The influence of some aminoacids on the Krebs cycle dehydrogenases in *Fusarium graminearum* Schwabe (telemorphe - *Gibberella zeae* Schwein.) Petch parasite on wheat

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Abstract

In this paper, the authors present the influence of some aminoacids (glutamic acid, serine, leucine, methionine, histidine, lysine, asparagine, valine, and alanine) on the dehydrogenases activity in Fusarium graminearum (telemorphe - Gibberella zeae) parasite on wheat. The dehydrogenases activity was inffluenced both by the type of aminoacid from the culture medium and by culture age.

Keywords: *Fusarium graminearum,* aminoacids, dehydrogenases

Introduction

Mycotoxins are toxic secondary metabolites of fungi that represent numerous and diverse chemical classes. Usually, those are protein substances with great molecular weight, elaborated outside (exotoxins) in some nutriţion, temperature or pH condiţions [6].

F.A.O estimated that ≈ 25% of the world crops are infested by mycotoxins, which produce serious trouble on the population's health and on the domestic animal's [19]. There are five genera of fungi (*Aspergillus, Fusarium, Penicillium, Alternaria* and *Claviceps)* that represent the origin of the most important part of synthesised mycotoxins' diversity and also of the simptomes associated to them.

In 1996, from more samples of wheat, harvested from different regions of Canada, ≈ 20% contained different mycotoxins: trichothecenes, dezoxynivalenol (DON, also known as vomitoxin), zearalenone, T_2 toxine, HT2 toxine etc. [20].

The *Fusarium* species are generally considered to be field fungi and are thought to proliferate before harvest [7]. However, *Fusarium* species may also grow and produce mycotoxins under certain storage conditions. Recently, A. E. Desjardins has published a monography about *Fusarium* mycotoxins, regarding chemistry, genetics and biology of them [8].

Fusarium graminearum produced head blight (FHB) or scab with impact in wheat production. Severe FHB epidemics have occurred all over the world, resulting in major yield and quality losses that cause problems to producers and to various industries that use grain as raw material. *Fusarium graminearum* reduces yield through florest sterility and poor seed filling, and severely reduces seed germination. Infection by *Fusarium graminearum* lowers grain quality due to reductions in storage protein, cellulose and amylose [4]. In contrast to seeds used for feed or miling, seeds planted to regenerate the crop must be alive and possess those physiological traits that allow germination and seedling establishment. Consequently, a FHB epidemic can be a serious problem for seed producers. Research recently about *Fusarium graminearum* was effectued by Bai, G. and Shaner, G. [3], Boshoff, W. H. P. & al. [5], Dill- Macky, R. and Jones, R. [9], Gilbert, J. and Tekauz, A. [10], Goswami, R. S. and Kistler, H. C. [11], Kriel, W. M. and Minnaar - Ontong, A. [12], Leonard, K. J. and Bushnell, W. R. [13].

The knowledge of the *Fusarium* biology is very important and this is the reason because in our laboratory has initiated some research on *Fusarium* species that produced mycotoxins, the purpose being to clarify certain aspects regarding the biology of these parasites fungi.

The objective of this paper was to determine the influence of some aminoacids: glutamic acid, serine, leucine, methionine, histidine, lysine, asparagine, valine, alanine, on the dehydrogenases activity: glucose 6 - phosphatedehydrogenase (E.C.1.1.1.49), isocitratedehydrogenase (E. C. 1. 1.1. 42), malatedehydrogenase (E. C. 1. 1. 1. 37), α - cetoglutaratedehydrogenase (E. C. 1. 2. 4. 2) in *Fusarium graminearum*.

In Biological Research Institute Iaşi was effectued a lot of study regarding the influence of the different factors on the dehydrogenases evolution at fungi [1,14,15,16,17,18].

Materials and Methods

The investigations have been performed in *Fusarium graminearum*, harvested from the the experimental field of the Agricole Research Station Podu Iloaiei, county Iaşi. In laboratory the fungus was cultivated on Brown medium: glucose - 30g, asparagine - 1g, $MgSO_4 \cdot 7H_2O$ - 0,5g, K_2HPO_4 - 1,5g, distiled water - 1000 ml. In this medium we substituted asparagine with 1g from the following aminoacids: glutamic acid, serine, leucine, methionine, histidine, lysine, valine, alanine; it was also used a control without aminoacids. The dehydrogenases activity was determined from mycelium, trough Sîsoev and Krasna method, partially modified by Artenie [2], at 21 days and 28 days after the inoculation.

Results and Discussion

The results of the investigations dealing with the influence of some aminoacids on dehydrogenases activity are presented in the figures 1- 4. Thus, in figure 1 is presented the activity of glucose 6 - phosphatedehydrogenase, concluding that at 21 days after the inoculation, the smallest value - 0,0886 mg formasane/ g mat.- was registered in V_2 (serine), followed in increasing order by V_6 (asparagine) - 0,1296 mg formasane/ g mat., V_7 (lysine) - 0,1512 mg formasane/ g mat., V_9 (alanine) - 0,1705 mg formasane/ g mat., V_3 (leucine) -0,2387 mg formasane/ g mat., V_4 (methionine) - 0,2660 mg formasane/ g mat., V_{10} (control) - 0,4001 mg formasane/ g mat., V_8 (valine) - 0,5415 mg formasane/ g mat., V_5 (histidine) -0,6822 mg formasane/ g mat. and V_1 (glutamic acid) - 0,7860 mg formasane/ g mat., it was observed that at the variants with glutamic acid, histidine, valine, the activity of glucose 6 - phosphatedehydrogenase was higher that the control, this being the showing the stimulating action of these aminoacids. At 28 days from inoculation, the activity of glucose 6 - phosphatedehydrogenase have had maximum value in V_{10} (control) - 0,3720 mg formasane/ g mat., following in decreasing order by V_2 (serine) - 0,2379 mg formasane/ g mat., V_3 (leucine) - 0,1893 mg formasane/ g mat., V_7 (lysine) - 0,0967 mg formasane/ g mat., V_4 (methionie) - 0,0733 mg formasane/ g mat., V_5 (histidine) - 0,0730 mg formasane/ g mat., V8 (valine) - 0,0657 mg formasane/ g mat., in V_1 (glutamic acid), V_6 (asparagine), V_9 (alanine) the activity of this enzyme was zero. At this period of time the activity of glucose 6 - phosphatedehydrogenase was not stimulated by the presence of the aminoacids in the culture medium.

Analyzing the dynamics of glucose 6 - phosphatedehydrogenase activity at the two time intervals studied, 21 and 28 days, was observed a decreasing at V_1 (glutamic acid) from 0,7840 mg formasane/ g mat. to 0, V_5 (histidine) from 0,6822 mg formasane/ g mat. to 0,0730 mg formasane/ g mat., V_8 (valine) from 0,5451 mg formasane/ g mat. to 0,0657 mg formasane/ g mat., V_{10} (control) from 0,4001 mg formasane/ g mat. to 0,3720 mg formasane/ g mat. V_4 (methionine) from 0,2660 mg formasane/ g mat. to 0,0733 mg formasane/ g mat., V_3 (leucine) from 0,2387 mg formasane/ g mat. to 0,1893 mg formasane/ g mat., V_9 (alanine) from 0,1705 mg formasane/ g mat. to 0, V_7 (lysine) from 0,1512 mg formasane/ g mat. to 0,0967 mg formasane/ g mat.,V_6 (asparagine) from 0,1296 mg formasane/ g mat. to 0, and in increasing in V_2 (serine) from 0,0886 mg formasane/ g mat. to 0,2739mg formasane/g mat.

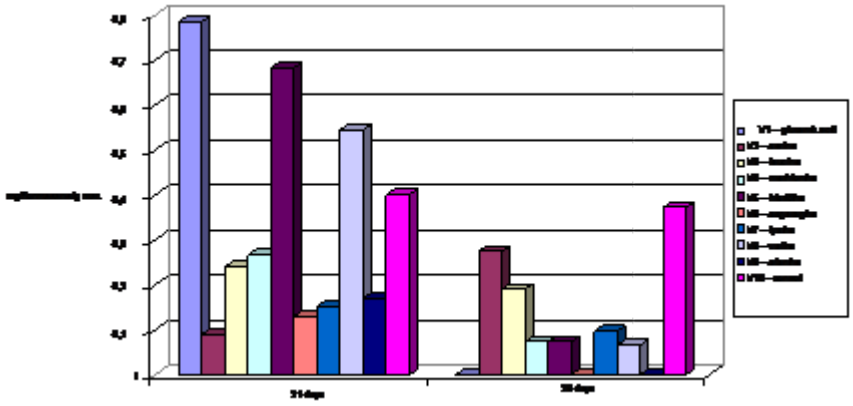

Figure 1. The influence of aminoacids on glucose 6- phosphatedehydrogenase activity

The data regarding the influence of the same aminoacids on isocitratedehydrogenase activity are presented in figure 2, from which results that at 21 days after inoculation, the highest value of this enzyme was registered in V_3 (leucine) - 0,9892 mg formasane/ g mat., followed in decreasing order by V_8 (valine) - 0,7163 mg formasane/ g mat., V_5 (histidine) - 0,6959 mg formasane/ g mat., V_{10} (control) - 0,6001 mg formasane/ g mat., V_1 (glutamic acid) - 0,5790 mg formasane/ g mat., V_9 (alanine) - 0,4707 mg formasane/ g mat.,V_6 (asparagine) - 0,3001 mg formasane/ g mat., V_4 (methionine) - 0,2865 mg formasane/ g mat., V_7 (lysine) - 0,2149 mg formasane/ g mat. and V_2 (serine) - 0,1023 mg formasane/ g mat. At 28 days from inoculation, it is observed that the enzyme had 0 activity in V_1 (glutamic acid), V_6 (asparagine) and V_9 (alanine), followed in increasing order by V_7 (lysine) - 0,1762 mg formasane/ g mat., V_4 (methionine) - 0,2063 mg formasane/ g mat., V_5 (histidine) - 0,2465 mg formasane/ g mat., V_{10} (control) - 0,3360 mg formasane/ g mat., V_8 (valine) - 0,3750 mg formasane/ g mat., V_3 (leucine) - 0,4154 mg formasane/ g mat. and V_2 (serine) - 0,9846 mg formasane/ g mat. From these data it was concluded that after 21 and 28 days from inoculation, leucine, histidine and valine have had a stimulating action on isocitratedehydrogenase activity in comparasion with the control.

Watching this enzyme in its dynamics, it was registered in increasing value for V_2 (serine) from 0,1023 mg formasane/ g mat. to 0,9486 mg formasane/ g mat. and a decreasing value for V_3 (leucine) from 0,9892 mg formasane/ g mat. to 0,4154 mg formasane/ g mat., V_8 (valine) from 0,7163 mg formasane/ g mat. to 0,3750 mg formasane/ g mat., V_5 (histidine) - 0,6959 mg formasane/ g mat. to 0,2465 mg formasane/ g mat., V_{10} (control) from 0,6001 mg formasane/ g mat. to 0,3360 mg formasane/ g mat.,V_1 (gutamic acid) from 0,5790 mg formasane/ g mat. to 0, V_9 (alanine) from 0,4707 mg formasane/ g mat. to 0, V_4 (methionine) from 0,2865 mg formasane/ g mat. to 0,2063 mg formasane/ g mat., V_6 (asparagine) from 0,3001 mg formasane/ g mat. to 0, V_7 (lysine) from 0,2149 mg formasane/ g mat. to 0,1762 mg formasane/ g mat.

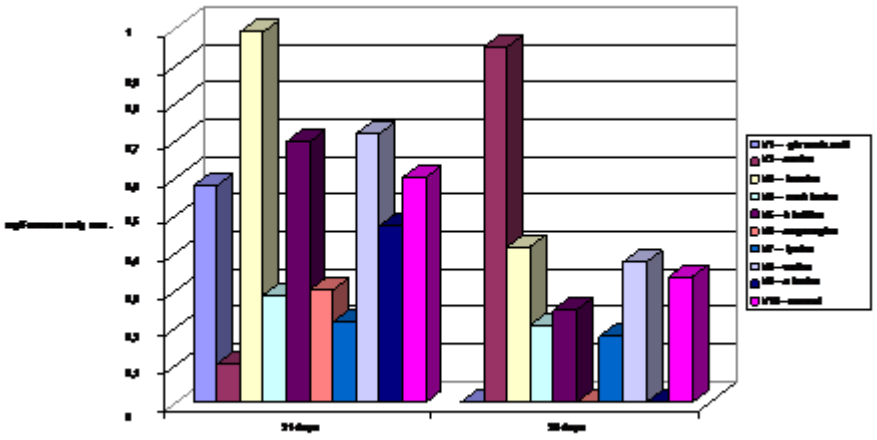

Figure 2. The influence of aminoacids on isocitratedehydrogenase activity

The malatedehydrogenase activity is presented in figure 3, from which is observed that at 21 days after the inoculation, the highest value was registered in V_3 (leucine) - 1,1240 mg formasane/ g mat., followed in a decreasing order by V_1 (glutamic acid) - 0,7840 mg formasane/ g mat., V_8 (valine) - 0,7504 mg formasane/ g mat., V_5 (histidine) - 0,6958 mg formasane/ g mat., V_{10} (control) - 0,6441 mg formasane/ g mat., V_4 (methionine) - 0,4980 mg formasane/ g mat., V_9 (alanine) - 0,3820 mg formasane/ g mat., V_6 (asparagine) - 0,3411 mg formasane/ g mat., V_7 (lysine) - 0,2010 mg formasane/ g mat. and V_2 (serine) - 0,1125 mg formasane/ g mat. At 28 days after the inoculation, the malatedehydrogenase activity registered the zero value in V_1 (glutamic acid), V_6 (asparagine), V_9 (alanine), followed in increasing order by V_7 (lysine) - 0,1878 mg formasane/ g mat., V_4 (methionine) - 0,2615 mg formasane/ g mat., V_{10} (control) - 0,3112 mg formasane/ g mat., V_5 (histidine) and V_8 (alanine) - 0,4272 mg formasane/ g mat.V_3 (leucine) - 0,5316 mg formasane/ g mat. and V_2 (serine) - 1,2515 mg formasane/ g mat. Analysing this enzyme regarding the age of the culture, it was observed in increased after 28 days from inoculation in comparison with the value registered after 21 days in V_2 (serine) from 0,1125 mg formasane/ g mat. to 1,2515 mg formasane/ g mat. and a decrease at V_3 (leucine) from 1,1240mg formasane/ g mat. to 0,5316 mg formasane/ g mat.,V_1 (glutamic acid) from 0,7840mg formasane/ g mat. to 0, V_8 (valine) from 0,7504mg formasane/ g mat. to 0,4272mg formasane/ g mat., V_5 (histidine) from 0,6958mg formasane/ g mat. to 0,4272mg formasane/ g mat., V_{10} (control) from 0,6401mg formasane/ g mat. to 0,3112mg formasane/ g mat.,V_4 (methionine) from 0,4980mg formasane/ g mat. to 0,2615mg formasane/ g mat., V_9 (alanine) from 0,3820mg formasane/ g mat. to 0., V_6 (asparagine) from 0,3411mg formasane/ g mat. to 0, V_7 (lysine) from 0,2012mg formasane/ g mat. to 0,1878mg formasane/ g mat. These data, as in the case of the isocitratedehyodrogenase activity, prove the stimulating action of the leucine, histidine and valine on the malatedehydrogenase activity, in comparasion with the control

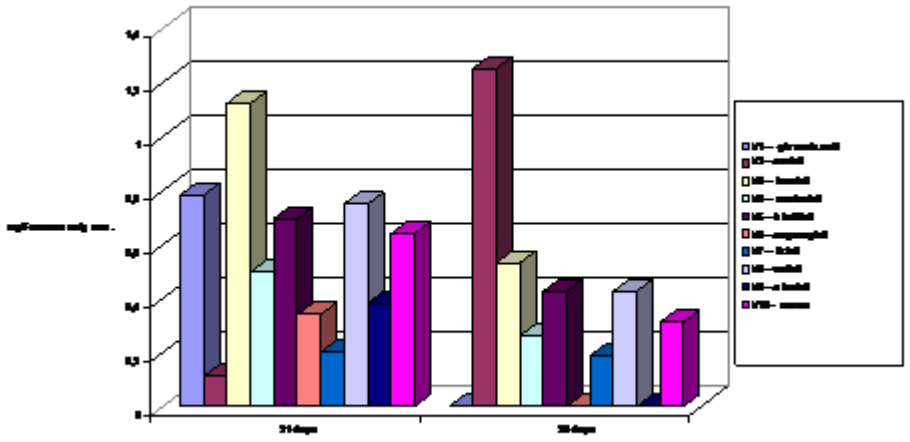

Figure 3. The influence of some aminoacids on malatedehydrogenese activity

Data relating of α - cetoglutaratedehydrogenase activity after 21 days from the inoculation are presented in figure 4, from which results that the lowest value was registered in V_2 (serine) - 0,0989 mg formasane/ g mat., followed in increasing order by V_7 (lysine) - 0,2456 mg formasane/ g mat., V_4 (methionine) - 0,3888 mg formasane/ g mat., V_9 (alanine) - 0,4264mg formasane/ g mat., V_8 (valine) - 0,5662 mg formasane/ g mat., V_3 (leucine) - 0,5719 mg formasane/ g mat., V_6 (asparagine) - 0,6413mg formasane/ g mat., V_{10} (control) - 0,6560 mg formasane/ g mat., V_5 (histidine) - 0,6754mg formasane/ g mat., V_1 (glutamic acid) - 0,7170 mg formasane/ g mat. Determining α – cetoglutaratedehydrogenase activity after 28 days from inoculation, it was observed that the highest value was registered in V_2 (serine) - 1,1966 mg formasane/ g mat., followed in a decreasing order by V_3 (leucine) - 0,7856 mg formasane/ g mat., V_5 (histidine) and V_8 (valine) - 0,4413 mg formasane/ g mat.,

V_{10} (control) - 0,3840 mg formasane/ g mat., V_4 (methionine) - 0,1519 mg formasane/ g mat., V_7 (lysine) - 0,1251 mg formasane/ g mat.; in V_1 (glutamic acid), V_6 (asparagine), V_9 (alanine) the activity of α - cetoglutaratedehydrogenase was 0. Glutamic acid and histidine have had stimulated action after 21 days from inoculation, in comparasion with the control; the variants with serine, histidine, valine have had stimulating action on the activity of this enzyme after 28 days from the inoculation.

Studying the dynamics of α - cetoglutaratedehydrogenase activity at the two time intervals, 21 and 28 days, it was observed that it decreased in V_1 (glutamic acid) from 0,7170 mg formasane/ g mat. to 0, V_5 (histidine) from 0,6754 mg formasane/ g mat. to 0,4413 mg formasane/ g mat., V_{10} (control) from 0,6560 mg formasane/ g mat. to 0,3840 mg formasane/ g mat., V_6 (asparagine) - 0,6413mg formasane/ g mat. to 0, V_8 (valine) from 0,5662 mg formasane/ g mat. to 0,4413 mg formasane/ g mat., V_9 (alanine) from 0,4264 mg formasane/ g mat. to 0, V_4 (methionine) from 0,3888 mg formasane/ g mat. to 0,1519 mg formasane/ g mat., V_7 (lysine) from 0,2456 mg formasane/ g mat. to 0,1251 mg formasane/ g mat. and it increased only at V_3 (leucine) from 0,5719 mg formasane/ g mat. to 0,7856 mg formasane/ g mat. and V_2 (serine) from 0,0989 mg formasane/ g mat. to 1,1966 mg formasane/ g mat.

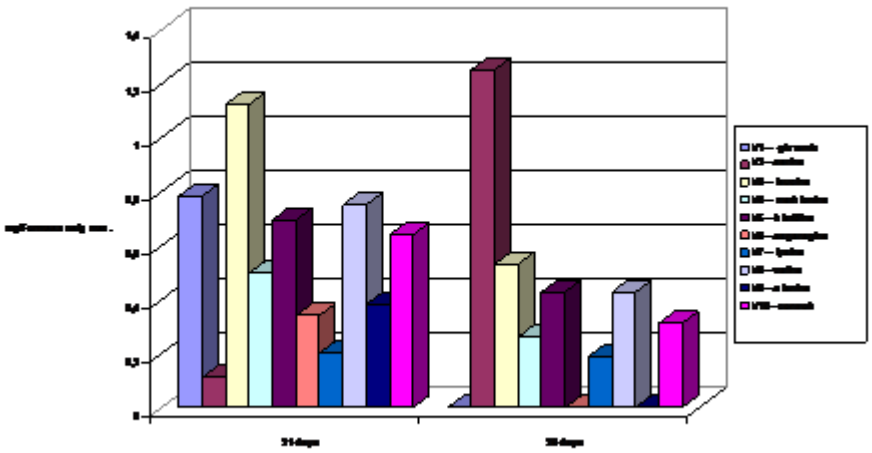

Figure 4. The influence of some aminoacids on α – cetoglutaratedehydrogenase activity

Conclusion

1. The glucose 6 - phosphatedehydrogenase activity was stimulated by introducing glutamic acid, histidine and valine in the culture medium after only 21 days from the inoculation; after 28 days, the glucose 6 - phosphatedehydrogenase activity was not stimulated by the presence of the aminoacids in the culture medium.

2. The leucine, histidine, valine have had stimulating action on the isocitratedehydrogenase activity, both at 21 and 28 days from inoculation.

3. The malatedehydrogenase activity was stimulated at 21 days after inoculation by the glutamic acid, leucine, histidine and valine and after 28 days by serine, leucine, histidine and valine.

4. The α - cetoglutaratedehydrogenase activity was stimulating by the glutamic acid and histidine, after 21 days from the inoculation. After 28 days from the inoculation serine, leucine, histidine and valine have had a stimulating action on the activity of this enzyme.

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