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The effect of cyclic hydroxamic acids on the growth of cucumber and maize and the underlying mechanizms

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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. These heterocyclic compounds consist of two hexamerous rings, where in one of the rings there are >N-OH and >C=O reactive groups.

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 (Wahlroos-Virtanen, 1959). The aglucones easily decompose to benzoxazolinones while formic acid releases (Brendenberg et al. 1962).

There are some publications in connection with the inhibitory effect of cyclic hydroxamic acids and benzoxazolinones on the germination and growth of plants (Nair et al. 1990; Chase et al. 1991; Perez-Ormeno-Nunez 1991,1993; Pethő 1993 a, b). The role of cyclic hydroxamic acids in allelopathy presents in higher concentration in form of glucosides, than that of the aglucones (DIBOA and DIMBOA) (Pethő 1993 b). Pethő and Kovács (1996) already published that the cyclic hydroxamic acid-glucosides in 1-3 micromole/L concentration have stimulatory effect on growth.

Materials and Methods

The role of cyclic hydroxamic acids in allelopathy was investigated in nutrient solutions. The following species were used: plants not producing cyclic hyroxamic acids: cucumber (*Cucumis sativus* cv. Joker F1; cv. Budai Korai) and bxbx maize mutant (*Zea mays* cv. bxbx), and a maize cultivar that produces cyclic hydroxamic acids (*Z. mays* cv. Norma).

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 some experiments the microelement concentrations were 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. The solutions were continuously aerated and exchanged in each 3rd day.

The application of the hydroxamic acid glucosides was carried out in two ways: 1) plants were incubated in a solution of cyclic hydroxamic acid glucoside for 1-6 hrs, and then they were put back into the respective, hydroxamate-deficient solutions (treatment for a determined period); 2) 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 hydroxamate was in contact with the plant roots for 3-6 days. For control treatments the cyclic hydroxamic acid was discarded. Following treatments, the shoots of the plants were harvested and dried. As samples a part of the shoot above the node of the maize coleoptile and a part of the shoot above the cucumber cotyledons were taken.

Results and Discussion

Upon addition of microelements in concentrations given in the mentioned publications (Cseh et al. 1982; Treeby et al. 1989) the cyclic hydroxamic acids in concentration of $3x10^{-5}$-10^{-4} mole/L resulted in no effect or in the decrease of dry weight of cucumber shoots with 3,9-11,9% according to the pH. Enhancing the pH the inhibitory effect increased. In concentration of $1\text{-}3x10^{-6}$ mole/L the hydroxamic acids had no inhibitory effect, even that metabolites increased the dry weight of shoots with 2-3%. According to the quantity of added GDIMBOA, the dry weight of shoots of maize variety, producing cyclic hydroxamic acids increased with 2-3%, or decreased with 2-8%. GDIMBOA, in concentration of $1x10^{-6}$ mole/L increased, but in concentration of $3\text{-}9x10^{-6}$ mole/L decreased the dry weight. In conclusion observable, that the cyclic hydroxamic acids in lower concentrations increase not only the growth of cucumber, but also the growth maize, producing cyclic hydroxamic acids too. I could not observe the inhibitory effect on growth of cyclic hydroxamic acids at the variety bxbx, even at higher concentration of GDIMBOA ($1\text{-}5x10^{-5}$ mole/L).

Upon addition of microelements in concentrations given in the mentioned publications (Cseh et al. 1982; Treeby et al. 1989) the addition of cyclic hydroxamic acids in continuous treatments and in 10^{-6} mole/L concentration increases the dry weight of the shoots of the cucumber and maize varieties 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 ($50x10^{-6}$ mole/L). The addition of cyclic hydroxamic acids for a determined period

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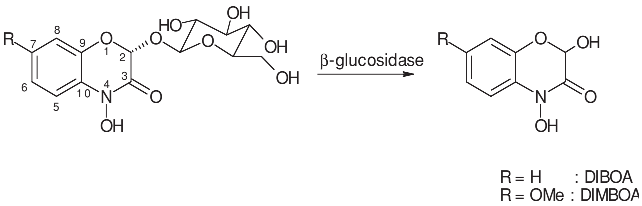

Figure 1. The structure of the cyclic hydroxamic acids and their glucosides.

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.

Upon addition of microelements in large excess the hydroxamate addition in continuous treatments resulted in the decrease of the dry weight of the cucumber shoots with 15,7-27,0% as compared to the treatments with the microelements exclusively. In experiments when the cucumber plants were treated with hydroxamic acids for a determined period, the decrease of the dry weight was lower: 1,1-11,9%. Within these data I observed that the inhibitory effect for growth was lower in the shorter treatments.

Upon addition of microelements in large excess the addition of cyclic hydroxamic acids – depending on the form of the iron supply – resulted in the decrease with 4,6% (in the case of Fe(III)-EDTA addition), or increased with 7,4% (in the case of $FeCl_3$ addition) of the dry weight of the maize shoots. According to the results of these experiments, in the case of $FeCl_3$ addition the inhibitory effect for growth of cyclic hydroxamic acids is effective only at higher hydroxamate concentrations, because $5x10^{-6}$ mole/L GDIMBOA has no inhibitory effect on the growth of the maize shoots.

Upon addition of microelements in large excess the effect of the addition of the cyclic hydroxamic acids and the microelements on the inhibition of plant-growth was combined in the given treatments.

Various mechanisms take part in the allelopathic effects caused by the cyclic hydroxamic acids. The benzoxazolinones inhibit the binding of auxines to the membrane receptors (Niemeyer, 1988). The cyclic hydroxamic acids react with the -SH groups of proteins (Niemeyer et al. 1982). Electrophil compounds can be formed from cyclic hydroxamic acids after 4-O-acetylation, which compounds react with the nucleophil parts of macromolecules (DNA, proteins) (Hashimoto and Shudo 1996). These types of reactions inhibit the various functions of macromolecules.

The cyclic hydroxamic acids support some of the peroxidative enzymes, which engaged in cell-wall synthesis (Gonzalez and Rojas 1999). The faster is the later process the slower is the growth.

The stimulation effect of cyclic hydroxamic acids on growth can be caused by their role in microelement uptake, which was proved by *e.g.* Pethő and Kovács (1996) or by Makleit and Pethő (1999).

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