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INFLUENCE OF VARIOUS STRESS EFFECTS ON ETHYLENE PRODUCTION IN WHEAT SEEDLINGS

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Abstract

Ethylene formation has been investigated under influence of water stress in various of wheat seedlings. The Change in ethylene production in the function of water deficit is described by a maximum curve. The position of the maximums dependes on the wheat variety. We have found that copper and cadmium ions promote ethylene prouction in the wheat seedlings investigated. The effect of copper ions depends on the pH. Ethylene production will decrease if the pH approaches 7. No correlation was observed between ethylene production and the pH in the presence of cadmium ions. In the presence of ions mentioned above an enhancement was obtained in the ACC formation. Ethylene production evoked by these ions may be inhibited by cobalt and zinc ions. Data presented in this paper show a significant difference in the ethylene production of infected and infection- protected seedling on the 9th days. Owing to serious damages in cells we found no ethylene production in the non protected seedlings on the 15th day.
Key words: ethylene, water stress, drought tolerance, copper ions, cadmium ions, downy mildew infection.

Introduction

In recent years the term "stress" has been used for all environmental factors, wich are potentially disadvantageous for living organism. In terminology "stress resistance" is used for expressing the ability of a plant to survive an unfavorable factor and even to grow in its presence (LEVITT, 1941).

In plants a stress effect can disturb metabolic processes and evoke a change, e.g. in the membrane transport mechanism as well. If the change of environmental factors is slow, the transport process coupled to the metabolism will be disturbed only in a small degree. In case of rapid changes the consequences might be severe. (ZSOLDOS et al., 1982).

It has been observed that plants subjected to stress produce ethylene in different quantities, depending on the time course and the strength of effects (ABELES, 1973; TINGEY et al., 1976).

Stress ethylene is produced only when a disturbance occurs in the metabolism of the plant cells but no decompartmentalization has been observed yet (ELSTNER and KONZE, 1976).

Plants under drought produce larger quantity of ethylene than the controls, with accompanied reduction in the growth rate. The role of the produced ethylene in the regulation of the growth process is unclear yet.

It has been suggested that ethylene is a by-product in the plant defensive mechanism (HUXTER et al., 1979).

Stress ethylene formation has among other factors investigated under water deficit (MCKEON et al., 1982; APELBAUM and YANG, 1981).

Ethylene production is enhanced in plants by injury, e.g. wounding, and also by chemical (MC GLASSON, 1964. BOLLER and KENDE, 1980; KENDE and BOLLER, 1981; YOU and YANG, 1980; HOFMANN, et al., 1982). Plant breeders have an important task to produce drought tolerant wheat varieties. therefore they have to get data connected with this problem, we have suggested that various wheat varieties provide different quantities of ethylene under water stress and also we have made experiments to study this effect.

The authors have established that heavy metal ions getting into the soil are noxious to the organs of animals and plants. These have received considerable attention over the years as a result of increased environmental dangers from industrial, agricultural, energy and municipal sources. The sources of heavy metals and their behavior in soils and plants have been reviewed by FOY et al. (1978). Stress ethylene production is also induced by aqueous solution of metal salts (RODECAP and TINGEY, 1981). We are particularly interested in the effect of cd^{2+} on the increase of ethylene production in wheat seedlings because we have shown in an earlier paper that these ions enhance ethylene production in tobacco callus tissues (GAÁL, 1985.

It has been established that Cd^{2+} is one of the dangerous environmental ions which inhibits photosynthetic activities in plants (FRIBERG et al., 1974; WIEGEL, 1985; MAUCH, 1984), stimulates ethylene production via the same pathway like basal ethylene and regulates it in a similar way (LIEBERMAN, 1979).

Similar results have been obtained in the presence of Cu^{2+} applied to mung bean hypocotyls. An enhancement was found in the ACC content and in the rate of ethylene production but no increase was observed in SAM content (FUHRER, 1982).

Since the role of Cd^{2+} and Cu^{2+} concerning ethylene production also belonged to our range of interest, it seemed resonable to study the action of these ions on wheat seedlings.

A common feature of many plant diseases is an increase in ethylene production (ABELES, 1973; PEGG, 1976). It has been observed that ethylene production increases largely in virus infected tobacco plants (LAAT et al., 1981; PRICHARD et al., 1975; LAAT et al., 1982).

According to some wheat breeders there are wheat warieties infected by downy-mildew and yet appearing to be healthy. Considerable deficit, however, can be found in the yield at harvest. In the opinion of many authors increased ethylene synthesis can be employed as an indicator for infection (BOLLER, 1982; PEGG, 1976; YANG and PRATT, 1978; MAUCH et al., 1984).

Our aim has been to search for correlation in the ethylene production at the protected and non protected wheat seedling infected by downy-mildew.

Materials and Methods

Winter wheat (*Triticum aestivum* L. cv. GK Szeged) seeds were washed in running tap-water for 4—6 h and germinated in Petri dishes for one day at 24°C. After germination the seeds were placed on a plastic sieve in suitable plastic vessels containing $5x10^{-4}$ M $CaSO_4$ solution. The solution was renewed after the third day of growth. The seedling were grown at 25°C on a 16 h photoperiod of 7000 lux. To study the effect of metal ions ethylene production lg of wheat seedlings was put into test tubes containing 1 ml of 0,005 M citrate buffer, pH 5,6 in which metal ions were dissolved in 10^{-2} M concentration.

There were no metal ions in the control solution. After incubation the seedling were transferred into test tubes closed by serum caps. Ethylene was determined by a Type Chrom 42 gas shromatograph equipped with an aluminium oxide column and a flame ionisation detector. ACC was assayed according to LIZADA and YANG (1979).

Seedlings were frozen in liquid N2 and ground in a mortar with pestle. Two ml of 80% ethanol was added per g fresh weight and the mixture stirred for 30 min at room temperature. The homogenate was centrifuged at 10000 g for 10 min and the ACC determined in the supernatant by the method as above.

In the water stress technique, seedlings (1g) were allowed to wilt while spread in Petri dishes at room temperature (20—22°C). When the seedlings reached the desired degree of water stress as indicated by loss of fresh weight they were inserted into glass test tubes which were then closed with serum caps for the desired period. Nonstressed seedlings used as controls were inserted in test tubes containing several drops of water to maintain high RH. The sealed tubes were kept at 22°C under light. gas samples (2,4 ml) were taken periodically from each tube for ethylene analysis.

To study effect of infection the wheat seedlings were cultured at 70—80 RH and 20—25°C in greenhouse. Then seeds were sown in each chambers and twenty chambers were used per the experiments. Ten of them represented the controls.

The procedure of infection: On the wheat varieties sensitive to downy-mildew infection the fungus was allowed to proliferate. From these infected seedlings the fungus was transferred on the experimental plants by shaking. Plants were infected by downy-mildew when they had two leaves.

Before infection the control plants were sprayed with a protecting material (Bayleton 25 WP, Bayer (Triadimefon)).

Plants were harvested for experiments after sowing on the 9th and 15th day.

Results and Discussion

Effect of pH

According to our results the rate of ethylene production shows no change between pH 3—7 in the presence of Cd^{2+} but decreases in the precense of copper ions when the pH approaches to 7 (Table 1). We propose the hypothesis that the copper ions from a complex or enhance the production of MACC conjugate from ACC as it has already been established by KIONKA and AMRHEIN. (KIONKA and AMRHEIN, 1984).

Effect of water stress

drought tolerant wheat varieties lose their water content slower than non-drought tolerant ones (private opinion of some wheat breeders). Data presented in this paper show that a connection can be found between water deficit and ethylene production in wheat seedlings (Fig 1.) Change in the rate of ethylene production depending on water deficit are described by a maximum curve. GK Szemes provides the highes and D—22 the lowest ethylene maximum.

Table 1. Effect of pH on ethylene production of wheat seedlings in presence of cadmium and copper ions

	Ethylene production nl/gh Treatments		
pH	Control	Cu^{2+}	Cd^{2+}
3	0.73 ± 0.2	17.5 ± 1.2	8.3 ± 0.7
4	0.95 ± 0.32	16.4 ± 1.6	7.3 ± 1.5
5	0.62 ± 0.4	16.7 ± 1.4	8.7 ± 0.97
6	0.76 ± 0.35	10.7 ± 0.6	9.1 ± 0.75
6,6	0.48 ± 0.26	7.6 ± 0.65	10.1 ± 0.97

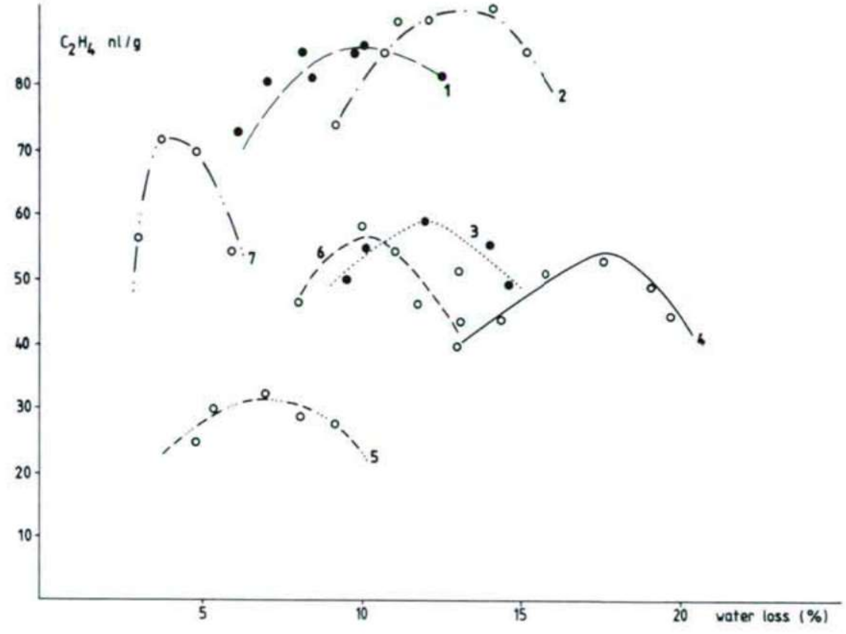

Fig. 1. Ethylene production of 9- day old seedlings of different wheat varieties in function of percentage water deficit.
1. GK Boglár; 2. GK Szemes ATK; 3. GK Kincső; 4. GK Minaret SE; 5. D-22. 6. Jubilejnaja 50; 7. GK Szeged.

The position of maximums deviates according to water loss, from one another. It can be concluded from these data that wheat variety GK Szemes represents the worst and Minaret SE the best one in terms of drought resistance. The result would be interesting for all who are engaged in the wheat production. It is be expected that plant breeders having this information can establish easier which are the best wheat varieties with a view to drought resistance.

Effect of metal ions

At a concentration higher then 10^{-4} M copper (II) ions seem to be a more powerful activator than cadmium (II) ions of ethylene evolution. (Fig. 2). The rate of ethylene production changes according to a maximum curve as a function of time and the position of the maximum is found about three hours (Fig. 3).

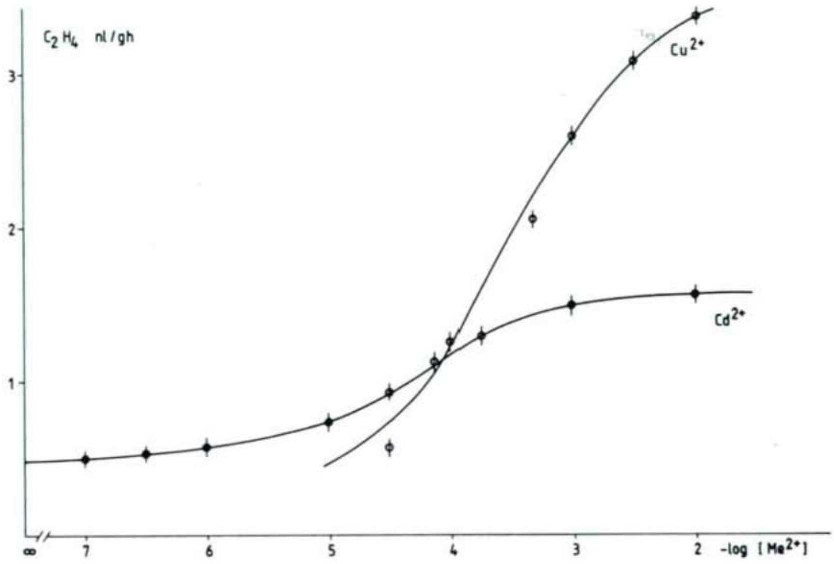

Fig. 2. Changes of ethylene production in function of metal ion concentration.

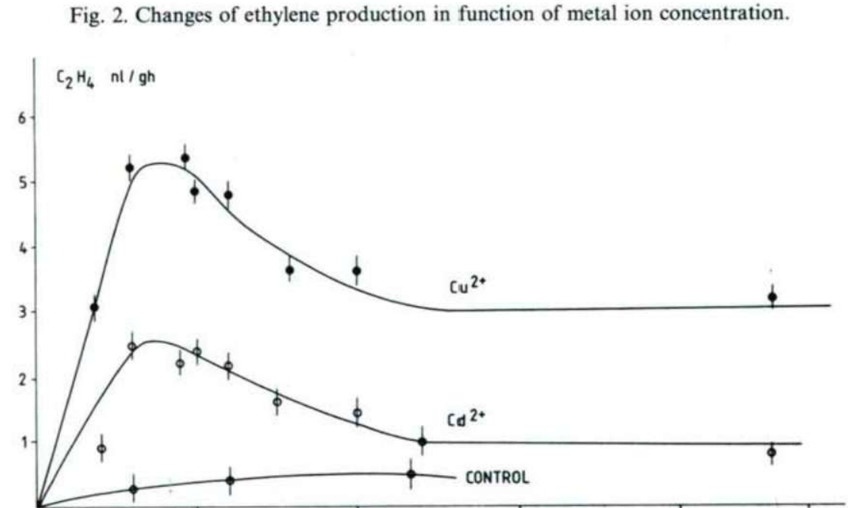

Fig. 3. Time course for effect of metal ions on ethylene production.
$\cdot = Cu^{2+}$; $o = Cd^{2+}$. $[Me^{2+}] = 10^{-2}$ M/l.

Our findings indicate that ethylene production provoked by Cu and Zn ions can be inhibited by Co^{2+} and Zn^{2+} as well (Figs. 4,5). Zn ions inhibit ethylene production also in the presence of 10^{-3} M exogenous ACC concentration (Fig. 6). Co^{2+} has already been shown to be an inhibitor of ethylene production from ACC

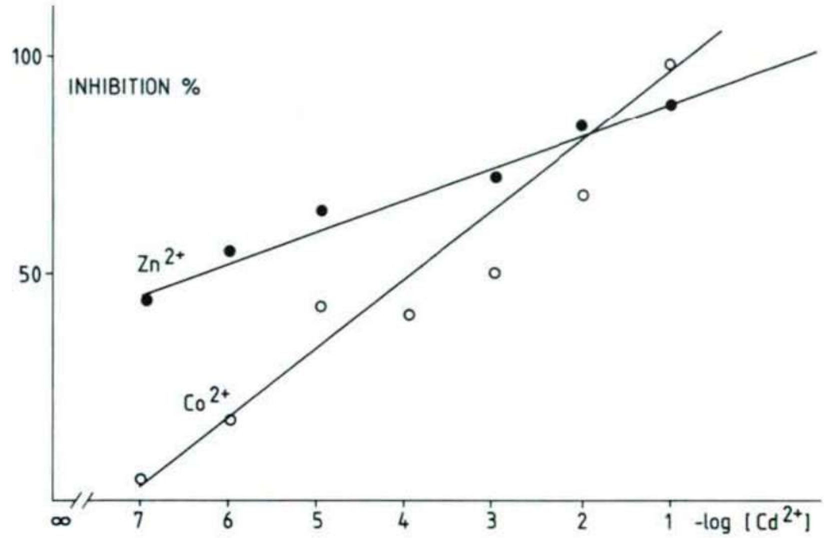

Fig. 4. Effect of cobalt and zinc ions on ethylene production evoked by copper ions. o = Co^{2+}; · = zinc ion.

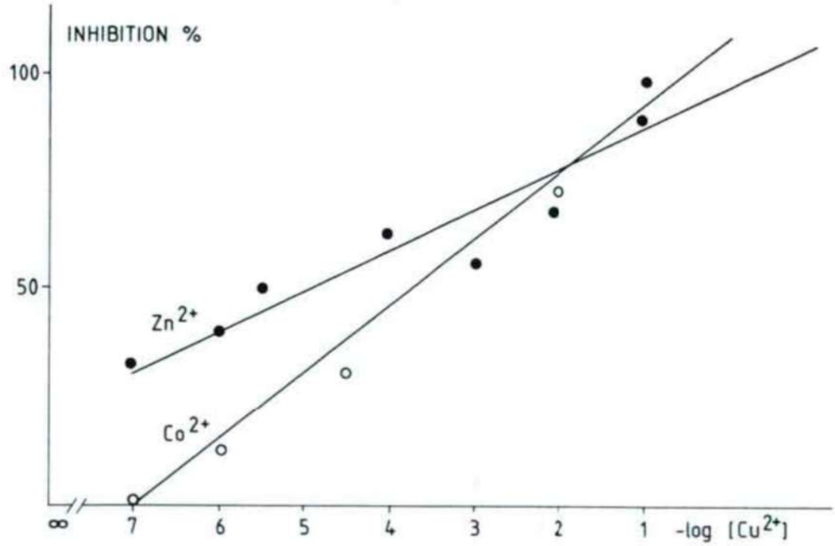

Fig. 5. Effect of cobalt and zinc ions on ethylene production evoked by cadmium ions. o = Co^{2+} · = zinc ions.

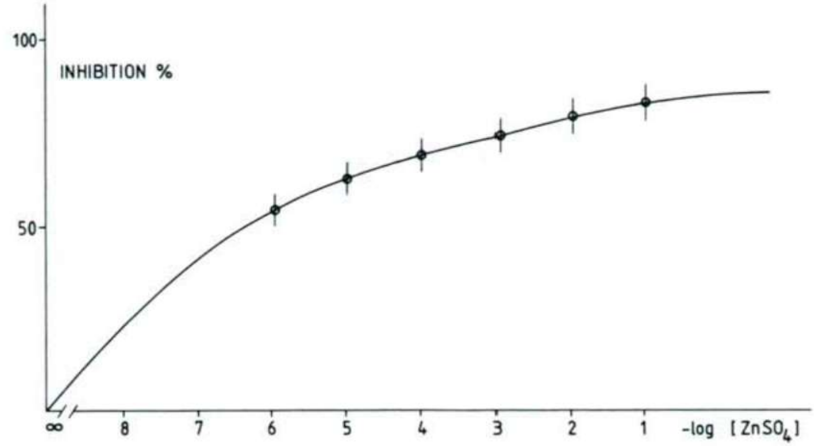

Fig. 6. Effect of zinc ions on ethylene production in the presence of exogenous ACC.

but no data could be found concerning this finding in the literature. It seems likely that cobalt ions can be substituted with Zn^{2+} in the inhibition process as it has already been observed in the case of metalloenzymes (KADDEN, 1974; KENEDDY, 1972).

Effect of the downy mildew infection

Our results demonstrate that downy-mildew produces ethylene in larges quantities on the days after infection. The infection of wheat seedlings can be prevented by triadimefon 25. In Tables 2—3 P stands for the protected, I the infected wheat seedlings in respect to ethylene production. D means the degree of infection. It can be established that a significant difference exists between the protected and unpro-

Table 2. Ethylene production of four wheat varieties on the 9th day of infection

		Ethylene production nl/gh			
Varieties	D	P	I	I—P	% change
GK Réka	0	0.36	0.33	0.04	—9.64
GK Ságvári	4	0.67	2.17	1,48	216.01
Mini Manó	2	0.61	1.1	0.48	78.31
Arthur	0	0.46	0.7	0.25	54.02
Average		0.53	1.1		102.6
SD 5% between main averages 0.13					
SD 5% between the varieties 0.19			in the same block		
SD 5% between any two values 0.27					

(Data in Table 2 are means of three experiments. D: degree of infection; P: protected; I: infected wheat seedlings)

Table 3. Ethylene production of four wheat seedling varieties on the 15th day of infection

		Ethylene production nl/gh			
Varieties	D	P	I	I—P	% change
GK Réka	0	0.37	0.26	—0.11	—30.58
GK Ságvári	4	0.54	0.50	0.04	—9.87
Mini Manó	2	0.40	0.50	0.10	24.30
Arthur	0	0.42	0.41	0.01	—3.21
Average		0.44	0.42		—4.75
SD 5% between main averages 0.086					
SD 5% between the varieties 0.122					
SD 5% between any two values 0.172					

(Data in Table 2 are means of three experiments. D: degree of infection; P: protected; I: infected wheat seedligs)

tected wheat seedlings in the rate of ethylene production on the 9th day after infection, however, no ethylene production was observed after infection on the 15th day because of the serious damage of cells responsible for ethylene production.

The results show that a downy-mildew infection can be assumed if the ethylene production increases in wheat seedlings and this provides a possibility for protection in time.

Abbreviations: ACC= 1-aminocyclopropane-1-carboxylic acid
MACC= 1-(malonyl-amino) cyclopropane. carboxylic acid
ATK= mixture of cultivars
SE= Super Elite

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