the concentration of the hydroxamate, $FeCl_3$, and $NiSO_4$ was $3x10^{-5}$, $5x10^{-5}$ and 10^{-6} mole/liter, respectively. The microelement contents and the dry weight of the 21 days old cucumber shoots are summarized in Table 2 and in Figure 3 and 4.

Table 2.

The effect of GDIMBOA on the microelement content and the dry weight (mg/kg dry weight; mg/piece of shoot) of the 21 days old cucumber shoots

Treatment				
Element	4,0	4,0 GDM	6,0	6,0 GDM
Cu	14,8	18,0	16,5	15,3
Fe	71,5	81,0*	79,5	64,5
Mn	215,0	248,0	221,0	256,0
Zn	74,1	58,6	53,4	61,4
Ni	24,7	19,4	25,9	25,7 n.s.
Dry weight	24,6	22,1	22,7	20,0

Note: The values and the abbreviation GDM in the second row relate to the pH and the addition of GDIMBOA, respectively.

At pH=4.0 the addition of GDIMBOA increased the Fe, Cu and Mn content, and enhancement of the Mn and Zn content was also observed at pH=6.0. Addition of the hydroxamate decreased the Ni and Zn content at pH=4.0, and lowered the Fe content at pH=6.0.

The addition of GDIMBOA resulted in the decrease of dry weight of shoots at pH=4,0 and 6,0 with 10,2 and 11,9%. At higher pH the inhibitory effect of hydroxamate addition was higher in case of GDIMBOA too.

3.1.2. Examination of the microelement uptake by the maize plants cultivated in hydroxamic acid-containing nutrient solutions for a longer period

To the nutrient solution of the ten days old maize plants GDIMBOA was added in 1, 3, and $9x10^{-6}$ mole/liter concentration, and addition of the same amounts of this substance was repeated at the 14^{th} day following exchange of the nutrient solution. The volume of $FeCl_3$ in the medium was 10^{-6} mole/liter. The microelement content and the dry weight of the 16 days old plants are shown in Table 3 and in Figure 5 and

6.

Table 3.

The effect of the GDIMBOA concentration on the microelement content and the dry weight (mg/kg dry weight; mg/piece of shoot) of the 16 days old maize plants

Element	Control	1×10^{-6} mole/liter	3×10^{-6} mole/liter	9×10^{-6} mole/liter
Fe	61,5	75,4	96,0	106,0*
Mn	191,0	208,0	196,0	165,0
Cu	21,8	21,2*	18,2	28,2
Zn	172,0	179,0 n.s.	152,0	180,0 n.s.
Cu	21,8	21,2*	18,2	28,2
Dry weight	58,8	60,2	56,8	57,4

We observed that the iron content was increasing by the hydroxamate addition, and enhancement of the copper content showed up at 9×10^{-6} mole/liter concentration.

The 1×10^{-6} mole/l hydroxamic acid addition increased with 2.4%, while the 3×10^{-6} mole/l hydroxamic acid addition with 3.4% and the 9×10^{-6} mole/l hydroxamic acid addition with 2.4% decreased the dry weight of the shoots corresponding to the control treatment.

In another experiments I cultivated maize plants in nutrient solutions free from iron for 8 days. Then at the 8th and 11th days the following treatment was applied: control - free from iron, 5×10^{-6} mole/liter $FeCl_3$, and 5×10^{-6} mole/liter $FeCl_3$ + 5×10^{-6} mole/liter GDIMBOA. The iron content and the dry weight of shoots of the 14 days old maize plants are shown in Table 4 and in Figure 7.

Table 4

The effect of GDIMBOA on the iron content and the dry weight of shoots (mg/kg dry weight; mg/piece of shoot)

	Control	$FeCl_3$	$FeCl_3$ + GDIMBOA
Fe-content	26,2	51,0	91,0
Dry weight	48,0	51,6	47,5

As an effect of the GDIMBOA addition the iron content of the shoots increased, but the dry weight decreased with 7,9%.

3.1.3. Iron uptake of the bxbx maize mutant

The bxbx maize mutant does not produce cyclic hydroxamic acids, therefore it is a suitable experimental plant for clarifying the role of cyclic hydroxamic acids in the microelement uptake and tolerance.

After I presented with the procedure of ZHANG *et al.* (1989) that this mutant does not compensate the lack of hydroxamic acid release with higher mugineic acid excretion I investigated the influence of the GDIMBOA treatment on the iron content of the shoots of this variety. In one type of the experiments the 11 days old plants, pre-cultivated in an iron-free nutrient solution, were divided into two groups. The representatives of the first series were placed onto a $5x10^{-5}$ mole/liter $FeCl_3$ solution for 6 hrs and the members of the other series were cultivated for 6 hrs in a nutrient solution which contained $5x10^{-5}$ mole/liter quantity of the Fe(III) complex of GDIMBOA. Following such treatments, the roots of the plants were soaked in distilled water for 1 hr, rinsed thoroughly, and then put back into the iron-free nutrient solution. The shoots were harvested after one and four days, dried until constant weight and the iron contents were determined. The results of these experiments are summarized in Table 5 and in Figure 8.

Table 5.

The effect of a 6 hour GDIMBOA treatment on the iron content and dry weight (mg/kg dry weight; mg/piece of shoot) of the maize shoots

Date of taking the sample	Fe-content		Dry weight	
	-GD	+GD	-GD	+GD
1 day after treatment (12 days old)	101,5	156,0	19,9	21,3
4 days after treatment (15 days old)	144,0	165,0	25,4	27,9

Note: The abbreviations of +GD or -GD in the second row relate to the addition or the lack of addition of GDIMBOA respectively.

In the bxbx mutant the iron content increased in both data of observation following treatment with GDIMBOA for a defined period (6 hrs), therefore this glycoside facilitated the uptake of iron. The differences are more apparent after treatment for 4 days, indicating that translocation of iron into the leaves takes time.

One day after treatment the amount of the cyclic hydroxamic acid glucosides in the shoots and the roots was determined. In the roots of the bxbx maize mutants a small amount of GDIMBOA was detected, which means that the plant consumes GDIMBOA, thus there is a chance for iron uptake - at least in part - in the form of a GDIMBOA-Fe(III) complex.

The addition of GDIMBOA in concentration of $5x10^{-5}$ mole/liter resulted in the increase of dry weight of mutant in both data of observation following treatment with GDIMBOA: one day after the treatment with 7,0%, and after 4 days with 9,8%.

3.2. Microelement tolerance

3.2.1. The role of the DIBOA glucoside in the nickel tolerance of the cucumber plant in case of the administration of high amounts of nickel

In larger amounts, the plants as a human originated soil contaminant may take up nickel. This metal is considered potentially toxic, and since cyclic hydroxamic acids produce complexes with nickel, I thought interesting to investigate the influence of GDIBOA on the nickel uptake of cucumber.

The cucumber plants were grown in nutrient solutions free from nickel for 10 days, and then the plants were divided into three groups. One of the groups did not received nickel at all (control), and the other two groups were treated with $NiSO_4$. Besides the nickel salt, the representatives of one of these latter two groups were also treated with GDIBOA in concentrations equal to that of $NiSO_4$. In parallel studies the plants were grown under continuous treatment conditions, and were also treated for a defined period (2 and 4 hours), and then samples were taken after three days. The results of th se experiments are summarized in Table 6 and in Figures 9-10-11.

Table 6.

The effect of GDIBOA on the nickel content and dry weight (mg/kg dry weight; mg/piece of shoot) of the cucumber plants

Treatment	Control	0.3×10^{-6} mole/liter $NiSO_4$	0.3×10^{-6} mole/liter $NiSO_4$+0.3×10^{-6} mole/liter GDIBOA
Ni (14 days old shoots, continuous treatment)	1.3	27.6	15.4
Dry weight	19,5	20,0	15,3
Treatment	Control	10^{-6} mole/liter $NiSO_4$	10^{-6} mole/liter $NiSO_4$ + 10^{-6} mole/liter GDIBOA
Ni (20 days old shoots, 2 hrs)	1.0	8.9	8.2*
Dry weight	23,9	18,9	18,7*
Ni (23 days old shoots, 4 hrs)	1.0	7.6	6.1
Dry weight	23,9	20,2	17,8

The addition of GDIBOA resulted in a decrease of the nickel content of the shoots in the case of both types of treatments (see also Table 1 and 2).

The addition of GDIBOA and nickel together as compared to an exclusive addition of nickel resulted in the decrease of the dry weight of cucumber shoots with 23,5% in the continuous treatment, and with 1,1% in the treatment for two hours, and with 11,9% in the treatment for four hours.

3.2.2. The role of the DIBOA glucoside in the microelement tolerance of the cucumber plant in case of the administration of high amounts of microelements

Cucumber plants were grown in nutrient solutions deficient in copper or zinc for 19 days, and the media were complemented with $4x10^{-6}$ mole/liter of Fe(III)-EDTA. Then one-one group of the plants were further grown in solutions deficient in the above elements (control), and the other plants were treated on the 19th and 22nd days with the respective missing microelement (copper and zinc) in form of the sulfate salts. Of these plants 1-1 group grown in the absence of copper or zinc received also GDIBOA in an amount correspondent to that of the added copper or zinc sulfate. The quantity of copper and zinc in these experiments was 62.5 and 26.3 times higher than that suggested by CSEH *et al*. (1982). The microelement content and the dry of shoots of the 24 days old plants are shown in Tables 7 and 8 and in Figures 12 and 13.

Table 7.

The effect of GDIBOA on the copper content and the dry weight (mg/kg dry weight; mg/piece of shoot) of the 24 days old cucumber shoots pre-cultivated in the absence of copper

Treatment	Control	$5x10^{-6}$ mole/liter $CuSO_4$	$5x10^{-6}$mole/liter $CuSO_4$ + $5x10^{-6}$mole/liter GDIBOA
Copper-content	4,4	38,5	18,5
Dry weight	31,0	31,9	26,9

Table 8.

The effect of GDIBOA on the zinc content and the dry weight (mg/kg dry weight; mg/piece of shoot) of the 24 days old cucumber shoots pre-cultivated in the absence of zinc

Treatment	Control	$5x10^{-6}$ mole/liter $ZnSO_4$	$5x10^{-6}$mole/liter $ZnSO_4$ + $5x\ 10^{-6}$mole/liter GDIBOA
Zinc-content	24,4	357,2	128,8
Dry weight	34,8	35,2	25,7

The administration of a large excess of GDIBOA significantly decreased the zinc and copper content of the cucumber shoots.

The addition of GDIBOA in case of the administration of high amounts of copper resulted in the decrease of the dry weight of shoots with 15,7% and in the case of the administration of high amounts of zinc with 27,0%.

3.3. Comparison of the release of cyclic hydroxamic acids in various plant species

The cyclic hydroxamic acid excretion of the following species was investigated: maize (species: Norma

SC), cultivated wheat (species: Mv 16) and rye (species: Kisvárdai). Harvest of the root exudates was done at the 12th day of age by the addition of 10^{-5} mole/liter of Fe(III)-EDTA, since - in contrast to $FeCl_3$ - the quantity of this complex did not influence the volume of hydroxamate excretion. In accord with the daily cycle of excretion, the release of hydroxamic acid was measured in the morning hours, between 8-12 a.m. = 4 hours incubation time (note that the release of hydroxamic acids gradually decreases upon illumination).

In studies carried out earlier at this Department the release of the mugineic acid-type phytosiderophore of the above three plant species and varieties in the presence of sufficient iron supply was investigated using the procedure of ZHANG *et al*. (1989). These data were taken from the publication of PETHŐ (2000, who kindly provided them at my disposal. The results are summarized in Table 9 and in Figure 14, and the data taken from the above communication are marked with an asterisk (*).

Table 9.

Excretion of mugineic acid and cyclic hydroxamic acids (micromole/g dry weight/4 hours) of various plant species

Release of substance				
Plant	MA*	DIMBOA	DIBOA	cHx/MA
Wheat	0.590	0.020	in traces	0.034
Rye	1.070	0.050	in traces	0.047
Maize	0.125	0.530	0.200	5,840

Note: MA = mugienic acid

In contrast to maize, upon Fe(III)-EDTA addition wheat and rye release the DIBOA-glucoside only in traces. By the decrease of the mugineic acid-type phytosiderophore, an enhancement of the release of hydroxamic acids can be observed. Therefore, the cyclic hydroxamic acid/mugineic acid ratio is very small in the case of rye and cultivated wheat, but it is rather high for the maize plant. This fact also emphasizes the role of hydroxamic acids in the microelement uptake of maize. Consequently, and in accordance with the conclusion of PETHŐ (1994, 2000), it is quite probable that in the case of maize, hydroxamic acids may take part in the uptake of the microelements.

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4. Summary

1. In experiments carried out in nutrient solutions, the contributive or inhibitory effect of the cyclic hydroxamic acids on the microelement uptake is essentially dependent on the pH, on the quantity and ratio of the potentially complex-producing ions, and on the nature of the applied cyclic hydroxamic acid.

2. Upon treatment for a defined period of time, the two frequently occurring cyclic hydroxamate glucosides GDIBOA and GDIMBOA influence the microelement uptake of the cucumber plant differently: GDIBOA facilitates the uptake of zinc, and both iron and copper at acidic and neutral pH, respectively. At the same time, GDIMBOA enhances the zinc uptake at pH’s close to neutral, and the iron and copper uptake at acidic pH. Both hydroxamate glucosides facilitate the uptake of manganese in media with pH’s close to neutral or acidic.

3. In combination with a supply with microelements in the required quantity and ratio, the continuous treatment with hydroxamate enhances the uptake of iron and copper both in the cucumber plant, and in the maize species producing cyclic hydroxamic acids.

4. A continuous treatment with hydroxamate, or that carried out for a defined period of time, together with an exclusive addition of iron, increases the iron content of the shoots of the investigated maize plants independently of whether the plant does or does not produce cyclic hydroxamic acids. There is a possibility for the uptake of iron, at least in part, in form of a Fe(III)-cyclic hydroxamic acid complex, because after addition of this complex not only the increase of the iron content can be observed, but we could detect cyclic hydroxamic acids even in the roots of the bxbx mutant which does not produce cyclic hydroxamic acids at all.

5. Upon Fe(III)-EDTA administration maize compensate the low mugineic acid release with an increased hydroxamic acid excretion. In contrast with the maize, rye and wheat produce high amounts of mugineic acid type phytosiderophores, but only a minimal volume of cyclic hydroxamic acids. This fact supports the alternative phytosiderophore function of cyclic hydroxamic acids in the microelement uptake of maize.

6. In the experiments aimed at studying the microelement uptake, the addition of cyclic hydroxamic acids in continuous treatments and in 10^{-6} mole/liter concentration increases the dry weight of the shoots of the cucumber and maize variants which produce cyclic hydroxamic acids. The growth of the bxbx mutant (which does not produce cyclic hydroxamic acids) was facilitated by the addition of cyclic hydroxamic acids even at higher concentration (50×10^{-6} mole/liter). The addition of cyclic hydroxamic acids for a determined period in higher concentrations has an inhibitory effect on the growth of cucumber. The level of the inhibition increases with the increase of the pH of the nutrient solution, and the length of time of administration with the cyclic hydroxamic acids.

7. Upon addition of the microelements in excess, the cyclic hydroxamic acids inhibit the uptake of iron, manganese, zinc, copper, and nickel by the cucumber plants.

8. The addition of cyclic hydroxamic acids leads to a decrease of the manganese, zinc, copper, and nickel content of the maize plants in the case of enhanced microelement supply.

9. Cyclic hydroxamic acids inhibit the uptake of certain elements (such as nickel) which do not occur in nature and therefore not considered as nutritional, but form complexes with the cyclic hydroxamic acids.

10. In the experiments aimed at studying the microelement tolerance, the cyclic hydroxamic acids have an inhibitory effect on the growth of cucumber. Depending on the experimental conditions, it is due to the high concentration, or the long time of the administration of the cyclic hydroxamic acids, or because of the combined effect of the application of the cyclic hydroxamic acids and the microelements in a large excess. In the experiments aimed at studying the microelement tolerance, the effect of the cyclic hydroxamic acids on the growth of maize is dependent on the form of the added iron. Addition of the cyclic hydroxamic acids in 5×10^{-6} mole/liter concentration, and addition of iron in form of $FeCl_3$ increase the dry weight of the maize shoots.

11. The inhibitory effect of the cyclic hydroxamic acids on the microelement uptake appears, first of all, during overfeeding with microelements. The produced complexes inhibit the quick micronutrient uptake in a large portion, and thus inhibit accumulation of the excess of the microelement(s) onto a toxic level. Therefore, the released cyclic hydroxamic acids promote the plant to tolerate higher microelement concentrations, and thereby possess a preventive function.

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5. New scientific results and novelties

1. I demonstrated that the facilitating effect of the cyclic hydroxamic acids on the microelement uptake is dependent on the type of the hydroxamic acid, on the quantity and ratio of the metal ions potentially involved in the complexation, as well as on the pH of the nutrient solution.

2. By using maize mutants which do not produce cyclic hydroxamic acids, I established the role of the cyclic hydroxamic acids in the iron uptake, and confirmed the assumption that the iron uptake may proceed in form of hydroxamate complexes.

3. I confirmed that the administration of cyclic hydroxamic acids in micromole concentrations (10^{-6} mole/L) facilitates the growth of both of the monocotyledons and dicotyledons (maize and cucumber).

4. I confirmed that at concentrations which with orders of magnitude higher than the micromole concentration, the cyclic hydroxamic acids generally inhibit the growth of the plants. I established that this inhibitory effect increases with the increase of the pH of the nutrient solution.

5. I demonstrated that cyclic hydroxamic acids play a role in microelement tolerance, and this role comes into prominence in two cases. One is when the microelements potentially involved in the complexation are administered in higher amounts than those given in the literature. The second case is when the plant is treated with a microelement which does not occur in nature, but able to form a complex with the cyclic hydroxamic acids.

6. By means of administration with Fe(III)-EDTA I established that in the case of maize the release of a small quantity of a mugineic acid-type phytosiderophore is compensated by the release of a significant volume of cyclic hydroxamic acids. The cyclic hydroxamic acid release of cultivated wheat and rye (which produce significant amounts of mugineic acid-type phytosiderophore) is low, as compared to that of the maize plant.

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