The interaction of calcium with ethylene and abscisic acid in the pathogenesis of wheat seedlings caused by *Fusarium culmorum*

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S u m m a r y

The influence of ethylene and abscisic acid on the infection of wheat seedlings by *Fusarium culmorum* depending on the content of calcium in the medium was investigated. It has been established that when this element does not occur in the medium ABA stimulates, whereas ethylene inhibits the development of a disease. Adding calcium to the medium decreases considerably the stimulating influence of ABA, whereas it increases the inhibiting action of ethylene in this process. The influence of ethylene and abscisic acid on the growth and development of *Fusarium culmorum* depending on the content of calcium in the medium, was also investigated.

Basing on the above results it can be concluded that calcium in media enhances the inhibitory effect of ethylene and reduces the stimulatory effect of abscisic acid on the growth and development of *Fusarium culmorum* and also on the infection of the seedlings with that pathogen.

Key words: plant infection, calcium, abscisic acid, ethylene, *Fusarium culmorum*

INTRODUCTION

Abscissic acid and ethylene are natural growth regulators, produced by plants and by many parasitic fungi. They play an essential role in plant growth and development as well as in host-pathogen relationships (Ables et al., 1992; Davies et al., 1991).

The results of earlier experiments carried out in our laboratory indicate that the important role in the pathogenesis of wheat seedlings caused by *Fusarium culmorum* is played by endogenous growth regulators, especially ABA which increases plant sensitivity to that pathogen, and ethylene which decreases this sensitivity (Michniewicz et al., 1990).

It is evident that the changes in the calcium content in the environment affect the plant susceptibility to fungal pathogen (Nizioł, Różej, 1993; Elad, Volpin 1993; Conway et al., 1994; Biggs et al., 1994; Wisniewski et al., 1995; Drobny et al., 1997).

The aim of this work was to investigate the interaction of calcium with ethylene and abscissic acid in the pathogenesis of wheat seedlings infected by *Fusarium culmorum*.

MATERIAL AND METHODS

The material for experiments were winter wheat seedlings cv. Grana and fungus *Fusarium culmorum* (W. G. Sm.) Sacc. isolated from the roots of wheat (F_1 isolate).

Wheat kernels were germinated on moist filter paper in Petri dishes in darkness at 22°C for 3 days. After that time the kernels were selected and to each Petri dish the Hoagland nutrient solution containing different calcium concentration (0, 2, 4, 8 mM) was added.

2-chloroethylophosphonic acid (Ethrel, Amchem), an ethylene-releasing compound, at the concentrations of 100 mg dm⁻³ or ABA (Sigma) at the concentrations of 0.1 µM were added to sterile media. These concentrations were selected basing on the results of our previous experiments (Michniewicz et al., 1983; Michniewicz et al., 1984; Nizioł, Różej 1993). The variants without growth regulators were used as control material.

The germs were inoculated with *Fusarium culmorum* spores by applying a drop of spore suspension (averaging 960000 spores per 1cm³) onto the coleoptile base. On the 7th day after inoculation the percentage of coleoptile area showing symptoms of fungal infection was estimated with methods described earlier (Michniewicz et al., 1986).

In other experiments the fungus was grown in darkness at 22°C in liquid or agar Czapek-Dox medium. The medium contained the extract of wheat leaves (20 g·dm⁻³ of the medium) and Ca at different concentrations (0, 2, 4, 8 mM). 2-chloroethylophosphonic acid (Ethrel, Amchem), at the concentration of 100 mg dm⁻³ or ABA (Sigma) at the concentration of 0.1 µM were added to sterile media. The growth regulators and plant extracts were filter-sterilized (Milipore, pore size 0.47 µm).

The measurement of the surface of fungi colonies on an agar medium, the number of spores in a liquid medium and the methods of measurement of spores germination as well as other methodical details were described earlier (Michniewicz et al., 1983).

All experiments were performed in triplicate. The significance of differences was determined by Student's test at $P=0.05$ and standard errors (shown in figures as vertical bars) were estimated.

RESULTS AND DISCUSSION

The results presented in Table 1 and in Fig. 1 show that calcium present in the medium modifies the effect of ABA and ethylene on the infection of wheat seedlings by *Fusarium culmorum*. Adding ABA ($0.1 \mu\text{M}$) to full Hoagland solution (4mM Ca) caused increasing the infection of seedlings by 50% in relation to control plants (seedlings growing in Hoagland solution, without ABA). It has been observed that increasing the amount of calcium in the medium weakened the stimulating action of ABA. The seedlings growing on the medium containing 8 mM of calcium were infected to a similar degree as control seedlings, which mean that calcium contained in the medium reduced the action of this growth regulator. However, with a decreased content of Ca in the medium (2mM) the infection of seedlings stimulated with ABA was increased considerably.

Adding ethrel (100 mg dm^{-3}) to the medium inhibits considerably (by about 50%) the infection of plants caused by *Fusarium culmorum*. Increasing the amount of calcium in Hoagland medium intensifies the inhibitory action of ethylene, whereas decreasing its content causes the extension of infection.

The data presented in Table 2 and in Fig. 2 show that Ca influences also the action of investigated regulators in the process of growth and development of *Fusarium culmorum*. In absence of Ca in the medium, ABA markedly stimulates the growth of the mycelium of fungi. The stimulatory action of ABA became weaker with the increase of Ca concentration in the medium. Calcium at the concentration of 8 mM reduced completely the influence of ABA and markedly inhibited the growth of the mycelium of fungi in comparison to control. The effect of Ca on the action of ABA proved to be the strongest during the first and second day of cultivation and afterwards it gradually decreased. On the last day the differences between the combinations were insignificant.

Ethrel (Table 2 and Fig. 2) inhibited the mycelium growth of *Fusarium culmorum*. During the first day of cultivation this inhibition was the strongest (43,7% in relation to control) and it decreased with time, though the differences in relation to control proved to be essential during the whole time of an experiment. Adding calcium to the medium enhanced the inhibitory action of ethylene. The inhibition of the growth of the mycelium of fungi caused by ethylene was stronger with the increasing of the concentration of Ca in the medium.

The results concerning the influence of Ca on the activity of abscisic acid and ethrel in the process of development of *Fusarium culmorum* have been gathered in Table 3 and in Fig. 3 and 4. The presence of ABA in the medium markedly stimulates the process of sporulation and the germination of *Fusarium culmorum* spores, where-

as ethylene inhibits these processes. Calcium present in the medium weakens the stimulatory influence of ABA both on the process of sporulation and spore germination. The concentration of 8 mM of Ca completely reduces the stimulating influence of ABA and causes a considerable inhibition of investigated processes (in relation to controls by about 50%).

Calcium modifies also the action of ethylene in the process of *Fusarium culmorum* development. This element intensifies markedly the inhibiting influence of ethylene on the sporulation and spore germination. The inhibition of investigated processes was stronger with the growth of Ca content in the medium.

The results obtained in this study confirm our earlier data that ethylene inhibits (Michniewicz et al., 1983), while ABA stimulates (Michniewicz et al., 1984) the growth and development of *Fusarium culmorum*. They are also in accordance with our previous results (Michniewicz et al., 1990) indicating that ethrel treatment reduced, while ABA stimulated, the development of infection in wheat seedlings caused by that fungus, and the effects of the two growth regulators were antagonistic.

The inhibiting influence of ethylene and the stimulating action of abscisic acid on the infection of wheat seedlings by *Fusarium culmorum*, as on the growth and development of this pathogen find the confirmation in literature concerning other host-pathogen relationships. Application of ABA has been shown to increase the susceptibility of various plant to fungal infection e.g. of barley to *Erysiphe graminis* (Edwards, 1983), of soybeans to *Phytophthora megasperma* (Ward et al., 1989), of tobacco to *Peronospora tabacina* (Salt et al., 1986), tomato to *Botrytis cinerea* (Kettner, Dörfling, 1995), of pine seedlings to *Cylindrocarpon destructans* (Kriesel, 1997). Similarly the leaves of beans under the influence of ABA were more susceptible to *Uromyces vigne* – the race of rust which was not a pathogen for beans (Li, Heath, 1990).

As far as the influence of ethylene on the plant pathogenesis is concerned, there are data in literature which point out to the fact that applying exogenous ethylene may increase, decrease or may not effect the resistance of plants to fungal pathogens. Data on this subject was presented in our earlier paper (Michniewicz et al., 1986).

The literature data concerning the effect of ethylene on the growth and development of fungi are also very scarce and controversial. Some authors observed an inhibitory effect of ethylene on *Helminthosporium sativum*, *Sclerotium rolfsii* (Smith, 1973), *Ceratocystis fimbriata* (Stopińska, Kuik, 1991), *Botrytis cinerea*, *Monilia fructigena* and *Penicillium expansum* (Zviagintseva, 1982). Some authors, however, obtained the results showing the stimulating effect of ethylene on the development of fungi (Kępczyńska, Kępczyńska, 1977; El-Kazzaz et al. 1983). The indispensability of ethylene for germination and growth of *Botrytis cinerea*, the main cause of apple decay in storage was stated by Kępczyńska (1989, 1993).

There are also reports on the lack of influence of ethylene on the growth and spore germination or sporulation processes (El-Kazzaz et al., 1983). This discrepant influence on the above mentioned processes may be explained by the distinct character of species and different susceptibility of individual pathogens to this compound.

Table 1. The effect of calcium on activity of ABA and ethrel (E) in the process of infecting wheat seedlings by *Fusarium culmorum*. The degree of infection expressed as a percentage of infected coleoptile surface.

Measurement	Concentration of calcium in Hoagland medium [mM]											
	0			2			4 (*)			8		
	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E
Length of coleoptiles [cm]	2.35	2.35	2.10	2.20	2.22	2.11	2.19	2.29	2.04	2.19	2.19	1.85
Length of injured area of coleoptiles [cm]	1.50	1.68	0.52	1.09	1.46	0.39	0.69	1.10	0.30	0.51	0.78	0.22
Percentage of injured area of coleoptiles [%]	63.8	71.5	24.7	49.5	65.8	18.5	31.5	48.0	14.7	23.3	35.6	11.9

(*) – amount of Ca in ful Hoagland nutrient solution.

K – Hoagland medium without ABA and ethrel

+ABA – ABA (0.1 μ M) was added to Hoagland medium,

+E – ethrel (100 mg·dm⁻³) was added to Hoagland medium

Fig. 1. The effect of calcium on the action of ABA (A) or ethrel (B) in the process of infecting wheat seedlings by *Fusarium culmorum*. The data expressed in percentages in relation to control (the degree of infecting the plants growing on a stable Hoagland medium, without hormones was assumed as 100%).

Table 2. The effect of calcium on activity of ABA and ethrel (E) on the mycelium growth of *Fusarium culmorum* (the area of colonies in cm²).

Day	Concentration of calcium in the medium [mM]											
	0			2			4			8		
	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E
1	0.36	0.57	0.16	0.29	0.50	0.12	0.22	0.40	0.10	0.12	0.29	0.06
2	3.85	4.49	2.37	3.25	4.36	1.83	3.09	3.56	1.79	2.98	3.09	1.03
3	12.62	14.69	8.35	12.10	14.40	7.72	11.59	12.42	7.14	11.19	12.10	5.03
4	29.89	33.71	22.81	29.23	32.27	20.15	28.59	30.84	19.58	27.26	30.19	15.36
5	48.50	53.46	39.33	48.49	52.09	35.84	45.63	51.13	34.39	44.41	49.25	33.13

K – Czapek-Dox medium without ABA and ethrel,
+ABA – Czapek-Dox medium with ABA (0.1 μM),
+E – Czapek-Dox medium with ethrel (100 mg·dm⁻³),

Fig. 2. The effect of calcium on ABA (A) and ethylene (B) activity on the growth of the mycelium of *Fusarium culmorum*. The data expressed in percentages in relation to controls (the growth of the mycelium of fungi on the medium without calcium and hormones was assumed as 100%).

Table 3. The effect of calcium on activity of ABA and ethrel (E) on the sporulation and spore germination of *Fusarium culmorum*.

Measurement	Concentration of calcium in the medium [mM]											
	0			2			4			8		
	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E	K	+ABA	+E
Sporulation (*)	671,4	843,8	495.5	639,4	800,6	439.8	615,0	781,9	292.7	157,3	350,6	117.5
Germination of spores (**)	59.4	70.8	36.12	49.0	66.4	32.8	33.8	57.6	25.9	28.2	31.4	21.62

(*) – the amount of spores in 1 cm³ of the medium expressed in thousands,

(**) – percentage of germinated spores.

Fig. 3. The effect of calcium on activity of ABA on the sporulation (**A**) and spore germination (**B**) of *Fusarium culmorum*. The data expressed in percentages in relation to controls (the sporulation and the germination of spores of the fungus on the medium without Ca and ABA was assumed as 100%).

Fig. 4. The effect of calcium on activity of ethrel on the sporulation (A) and spore germination (B) of *Fusarium culmorum*. The data expressed in percentages in relation to controls (the sporulation and the germination of spores of the fungus on the medium without Ca and ethrel was assumed as 100%).

The inhibiting influence of calcium in the pathogenesis of wheat seedlings elicited by *Fusarium culmorum* finds also the confirmation in literature concerning different plants and fungal pathogens. Calcium inhibits the infection of plants caused by *Phytophthora* (K o, K a o 1989), of peaches by *Leucostoma peroonii* (B i g g s et al., 1994), of apples by *Botrytis cinerea* and *Penicillium expansum* (W i s n i e w s k i et al., 1995) and *Penicillium digitatum* (D r o b y et al., 1997). A similar effect of calcium, causing a decrease in the susceptibility of rose flowers (V o l p i n, E l a d, 1991), of *Ruscus* (E l a d, K i r s h n e r, 1992), of beans and tomatoes (E l a d, V o l p i n, 1993), of eggplant, pepper and cucumber (E l a d et al., 1993) to the grey mould disease caused by *Botrytis cinerea* has been reported. In our previous papers we have already analysed some possible mechanisms by which calcium reduced infection of plant (N i z i o ł, R o ż e j, 1993; Z i e l i ń s k a, M i c h n i e w i c z, 2001).

This work has supplied essential data concerning the interaction of calcium with ABA and ethylene in the pathogenesis of wheat seedlings elicited by *F. culmorum*. Adding calcium to the medium weakened the stimulating action of ABA on the above processes. Thus, it may be stated that Ca and ABA act antagonistically, and the inhibiting influence of calcium is so strong that it weakens or even completely reduces the stimulating action of this regulator both on the growth and development of *F. culmorum* as well as the infection of seedlings by this pathogen. In our previous papers (Z i e l i ń s k a, M i c h n i e w i c z, 2001; M i c h n i e w i c z et al., 1990) we shown that calcium increased ethylene production but ABA decreased ethylene level in leaves. As it was demonstrated in our previous papers ethylene inhibits the growth and development of that fungus (M i c h n i e w i c z et al., 1983).

It follows from the conducted experiments (Fig.1, 2, 4) that adding calcium to the medium enhances the inhibiting influence of ethylene in the investigated processes, which means that these compounds are synergistic. This fact may undoubtedly find practical application in protecting of the plant from infection by this fungus and may, beside the application of ethylene, be one of the methods in the fighting against disease caused by *F. culmorum*.

The reduction of disease development in wheat seedlings infected by *F. culmorum* after treatment with calcium and ethylene may be connected with the influence of the applied elements on the production of ethylene by plants. The data obtained by us (unpublished so far) point out to such possibility. The seedlings treated with calcium and ethylene are characterised with the increased production of ethylene. Taking into consideration the fact that ethylene inhibits the growth and development of *F. culmorum*, it may be assumed that the production of ethylene increased under the influence of calcium and ethylene in wheat seedlings causes the reduction of growth and development of pathogen and as a result it decreases the extent of infection of wheat seedlings by this fungus.

However the interaction of calcium both with ethylene as well as with ABA in the pathogenesis of wheat seedlings caused by *Fusarium culmorum* observed in this work is the problem which has not been discussed so far in literature and its elucidation needs further studies.

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Współdziałanie wapnia z etylenem i kwasem abscysynowym w patogenezie siewek pszenicy wywołany przez *Fusarium culmorum*

Streszczenie

Badano wpływ ethrelu i kwasu abscysynowego na porażenie siewek pszenicy przez *Fusarium culmorum* w zależności od zawartości wapnia w pożywce. Stwierdzono, że przy braku tego pierwiastka w pożywce ABA stymuluje, natomiast etylen hamuje rozwój choroby. Dodanie wapnia do pożywki wyraźnie osłabia stymulujący wpływ ABA, natomiast wzmacnia hamujące działanie etylenu w tym procesie. Badano również wpływ etylenu i kwasu abscysynowego na wzrost i rozwój *Fusarium culmorum*, w zależności od zawartości wapnia w pożywce. Na podstawie uzyskanych wyników można wnioskować, że wapń wzmacnia hamujące działanie etylenu, natomiast osłabia stymulujący wpływ kwasu abscysynowego na wzrost i rozwój *Fusarium culmorum* oraz na stopień porażenia siewek pszenicy przez tego patogena.