THE INFLUENCE OF TAPHRINA DEFORMANS (BERKELEY) TULASNE (PEACH LEAF CURL) ATTACK ON THE ACTIVITY OF SOME OXIDOREDUCTASES IN CULTIVAR *CARDINAL*

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Abstract: *Taphrina deformans (Berk.) Tul. is the causal agent of peach leaf curl, a fungal disease which in present is widespreaded in all places where peaches and other related species are cultivated. In this paper are presented the result concerning the influence of the attack of fungus Taphrina deformans on the activity of catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7) and polyphenoloxidase (EC 1.10.3.1) compared with the activity of this oxidative enzymes in healthy leaves at cultivar Cardinal. The determination of enzymes activity were carried out at 4.05.2009, 18.05.2009, 25.05.2009, 1.06.2009 and 8.06.2009. Catalase activity was higher in diseased leaves at the beginning of the infection and decreased with the evolution of disease simptoms. Peroxidase and polyphenoloxidase activities were found higher in diseased leaves when compared with enzymes activity from healthy leaves, at all dates of the determination. Polyphenoloxidase dicreased with leaves age, but remained considerably grater in infected tissues. This study suggest that the accumulation of reactive oxygen species in peach tissues, as a response at fungus attack, increased activities of catalase, peroxidase and polyphenoloxidase, this enzymes beeing involved in host defence mechanisms.*

Keywords: *catalase, peroxidase, polyphenoloxidase, Persica vulgaris, diseased leaves, healthy leaves*

1. Introduction

Taphrina deformans (Berk.) Tul. is a parasitic fungus that causes the disease named „peach leaf curl" and it affects peaches (*Persica vulgaris* Mill.) but also almonds, apricots and necatarines. The disease was observed for the firt time in England in 1821 [1]. In Romania, the presence of *Taphrina deformans* was mentionated for the first time in 1929 by Traian Săvulescu [2]. In present this disease is widely distributed in all the countries where peaches are cultivated. Peach leaf curl affects especially leaves but it also can affect sprouts, twigs and rarely flowers and fruits. The leaves infected by *Taphrina deformans* became red, thickened and curled as they develop and eventually become brown and fall prematurely.

The disease occurrs in springtime when the temperature is oscillateing between 15°C and 20°C (optimum of 18 °C) and the weather is wet in late February until early March [3]. The attack of *Taphrina deformans*, if untreated, can affect the quality and quantity of the crop and lead to the death of trees in a few years. *Cardinal* is a peach cultivar (cv.) obtained in 1941 at Fort Valley, Georgia (SUA) by seflpollination of cv. *Halehaven.* In our contry this peach cultivar was introduced in 1962; it's a cultivar with an average vigour and hight resistance at frost and drought but susceptible at *Taphrina deformans* and other peach pathogens such

as *Fusicoccus amygdali* and *Pseudomonas mors-prunorum* [4].

2. Materials and methods

The material used in this study was represented by fresh healthy leaves (as control) and leaves infected by fungus *Taphrina deformans* collected from cv. *Cardinal* cultivated in Didactic Orchard „Vasile Adamachi" Iaşi. The vegetal material was collected starting to the beginning of May until early June in 2009. The determination of enzymes activity was carried out in the day of collecting.

Catalase activity was estimated using the iodometric method with sodium thiosulfate; this method is measureing the cantity of hydrogen peroxide remained after the intrreruption of catalase activity on it [5].

Orto-dianisidine method was used for estimateing of peroxidase activity; the method is measureing the optic density of orto- dianisidine (as hydrogen donor) oxidation product with the participation of peroxide under the action of peroxidase [5]. The results were expressed in peroxidase units (UP) and it represent the quantity of peroxidase which decompose 1µmol of peroxide in one minute in optimum conditions. Determination of polyphenoloxidase activity, a copper containing enzyme, was performed using the method with pyrocatechol described by Ermakov quoted by Roşu Crăiţa Maria [6]. The results were expresses in polyphenoloxidase units (U PPO). Polyphenoloxidase is a ubiquitous enzyme involved in the oxidation of endogenous phenols and it is responsible for browning in plants.

3. Results and Discussion

In Fig. 1 are presented the results concerning catalase activity in healthy leaves and in leaves infected by *Taphrina deformans*. In healthy leaves catalase activity registered the highest value - 218,1818 U C/g/min at 25.05.2009, followed in decreasing order by the next values: 130,3167 U C/g/min (1.06.2009), 112,0043 U C/g/min (8.06.2009), 76,5957 U C/g/min (4.05.2009) and 71,3286 U C/g/min (18.05.2009).

In the leaves attacked by *Taphrina deformans* catalase activity recorded the highest value -171,4285 U C/g/min at 25.05.2009 and it was followed in decreasing order by the values registered at: 18.05.2009 (142,2924 U C/g/min), 4.05.2009 (120,9302 U C/g/min), 1.06.2009 (119,502 U C/g/min), 8.06.2009 (107,8632 U C/g/min). Catalase activity recorded the maximum value in healthy and diseased leaves at 25.05.2009.

From analyseing the dynamics of catalase activity at cv. *Cardinal* it has been found that catalase is increasing its enzymatic activity in diseased leaves at the beginning of the fungal infection - 4.05.2009 (Diseased/Healthy = 1,6953), 18.05.2009 (D/H= 1,8577)- but with the evolution of the disease this oxidoreductase is recording a decreasing activity in diseased leaves when compared with the activity from healthy leaves: 25.05.2009 (D/H=0,7857), 1.06.2009 (D/H=0,9170), 8.06.2009 (D/H=0,9630).

At the appearance of the first simptoms of disease, catalase activity is considerably higher in the leaves infected with *Taphrina deformans* comparatively with the enzymatic activity from the healthy leaves, this situation can be explain by the role of catalase which is well known that it occurs in regulateing the hydrogen peroxide content resulted from intense methabolic proceses and to stop the toxic effect of this product upon the plant tissues [7]; as the infection progress, and peach leaves are aging, catalase activity decrease in diseased leaves, the situation can be correlate with the intensification of peroxidase activity observed in the diseased tissues of cv. *Cardinal*. At the

beginning of fungus attack, catalase is more active in diseased leaves due to the higher amount of hydrogen peroxide, then the enzyme remains without its specific oxidation substrate because of the peroxidase activation which can be active at a smaller amount of hydrogen peroxide. From the catalase reaction we can say that it is involved, beside other enzymes, in the host defence mechanismes agains the pathogen. The results obtain at cv. *Cardinal* in 2009 are similar with those observed by Roşu Crăiţa Maria in the case of foliar attack of *Cercospora beticola* at sugar beet [6]; the same dynamics in catalase activity was observed and by Elźbieta Kużniak et Maria Skłodowska at foliar infection of *Lycopersicon esculentum* with *Botrytis cinerea* [8]. Similar result concerning the dynamics of catalase and peroxidase activity as in the present experimental study were mentionat also by Arun *et al.* at *Pennisetum glaucum* plants infected with *Sclerospora gaminicola* [9], by Mahmoud *et al.* in the case of the attack of *Botrytis fabae* at *Vicia faba* [10] and by Garcia-Limones *et al.* who observed an intensification of catalase and other oxidative enzymes in the case of the attack of *Fusarium oxysporum* f. sp. *ciceris* produced at *Cicer arietinum* [11].

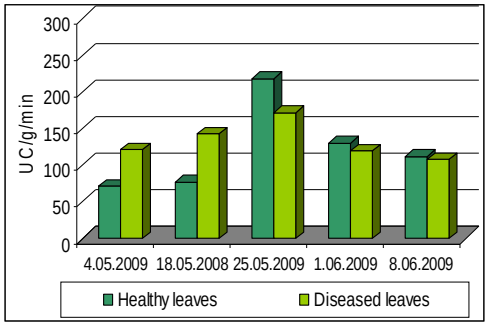

Figure 1 Catalase activity in healthy leaves and in leaves infected by *Taphrina deformans* at cv. *Cardinal*

The result concerning peroxidase activity in healthy leaves and in leaves infected by *Taphrina deformans*, are presented in Fig. 2, from which it can be observed that in healthy leaves the highest value of peroxidase activity -1,0024 UP/g min was recorded at 25.05.2009, followed in decreasing order by next values: 0,965 UP/g min (4.05.2009), 0,8487 UP/g min (1.06.2009), 0,8125 UP/g min (18.05.2009) şi 0,6765 UP/g min (8.06.2009).

In diseased leaves peroxidase activity had the highest value-1,44419 UP/g min at 8.06.2009, followed in decreasing order by the next values: 1,3704 UP/g min (1.06.2009), 1,088 UP/g min (18.05.2009) and a minimum value - 0,5517 UP/g min was recorded at 4.05.2009.

The D/H (Diseased/Healthy) ratio of peroxidase activity is subunit at the beginning of the attack 4.05.2009- 0,5717 and with the intensification of the attack it take place a intensification of enzymatic activity in diseased leaves when compared with the activity recorded in healthy leaves, the ratio D/H had values higher then the control (healty leaves): D/H=1,339 at 18.05.2009; D/H=1,1002 (25.05.2009), D/H= 1,6147 (1.06.2009) and D/H= 2,1314 (8.06.2009). This increasing of peroxidase activity at one time with the intensification of the fungus attack demonstrates the rol played by this enzyme in the defensive mechanisms of peach against oxidative stress caused by *Taphrina deformans* attack.

In 2009 at cv. *Cardinal* it take place an intensification of the peroxidase activity at one time with the evolution of *Taphrina deformans* infection in compare with the activity from healthy leaves; this intensification of the activity in diseased leaves take place because peroxidase is involved together with catalase in reduction of harmful effect of reactive oxygen species resulted as an effect of increasing of metabolic processes due to fungal infection, but also to peroxidase capacity to reduce small amounts of hydrogen peroxide from plant tissues, because peroxidase can act only in this

special conditions; the results obtained in this experiment can be compared with those observed in the case of wheat infected by different pathogenic fungi by Johnson et Cunningham and Flott *et al.* in the case of *Puccinia recondita* attack [12, 13], by Diani *et al.* in infection with *Septoria tritici* [14], by Patykowsky *et al.* in *Erysiphe graminis* attack [15], by Kerby et Somerville at barley infected with *Erysiphe graminis* f.sp. *horedi* [16]; the same dynamic of peroxidase activity was observed by Grzelinska et Sierakowska at tomatoes plants infected with *Verticiliium albo-atrum* [17] and by Reuveni et Ferreira who observed an increasing in peroxidase activity from tomatoes leaves infected with *Verticillium dahliae* [18]; by Reuveni et Bothma at *Cucumis melo* infected by *Sphaerotheca fuliginea* [19],

Catalase and peroxidase are two defensive enzymes which act synergistically and degradate the hydrogen peroxide to help to protect plant tissues against its toxic effect.

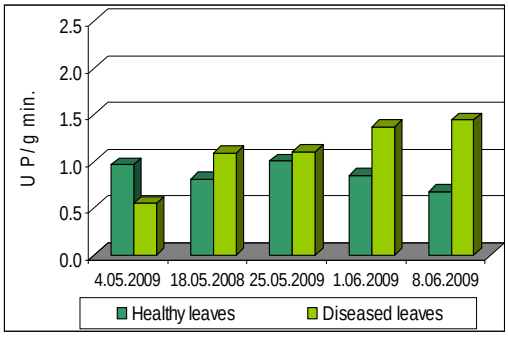

Figure 2 Peroxidase activity in healthy leaves and in leaves infected by *Taphrina deformans* at cv. *Cardinal*

In Fig. 3 are presented the results of the researches concerning the polyphenoloxidase activity in healthy and diseased leaves at cv. *Cardinal*.

The maximum polyphenoloxidase activity in healthy leaves, occurred at 18.05.2009 (4,2551 U PPO/g/min), followed in decreasing order by the values recorded at: 1.06.2009 (3,6176 U PPO/g/min), 8.06.2009 (3,4072 U PPO/g/min), 4.05.2009 (2,9285 U PPO/g/min) and at 25.05.2009 was recorded the smallest value- 2,7961 U PPO/g/min.

Polyphenoloxidase activity from diseased leaves recorded the highest value at 4.05.2009 - 9,1780 U PPO/g/min, followed in decreasing order by next values: 5,2439 U PPO/g/min (18.05.2009), 3,7319 U PPO/g/min (25.05.2009), 3,7313 U PPO/g/min (1.06.2009) and the smallest value- 3,6949 U PPO/g/min was recorded at 8.06.2009.

The dynamics of polyphenoloxidase activity recorded in diseased leaves at cv. *Cardinal*, in 2009, showed higher values compared with those observed in healthy leaves, the ratio Diseased/Healthy had the following values: D/H=3,1339 (4.05.2008), D/H=1,2323 (18.05.2009), D/H=1,3346 (25.05.2009), D/H=1,0314 (1.06.2009) and D/H=1,0844 at 8.06.2009.

Polyphenoloxidase activity was higher in diseased leaves at all periods of the study when compared with the activity recorded in healthy leaves. The higher activity of this oxidoreductare in diseased peach leaves is probably due to its role in the oxidation of phenolic compunds (which are toxic for the fungi) to quinones which are more reactive and more toxic to the pathogen. By increasing its polyphenoloxidase activity the host plant attempts to limit or to stop the pathogen penetration. The increased activity of polyphenoloxidase is accompanied at cv. *Cardinal* by an increased activity of peroxidase, this indicates the role of this two enzymes in disease resistance of peaches. In diseased leaves polyphenoloxidase activity is decreasing with the leaves age; in healthy leaves the enzyme activity recorded specific variation to one date to another and remains lower then the activity from attacked tissues.

The results obtained in this study are confirmed by those mentionated by El-Fiki *et al.* in the case of sugar beet leaves infected by *Uromyces betae* and they point out the role played by catalase, peroxidase and polyphenoloxidase which are implicated in defensive mechanisms of host plant against the fungal pathogen [20]; the results are similar with those also observed by Tyagi *et al.* at some wheat varieties with different degrees of susceptibility at the attack of *Alternaria triticina* [21]; increasing activities of polyphenoloxidase and peroxidase in response of the attack of *Alternaria tenuis* on *Rosa centifolia* leaves were observed by Khatun *et al.* (2009) [22]; Arun *et al.* observed the same dynamics of this enzymes activity at *Pennisetum glaucum* plants infected by fungus *Sclerospora graminicola* [9].

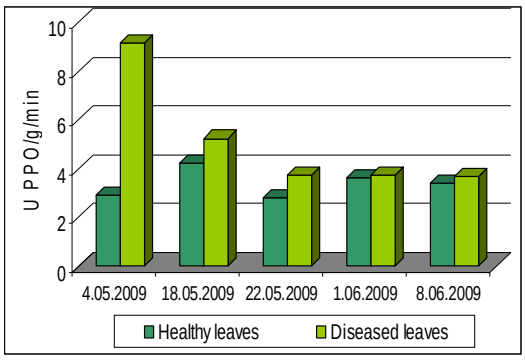

Figure 3 Polyphenoloxidase activity in healthy leaves and in leaves infected by *Taphrina deformans* at cv. *Cardinal*

4. Conclusion

The results recorded in this study showed that catalase, peroxidase and plyphenoloxidase activity was influenced by wheather contition, by the presence or the absence of *Taphrina deformans* attack and by the stage of infection.

Peroxidase activity was higher in diseased leaves then in the healthy ones; the researches indicated that catalase and peroxidase act synergistically to help to protect peach leaves against *Taphrina deformans* infection.

Results revealed that in cv. *Cardinal* polyphenoloxidase activity was higher in diseased leaves in all study periods; polyphenoloxidase activity in diseased leaves is decreasing at one time with the intensification of the attack of the pathogenic agent, probably due to the leaves ageing.

This study confirmed that catalase, peroxidase and polyphenoloxidase work together to protect plant tissues against oxidative stress induced by the attack of *Taphrina deformans* on *Persica vulgaris* leaves.

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