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The effect of passaging of *Fusarium culmorum* (W.G.Sm.) Sacc. on media containing calcium on the growth and development of this fungus and on disease development in wheat seedlings

MARLENA ZIELIŃSKA, MARIAN MICHNIEWICZ, BARBARA ROŻEJ

Department of Plant Physiology and Morphogenesis, Institute of General and Molecular Biology, Nicholas Copernicus University, 87-100 Toruń, Gagarina 9 Street, Poland

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Summary

Fusarium culmorum was transferred ten times on media with a different content of calcium (0, 2, 4, 8 mM) and then was used for inoculation of winter wheat seedlings (cv. Grana). It was found that the disease of wheat seedlings was weaker when they were infected with the suspension of mycelium of fungi passaged on media containing higher concentration of calcium ions. It was also found that passaging *Fusarium culmorum* on media containing calcium causes the inhibition of growth and development of this pathogen.

Basing on the results of these experiments it can be concluded, that passaging *Fusarium culmorum* on media with calcium reduces the pathogenicity of this fungus to wheat seedlings.

Key words: plant infection, calcium, *Fusarium culmorum*

INTRODUCTION

Calcium plays an essential role in the host – pathogen relationships. There are many data in literature showing that this element increases the resistance of plants to some diseases. It was stated in our previous works on *Fusarium culmorum* (N i z i o ł, R o ż e j, 1993) and in other publications devoted to other pathogens (E l a d,

Volpin 1993; Elad et al. 1993; Punja, Gaye 1993; Conway et al. 1994; Biggs et al. 1994; Wisniewski et al. 1995; Droby et al. 1997).

The results of our previous experiments have indicated that calcium may reduce fungal infection through the direct inhibition of growth and development of that pathogen (Niziołł, Rożej 1993).

The question was what would be response of the successive generations of fungi developing on plants to which calcium had been applied. Thus, we decided to test the effect of passaging *Fusarium culmorum* on media containing calcium on the susceptibility of the fungi to the treatment with calcium and on disease development in wheat seedlings.

MATERIAL AND METHODS

Fusarium culmorum (W.G.Sm.) Sacc. (isolate F_1) isolated from wheat roots was used as an experimental material. The fungus was grown in darkness at 22°C on agar Czapek-Dox medium containing calcium at the concentration 2, 4, 8 mM. The concentrations were selected basing on the results of our previous experiments (Niziołł, Rożej 1993). The variant without calcium was used as control. The colony was grown from one spore.

The sterile media were infected with one drop of spore suspension (average 960000 spores per 1 cm^3) and the fungi were cultured under the above-described conditions for 14 days until spores were formed. After that time they were transferred to new media containing calcium (0, 2, 4, 8 mM respectively). The passaging cycle was repeated 10 times. Then the fungi were transferred to media without calcium and the growth and development were estimated.

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

A separate experiment was carried out to study the effect of passaging *Fusarium culmorum* on media with calcium on the development of the infection of wheat seedlings (cv. Grana) caused by the fungus. Therefore, kernels were germinated under sterile conditions in Petri dishes lined with paper soaked with distilled water in darkness at 22°C. Each variant of the experiment contained six dishes with 100 kernels per dish. After 72 hrs the kernels were selected and to each Petri dish the full Hoagland solution was added. The seedlings were infected with *Fusarium culmorum* (passaged on media containing different calcium concentrations) by applying one drop of spore suspension (averaging 960000 spores per 1 cm^3) 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).

All experiments were performed in triplicate. LSD at P=0,001 and P=0,01 (t-Student«s test) and the standard errors (shown in figures as vertical bars) were estimated.

RESULTS AND DISCUSSION

The results presented in Table 1. and in Fig. 1. indicate clearly that passaging *Fusarium culmorum* on media with calcium significantly reduced the development of infection in wheat seedlings induced by this fungus. The plants infected with *Fusarium culmorum* passaged on media containing calcium were less injured in comparison with plants infected with the mycelium of fungi passaged on media without calcium. The higher concentration of Ca in the media on which the fungi were passaged, the lower degree of infection of plants.

Table 1.

The effect of passaging *Fusarium culmorum* on Czapek-Dox media with different contents of calcium on the degree of infection of wheat seedlings

Measurement	Calcium concentration in Czapek-Dox medium [mM]*			
	0	2	4	8
Length of coleoptiles [cm]	2.68	2.52	2.50	2.46
Length of injured area of coleoptiles[cm]	0.80	0.67	0.45 [a]	0.29 [a]
Percentage of injured area of coleoptiles [%]	29.85	26.59 [b]	18.00 [a]	11.79 [a]

* – calcium concentration in Czapek-Dox medium on which the mycelium of *Fusarium culmorum* was passaged.

Differences significant in relation to control at: a – P=0.001, b – P=0.01

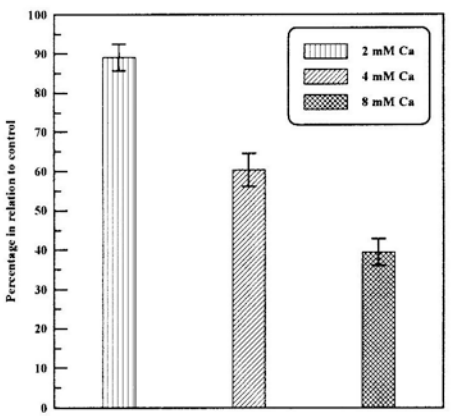

Fig. 1. The effect of passaging *Fusarium culmorum* on media with different calcium concentration on the infection of wheat seedlings with this pathogen. Data expressed in percentages in relation to control (the degree of infection of the plants with the mycelium of fungi passaged on media without calcium was assumed to be 100%)

The data presented in Table 2. and in Fig. 2. show that passaging *Fusarium culmorum* on media with calcium causes the inhibition of its growth. The mycelium of fungi passaged ten times on the media containing different concentrations of calcium and transferred on the medium without calcium was characterised with inhibited growth in relation to control (the mycelium of fungi passaged on media without calcium). The higher the concentration of Ca in the medium on which the mycelium of fungi was passaged, the stronger the inhibition of growth. The smallest surface of the mycelium of fungi was observed in case of the highest applied concentration of calcium, that is 8 mM. The inhibiting influence of calcium was strongest on the first day after transferring onto the medium without calcium, then it was decreasing. During the last day the differences between the variants were small.

Table 2.

The effect of passaging *Fusarium culmorum* on media with different contents of calcium on the growth of mycelium of this pathogen (the surface of colonies in cm^2)

Day	Concentration of calcium in the medium [mM]*			
	0	2	4	8
1	1.37	1.27	0.88 [a]	0.24 [a]
2	7.63	7.21	5,74 [a]	5.41 [a]
3	21.47	20.48	19.50 [b]	17.77 [a]
4	50.68	49.55	46.92 [b]	42.87 [a]
5	61.42	61.21	60.06	55.03 [a]

Differences significant in relation to control at: a – P=0.001, b – P=0.01

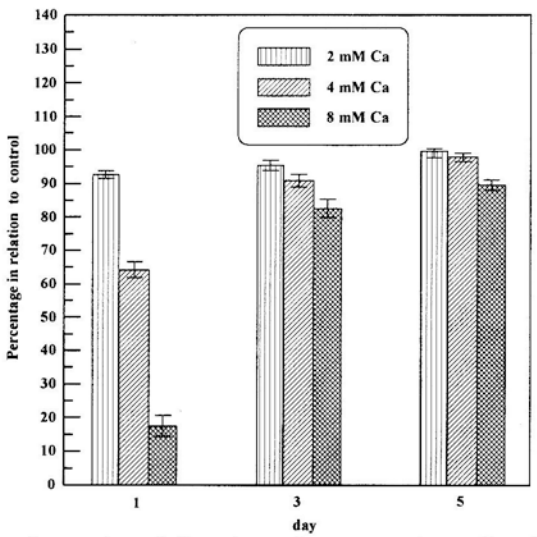

Fig. 2. The effect of passaging of *Fusarium culmorum* on the media with different calcium concentration on mycelium growth of this pathogen. The data expressed in percentages in relation to control (the growth of the mycelium of fungi passaged on the media without calcium was assumed to be 100%)

The results presented in Table 3 and in Fig. 3. indicate that passaging *Fusarium culmorum* on the media with calcium reduced its sporulation. The higher the concentration of Ca in the medium, the stronger the above-mentioned inhibition. In case of passaging the fungus on the media containing calcium at the concentration of 4 and 8 mM the strong inhibition of the sporulation was observed (19.4% and 3% in relation to control, respectively).

The inhibiting influence of calcium was also observed in case of spore germination (Table 3. and Fig. 4.). Passaging the mycelium of fungi on the media with calcium caused the decrease of spore germination by about 50% in relation to control (the fungus passaged on the medium without calcium).

Table 3.

The effect of passaging *Fusarium culmorum* on media with different contents of calcium on the sporulation and spore germination of this pathogen

Measurement	Concentration of calcium [mM]*			
	0	2	4	8
Sporulation (.)	215000	150000 [a]	41710 [a]	6540 [a]
Germination of spores (..)	62.4	38.4 [a]	36.6 [a]	28.2 [a]

(.) - the number of spores /1 cm^3 of medium,

(..) – the percentage of germinated spores,

Significant differences in relation to control at: a – P=0.001, b – P=0.01

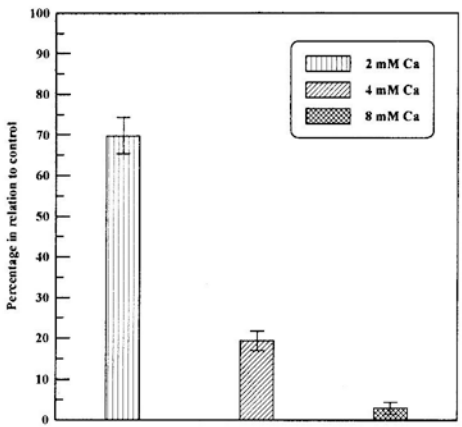

Fig. 3. The effect of passaging *Fusarium culmorum* on the media with different calcium concentration on the sporulation of this pathogen. The data expressed in percentage in relation to control (the sporulation of fungus passaged on the media without calcium was assumed to be 100%)

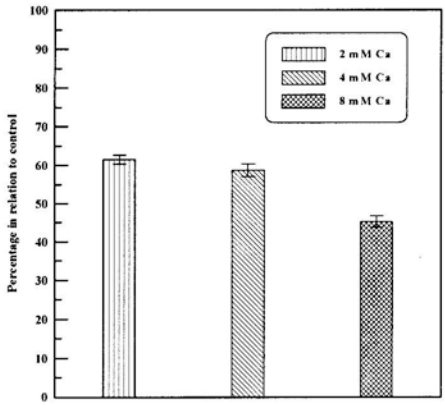

Fig. 4. The effect of passaging *Fusarium culmorum* on media with different calcium concentration on the germination of spores of this fungus. The data expressed in percentage in relation to control (the germination of spores of the fungus passaged on the media without calcium was assumed to be 100%).

The obtained results show that despite of the lack of calcium in the medium, the mycelium of fungi passaged previously on the media with calcium were characterised with the inhibition of growth and development (Fig. 2. - 4.). The higher the content of calcium in the media on which the mycelium of fungi was transferred, the stronger the inhibition. The results point that the effect of calcium could be transferred on to the next generations of the fungi.

On the base of the results of our work it may be concluded that repeated treatment of this pathogen with calcium inhibits its growth and development and it may be one of the reasons of limiting the development of the disease on wheat seedlings caused by *Fusarium culmorum.*

The inhibiting influence of calcium on *Fusarium culmorum* finds the confirmation in literature dealing with other pathogens. Calcium added to the medium inhibited the growth of *Botrytis cinerea* (V o l p i n, E l a d, 1991), germination and growth of *Botrytis cinerea* and *Penicillium expansum* (W i s n i e w s k i et al. 1995), *P. digitatum* (D r o b y et al. 1997). B i g g s et al. (1994) established that calcium salts inhibit the growth of *Leucostoma persoonii*, the fungus causing the peach canker. The greatest growth reduction (85%) was caused by calcium propionate, followed by calcium hydroxide (76%) and calcium silicate (73%). Calcium chloride had also an inhibiting influence, especially during the second day of cultivation (45,7%), and afterwards this effect was gradually weakened and on the 6th day it amounted to only 15,9%. The authors think that the pathogenicity of this fungus, in relation to peaches, lowered under the influence of calcium, results not only from the limitation of its growth, but may be also connected with the reduced production of polygalacturonase by this pathogen. B i g g s et al. (1994) also suggested, that Ca may act directly on the pathogen. Modification of membrane permeability, electron transport, and/or enzyme activity could cause reduced virulence or, in the extreme, fungistasis.

W i s n i e w s k i et al. (1995) suggest that Ca may decrease the fungal infection through direct influence on spore germination and growth of pathogen as well as through an effect on the activity of cell-wall-macerating enzymes. They also stated that calcium has a greater influence on the germination of spores and the growth of *Botrytis cinerea* than of *Penicillium expansum*, whereas the opposite was true for the effect of calcium on pectinolytic enzymes. This element decreased activity of pectinolytic enzymes by over 90% in *P. expansum* and by only about 20% in *B. cinerea*.

Similarly Q u i r o z et al. (1997) found that adding 10 mM of $CaCl_2$ to the medium inhibited (by about 50%) the germination of spores of *Sclerotinia sclerotiorum*, the pathogen of sunflower. Calcium also decreased the activity of polygalacturonase produced by this fungus.

The data from literature, however, concern calcium added to the medium, whereas in our experiments the fungi repeatedly treated with calcium were moved onto the medium without calcium. The effect of passaging on the media with calcium on the growth and development of pathogens and on their pathogenicity is the problem not encountered in literature, so far.

The experiments similar to those described above were conducted by M i c h n i e w i c z, R o ż e j (1991). These authors passaged *Fusarium culmorum* on the media with ethrel and established that long-lasting treatment of these fungi with above compound lowers the pathogenicity of the fungus in relation to wheat coleoptiles, and causes also the increased production of ethylene by these fungi. Perhaps, the repeated treatment of this pathogen with calcium causing the lowering of this pathogenicity, is also connected with the influence of the applied element on the production of ethylene by this fungus. The data obtained by us (unpublished so far) point out to such a possibility, that mycelium of fungi passaged on the media with calcium are characterised with the increased production of ethylene. It also resulted from our experiments (unpublished data) that the effect of calcium was transferred through repeated passaging on the media containing this element onto the next generations of fungi and increased the inhibiting action of ethylene in the pathogenesis of wheat seedlings caused by *Fusarium culmorum*. This fact may undoubtedly find practical application in protecting of the plant from infection by this pathogen and may, beside the application of ethrel, be one of the methods in the fighting against disease caused by *Fusarium culmorum*.

On the basis of the obtained data it may be concluded that passaging *Fusarium culmorum* on the media with calcium lowers the pathogenicity of this fungus in relation to wheat seedlings. However, the precise mechanism of the inhibition of fungal growth and development is not yet understood and further studies are necessary.

REFERENCES

Biggs A. R., El-Kholi M. M., El-Neshawy S. M. 1994. Effect of calcium on growth, pectic enzyme activity, and colonization of peach twigs by *Leucostoma persoonii*. Plant Dis. 78: 886-890.

Conway W. S., Sams C. E., Wang C. Y., Abbott J.A. 1994. Additive effects of postharvest calcium and heat treatment on reducing decay and maintaining quality in apples. J. Amer. Soc. Hort. Sci. 119(1): 49-53.

Droby S., Wisniewski M. E., Cohen L., Weiss B., Touitou D., Eilam Y., Chalutz E. 1997. Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue, and biocontrol activity of *Pichia guilliermondii*. Phytopathology 87: 310-315.

Elad Y., Volpin H. 1993. Reduced development of grey mould (*Botrytis cinerea*) in bean and tomato plants by calcium nutrition. J. Phytopathology 139: 146-56.

Elad Y., Yunis H., Volpin H. 1993. Effect of nutrition on susceptibility of cucumber, eggplant, and pepper crops to *Botrytis cinerea*. Can. J. Bot. 71: 602-608.

Michniewicz M., Czerwińska E., Rożej B., Bobkiewicz W. 1983. Control of growth and development isolates of *Fusarium culmorum* (W.G.Sm.) Sacc. of different pathogenicity to wheat seedlings by plant growth regulators. II. Ethylene. Acta Physiol. Plant. 5: 189-198

Michniewicz M., Rożej B. 1991. The effect of passaging *Fusarium culmorum* (W.G.Sm.) Sacc. on media containing ethrel or abscisic acid on the susceptibility of the fungus to treatment with these substances and on disease development in wheat seedlings. Bull. Pol. Acad. Sci., Biol. Sci. 39, 1: 47 - 55.

Michniewicz M., Rożej A., Czerwińska E., Kruszka G. 1986. Effect of Ethrel treatment of wheat on the susceptibility to fungal diseases and on the root zone mycoflora of this plant. Acta Agrobot. 39: 16-22.

Niziołł M., Rożej B. 1993. The effect of calcium on the extent of infection of wheat seedlings with *Fusarium culmorum* (W. G. Sm.) Sacc. and on the growth and development of that pathogen. Bull. Pol. Acad. Sci., Biol. Sci. 41, 3: 269-275.

Punja Z.K., Gaye M.M. 1993. Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis. 77: 989-995.

Quiroz F.J., Prioletta S.M., Pereyra V.R. Escande A.R., Flego D.C. 1997. Effect of calcium on germination, germ tube growth and extracellular enzymes activity of *Sclerotinia sclerotiorum*. Suppl. Plant Physiol. 87: 81.

Volpin H., Elad Y. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis blight*. Phytopathology 81: 1390-1394.

Wisniewski M., Droby S., Chalutz E., Eilam Y. 1995. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of *Candida oleophila*. Plant Pathol. 44: 1016-1024.

Wpływ pasażowania *Fusarium culmorum* na pożywkach z wapniem na porażenie siewek pszenicy oraz na wzrost i rozwój tego patogena

Streszczenie

Fusarium culmorum przeszczepiano dziesięciokrotnie na pożywkę (Czapek-Dox) o różnej zawartości wapnia (0, 2, 4, 8 mM), a następnie zawiesiną zarodników infekowano siewki pszenicy ozimej odmiany Grana. Zaobserwowano, że porażenie siewek pszenicy było mniejsze kiedy infekowano je zawiesiną zarodników z grzybni pasażowanej na pożywkach zawierających wyższe stężenia jonów wapnia. Stwierdzono również, sże pasażowanie *Fusarium culmorum* na pożywce z wapniem powoduje zahamowanie jego wzrostu i rozwoju.

Na podstawie uzyskanych wyników można wnioskować, że pasażowanie *F. culmorum* na pożywce z wapniem zmniejsza patogeniczność tego grzyba w stosunku do siewek pszenicy.