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CHANGES OF SOME DEHYDROGENASE ACTIVITIES IN THE LEAVES OF PEACH CULTIVAR *SPRINGCREST* NATURALLY INFECTED WITH THE FUNGUS *TAPHRINA DEFORMANS*

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Abstract: *The influence of Taphrina deformans (Berk.) Tul., associated with peach leaf curl disease,on glucose dehydrogenase (EC 1.1.99.10), isocitrate dehydrogenase (EC 1.1.1.42), α-ketoglutarate dehydrogenase (EC 1.2.4.2) and malate dehydrogenase (EC 1.1.1.37) activitities in the leaves harvested from peachcultivar Springcrest, was investigated. Samples of both healthy and diseased leaves were analyzed. The resultus of this study suggests that the leaves infection with the biotrophic fungus Taphrina deformans lead to the decreasing of glucose dehydrogenase and malate dehydrogenase activities and to a significantlly increasing of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase activities as an attempt of host plant tissues to limit the damages caused by the fungus attack. Data obtained in this study revealed significant differences in these enzymes activities depending on the type of theenzyme, the age of the leaves and the presence or absence of fungus attack.*

Keywords:*peach leaf curl, glucose dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase*

1. Introduction

Persica vulgaris Mill. is one of the major fruit crop in Romania, in present it is occuping the third place after apples and plums [1].
Peach leaf curl disease caused by *Taphrina deformans (Berk.) Tul.*is predominant in all the peach growing areas of the world [2] and is one of the most dangerous disease for peach because it can cause the defoliation and major crop loss at nearly all cultivars of peach trees.The infection is favoured by low temperature and high humidity from the time of bud swellen; the infection occurs mainly during a short period after the buds open when the new tissues are susceptible and as all organs grow older they become resistant to infection [3].
Dehydrogenases are oxidizing enzymes which catalyze the electron transfer from the donor to an acceptor other than molecular oxigen.
Glucose dehydrogenase (GHD, D-glucose: acceptor 1-oxidoreductase, EC 1.1.99.10) is a FAD-dependent enzyme. Glucose dehydrogenase is anoxidoreductase that catalyze the first hydroxyl group of glucose and other sugar molecules, utilizing FAD as primaryelectronacceptor. FAD GDHs utilize a variety of external electron acceptors, but not oxygen; glucose dehydrogenase has been found as extracellular enzyme in fungi, such as

Aspergillus sp., and it has ahigh specificityfor glucose [4]. Intracellular FAD-dependent GDH is involved inmetabolic pathways, such as the glycan metabolism and the biosynthesis of secondary metabolites; they are suggested to play a role in the pentose phosphate pathway involving glucose turnover for the production of NADH as reducing equivalents and pentoses as integral parts of nucleotides. The biological function of extracellular FAD-dependent glucose dehydrogenase is still unclear, but a role during fungal attack on the host-plant is proposed. By reducing quinones and phenoxy radicals glucose dehydrogenase is able to neutralize the action of plant laccases, phenoloxidases or peroxidases, which are used by infected plant tissues to limit the fungal attack [5].

Isocitrate dehydrogenase (IDH, EC 1.1.1.42) is a NADP-dependent enzyme, that controls the carbon flux between the Krebs cycle and the glyoxylate bypass via its activation and inactivation by the bifunctional IDH kinase/phosphatase. Thus, the activation of isocitrate dehydrogenase forces the flow through the Krebs cycle, causing a decrease in the intracellular isocitrate level and an increase in the α-ketoglutarate level [6].

α-ketoglutarate dehydrogenase (α-KGDH, EC 1.2.4.2), a key regulatory point of tricarboxylic acid cycle, plays vital roles in the multiple pathways of energy metabolism and biosynthesis [7]. α-ketoglutarate dehydrogenase is an enzyme which catalyses the non-equilibrium reaction converting α-ketoglutarate, coenzyme A and NAD^+ to succinyl-CoA, NADH and CO_2, requiring thiamine pyrophosphate as a cofactor [8]. It transfers four-carbon aldehyde group from α-ketoglutarate to thiamine phyrophosphate to form hydroxyethyl-thiamine phyrophosphate.

Malate dehydrogenase (MDH, L-malate: NAD^+ oxidoreductase, EC 1.1.1.37) catalyzes the conversion of oxaloacetate and malate utilizing the NAD or NADP coenzyme system. Malate dehydrogenase is found in cytosol where it participates in malate/aspartate shuttle and in the mitochondrial matrix where it has a key role in the citric acid cycle and are NAD-dependent enzymes; the malate dehydrogenase found in plant chloroplasts has NADP as coenzyme [9].

2. Materials and methods

Vegetal material used in this study was represented by fresh healthy leaves and leaves naturally infected with the fungus *Taphrina deformans*, harvested, starting to middle of April until late June in year 2008, from peach cv. *Springcrest*, from the experimental orchard "Vasile Adamachi" Iaşi. The determinations of the dehydrogenases activity were made at: 19 April (I), 7 (II), 19 (III) and 27 (IV) May, 3 (V), 10 (VI) and 22 (VII) June.

Springcrest cv. is considered to be very susceptilbe to this pathogen attack [10,11]. The leaves were harvested early in the morning and enzymes activity was estimated in the same day.

The dehydrogenases activity was determinated by Sîsoev and Krasna spectrophotometric method, modified by Artenie.

This method has at basis the ability of dehydrogenases to transfer hydrogen from various substrate (glucose, isocitric acid, *α*-ketoglutaric acid and malic acid) to 2,3,5-triphenil-tetrazolium-chloride (TTC) which is reduced to triphenyl formazan colored in red.

Samples collected were first washed with distilled water, then the enzymes were extracted using 3 ml of phosphate buffer pH-7,4. The assay mixture of dehydrogenases contained: 0,25 ml of

crude enzyme extract, 0,2 ml of specific substrate 0,2 M, pH-7,4, 0,75 ml distillated water and 0,2 ml of standard solution of 2,3,5-triphenil-tetrazolium-chloride 1%. In the control tests the specific substrate was replaced with the same quantity of phosphate buffer.

The tests were incubated 18 hours in a thermostat at 28˚C, then separated by centrifugation at 4000 rotations per minute; supernatants were discarded and in the tests was added 5 ml of dissolvent for the extraction of triphenyl formazan; samples were centrifugated again andthe absorbance was readed spectrophotometrically at 540 nm; the color intensity is proportional with dehydrogenases activity.

Dehydrogenase activities were expressed as μg formazan per gram fresh vegetal material[12].

3. Results and Discussion

The dynamics of glucose dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase activities have been studied in healthy and curled leaves of peach cv. *Springcrest* and are presented in Figs. 1-4.

The activity of glucose dehydrogenase at cv. *Springcrest* is presented in Fig. 1, from which it can be seen that in healthy leaves this enzyme had the highest value – 0,0640 μg formazan/g mat. in the last stage (VII) and it was followed in decreasing order by the values: 0,0356 μg formazan/g mat. (II), 0,0251 μg formazan/g mat. (IV), 0,0232 μg formazan/g mat. (VI), 0,0212 μg formazan/g mat. (III), 0,0182 μg formazan/g mat. (V), 0,0156 μg formazan/g mat. (I).

In the leaves infected by *Taphrina deformans*, glucose dehydrogenase activity recorded the smallest value in the third stage of infection - 0,0146 μg formazan/g mat., followed in increasing order by the values: 0,0156 μg formazan/g mat. (IV), 0,0166 μg formazan/g mat. (V), 0,0168 μg formazan/g mat. (I), 0,0193 μg formazan/g mat. (II), 0,0217 μg formazan/g mat. (VI), 0,0830 μg formazan/g mat. (VII).

The activity of glucose dehydrogenase in attacked leaves, recorded values higher than the control (enzyme activity in healthy leaves), in the first stage (D/H=1,0769) and in the last stage (D/H=1,2968); in stage II (D/H=0,5421), stage III (D/H=0,6886), stage IV (D/H=0,6215), stage V (D/H=0,9120) and stage VI (D/H=0,9353) glucose dehydrogenase activity in diseased leaves was smaller than the enzyme activity recorded in healthy ones.

Glucose dehydrogenase has an important role during fungal attack on the host plant, it can reduce quinones and phenoxy radicals and is able to neutralize the action of host plant peroxidases and polyphenoloxidase, which are used by plants to block the fungal attack [13]. Glucose dehydrogenase activity recordet at cv. *Springcrest*was higher in diseased leaves at the begining and at the end of fungal attack; at the other dates of the determinations glucose dehydrogenase activity was higher in healthy leaves when compared with the activity from attacked peach leaves; the results obtained in this study indicated that *Taphrina deformans* was not able to produce high amounts of glucose dehydrogenase and to stop the action of the enzymes responsible for plant protection against the oxidative stress caused by fungus attack; these results are in opposition with those mentionated in literature, which say that in injured plants the enzymes from pentose phosphate pathway are increasing their activity [14].

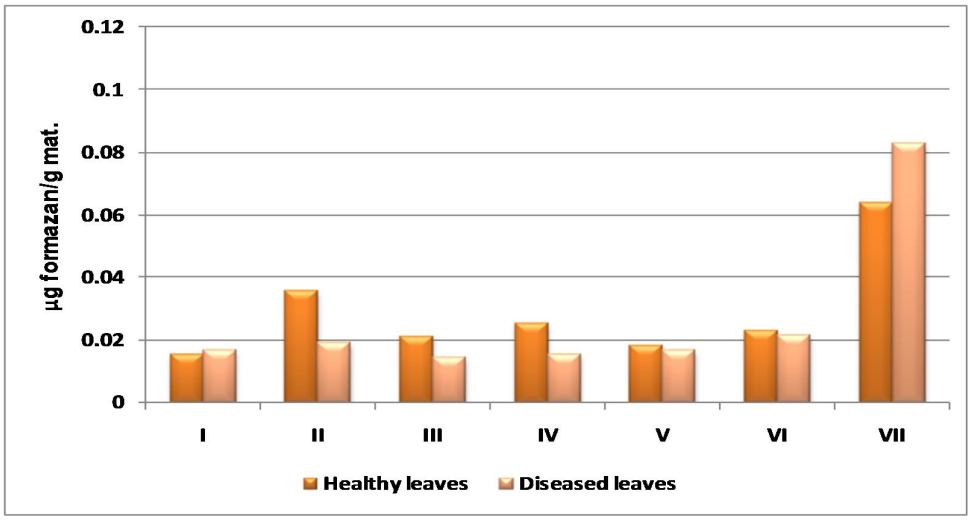


Figure 1 The influence of *Taphrina deformans* Berk. (Tul.) attack on the dynamics of glucose dehydrogenase activity

In Fig. 2 are presented the results concerning the isocitrate dehydrogenase activity in healthy and in infected leaves by *Taphrina deformans* in cv.*Springcrest.*

In healthy leaves, isocitrate dehydrogenase activity recorded the next values, presented in decreasing order: 0,0649 µg formazan/g mat. (I), 0,0514 µg formazan/g mat. (VII), 0,0368 µg formazan/g mat. (V), 0,0317 µg formazan/g mat. (VI), 0,0268 µg formazan/g mat. (IV), 0,0238 µg formazan/g mat. (III) and 0,0157 µg formazan/g mat. (II). Isocitrate dehydrogenase activity in healthy peach leaves, had the highest value at the begining of the fungus attack and the smallest value of it's activity was recorded in stage II.

In diseased leaves the activity of isocitrate dehydrogenase had the highest value– 0,0546 µg formazan/g mat. in the last stage (VII) and was followed in decreasing order by the values: 0,0512 µg formazan/g mat. (IV), 0,0455 µg formazan/g mat. (V), 0,0447 µg formazan/g mat. (VI), 0,0239 µg formazan/g mat. (I), 0,0159 µg formazan/g mat. (II), 0,0138 µg formazan/g mat. (III).

The activity of isocitrate dehydrogenase in the leaves infected by the fungus *Taphrina deformans* had smaller values in compare with those recorded in healthy ones at stages I (D/H=0,3682) and III (D/H=0,5798); at stages II (D/H=1,0127), IV (D/H=1,9104), V (D/H=1,2364), VI (D/H=1,4100) and VII (D/H=1,0622) the isocitrate dehydrogenase activity from diseased leaves was higher than the activity recorded in healthy leaves.

Isocitrate dehydrogenase activity is increasing in the leaves naturaly infected by *Taphrina deformans* as the disease simptoms develops, this enzymes is provideing the substratum necessary for α-ketoglutatate dehydrogenase activity, which recorded the same dymanic in it's activity. Isocitrate dehydrogenase is the enzyme that reflects the increased respiratory rate from diseased peach leaves.

The enhanced isocitrate dehydrogenase activity in the leaves infected by *Taphrina deformans* correlatd with a low photsynthesis activity [15] could be due to the synthesis of oxaloacetic acid by phosphoenolpyruvate carboxylase in the cytosol with subsequent transport into mitochondria where it serves as substratum for isocitrate dehydrogenaseactivity [16].

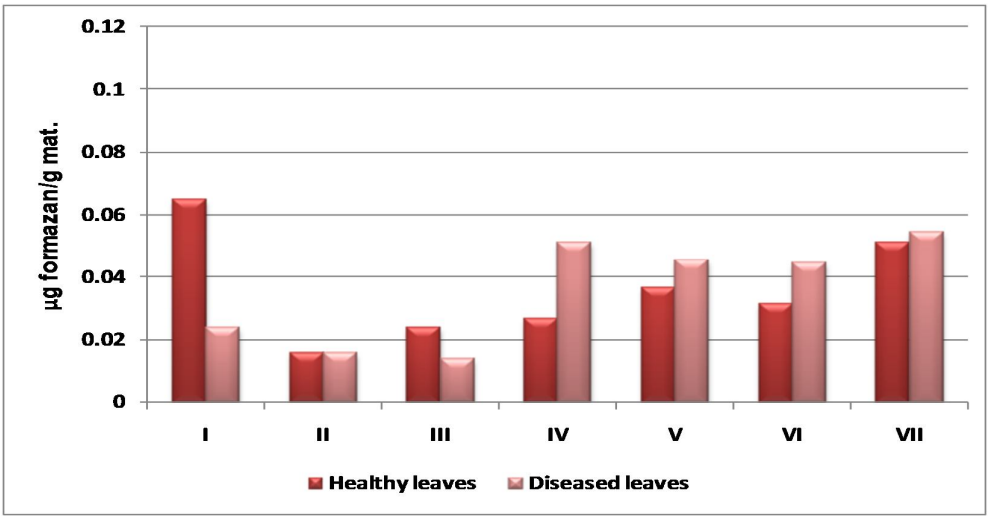


Figure 2 The influence of *Taphrina deformans*Berk. (Tul.) attack on the dynamics of isocitrate dehydrogenase activity

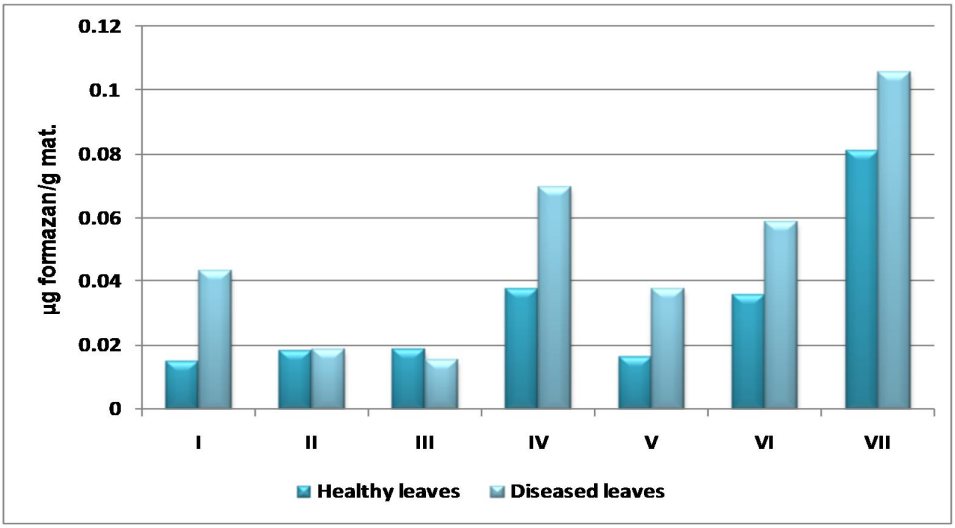
In Fig. 3 are presented the results concerning the activity of α-ketoglutatate dehydrogenase from which it can bee seen that the highest value of this enzyme activity, in non-infected leaves, was registered in the last stage (VII) - 0,0813 µg formazan/g mat. and it was followed in decreasing order by next values: 0,0376 µg formazan/g mat. (IV), 0,0359 µg formazan/g mat. (VI), 0,0188 µg formazan/g mat. (III), 0,0183 µg formazan/g mat. (II), 0,0162 µg formazan/g mat. (V), 0,0148 µg formazan/g mat. (I).

In diseased leaves were recorded the following values of α-ketoglutatate dehydrogenase activity: 0,1055 µg formazan/g mat. (VII), 0,0698 µg formazan/g mat. (IV), 0,0587 µg formazan/g mat. (VI), 0,0434 µg formazan/g mat. (I), 0,0377 µg formazan/g mat. (V), 0,0185 µg formazan/g mat. (II), 0,0153 µg formazan/g mat. (III).

The activity of α-ketoglutatate dehydrogenase had higher values in diseased leaves when compared with the enzyme activity from the healthy ones in the stages: I (D/H=2,9324), II (D/H=1,0109), IV (D/H=1,8563), V (D/H=2,3271), VI (D/H=1,6350), VII (D/H=1,2976); in stage III (D/H=0,8138) this dehydrogenase activity recorded a decreasing in its activity, which was higher in healthy leaves. This dehydrogenase activity is increasing in the same time with the disease development. α-ketoglutatate dehydrogenase it is found in it's soluble form in the mithocondrial matrix and it is considered one of the main center able to generate reactive oxygen species [17] in response to biotic stress caused by the pathogen attack.

The increased α-ketoglutatate dehydrogenase activity from infected leaves suggest an increase in respiratory rate, dependent of the age of the leaves and fungus, and an intense activity of the enzymes from the antioxidant defense line, knowing that a high metabolic rate is followed by the increase of oxidative stress markers that are responsible for the aging of mitochondria, which are the main source of reactive oxygen species [18] due to multiple reactions that transfer electrons. The decreased α-ketoglutatate dehydrogenase activity recorded at III in

diseased leaves could be due to the influence of reactive oxygen species, this enzyme in known to be one of the major target enzyme of this radicals, when inhibition of this enzyme take place and this limits the NADH availability and, as a result, the respiratory function of mitochondria [8, 19, 20, 21].



3 The influence of *Taphrina deformans* Berk. (Tul.) attack on the dynamics of α-ketoglutatate dehydrogenase activity

The last stage in the Krebs cycle, in which L malate is oxidize to oxaloacetate is catalysed by the malate dehydrogenase. The activity of malate dehydrogenase (Fig. 4), in healthy leaves had the smallest value – 0,0214 µg formazan/g mat. in the first stage of the determinations and it was followeed in increasing order by the next values: 0,0246 µg formazan/g mat. (III), 0,0293 µg formazan/g mat. (VI), 0,0333 µg formazan/g mat. (V), 0,0352 µg formazan/g mat. (IV), 0,0428 µg formazan/g mat. (II) and 0,1069 µg formazan/g mat. (VII).

In the leaves attacked by the fungus *Taphrina deformans*, malate dehydrogenase activity had the highest value – 0,0967 µg formazan/g mat. in the final stage of the attack (VII) and it was followeed in decreasing order by the values: 0,0512 µg formazan/g mat. (IV), 0,0492 µg formazan/g mat. (VI), 0,0221 µg formazan/g mat. (II), 0,0216 µg formazan/g mat. (V), 0,0127 µg formazan/g mat. (I), 0,0045 µg formazan/g mat. (III).

Malate dehydrogenase activityat peach cv. *Springcrest* recorded smaller values in diseased leaves in compare with the control in stages: I (D/H=0,5934), II (D/H=0,5163), III (D/H=0,1829), V (D/H=0,6486), VII (D/H=0,9045); in stages IV (D/H=1,4545) and VI (D/H=1,6791), this enzyme activity recorded higher values in leaves infected by the pathogenic fungus than the activity recorded in healthy leaves at the same dates.

Malate dehydrogenase activity recorded, in general, smaller values in curled leaves comparative with the values recorded in control, these results are similar with those presented in literature at *Nicotiana tabacum* plants infected by viruses, where it was observed a decrease of malate dehydrogenase activity in diseased plants

[22]. The decreased malate dehydrogenase activity from diseased leaves, can be correlated with the big amount of oxalate which is known to inhibit this enzyme activity [23].

The results obtained in this study show that the infection of peach leaves with *Taphrina deformans*, is followed by an enhancement of the activity of the enzymes of mitochondrial resiration except malate dehydrogenase activity.

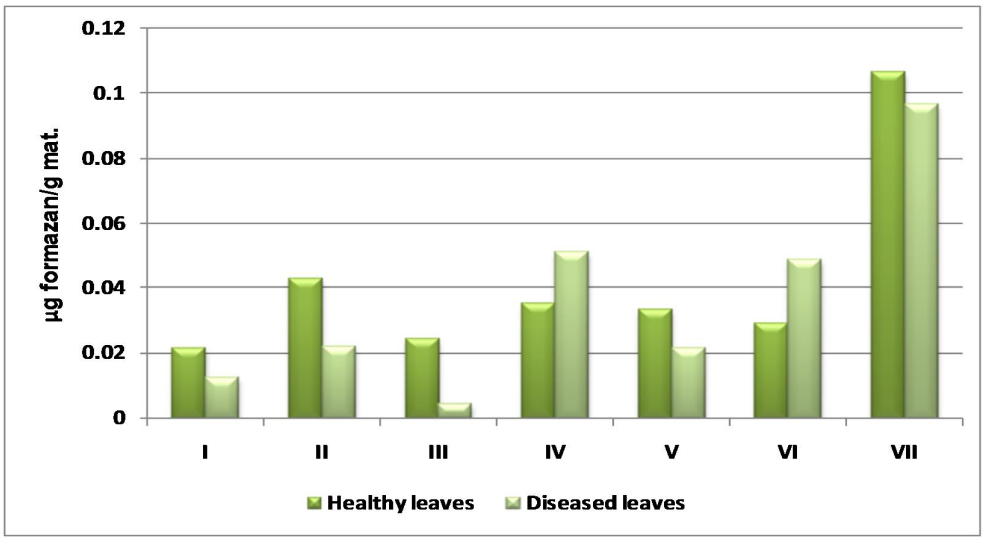


Figure 4 The influence of *Taphrina deformans* Berk. (Tul.) attack on the dynamics of malate dehydrogenase activity

4. Conclusions

Glucose dehidrogenase activity was smaller in diseased leaves, these results suggest that the host plant tissues were able to mobilize theirs defensive mechanisms against *Taphrina deformans* and to limit its attack.

Isocitrate dehydrogenase and α-ketoglutatate dehydrogenase activities recorded the same dynamics and were,in general, higher in the leaves infected by *Taphrina deformans*, than the activity recorded in healthy leaves.

Malate dehydrogenase activity recorded specific variations from one date to another, but the enzyme activity was in general, smaller in diseased leaves.

The results obtained in this study suggest that these dehydrogenases play important roles in defence machanisms against peach leaf curl infection; these reflects the ability of host plant to moblize its defensive enzymes and to limit the fungus attack and the damages produced by infection.

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