Effect of *Erwinia amylovora* infection on peroxidase enzyme activity in resistant apple cultivars

Kása, K.[1], Hevesi, M.[1], G.Tóth, M.[1] and Stefanovits-Bányai, É.[2]

[1] *BUESPA, Faculty of Horticultural Science, Department of Fruit Science 1118 Budapest, Villányi út 29–43., Hungary*

[2]*BUESPA, Faculty of Food Science, Department of Applied Chemistry 1118 Budapest, Villányi út 29–43., Hungary*

Summary: Two apple cultivars that display enhanced resistance to fire blight (causal agent: *Erwinia amylovora*) were selected. The aim of the present study was to characterize the peroxidase (POD) enzyme activity of 'Szemes alma' (a historical cultivar) and MR-03, (a Hungarian multiresistant hybrid of 'Prima') and compare them to susceptible 'Jonathan M 40' and resistant 'Remo' controls. Peroxides enzyme activity during *E. amylovora* infections was investigated in artificially infected apple shoots. Increases in enzyme activities were observed in a 'Jonathan M40' and in 'Remo', MR-03, 'Szemes alma' cultivars. There was a consistent relationship between total enzyme activity and fire blight disease severity. High activity of the peroxidase was positively correlated with the degree of resistance to fire blight. A general hypothesis that POD activity is related to fire blight susceptibility/resistance is supported by our results.

Key words: *Malus x domestica*, disease severity, fire blight, apple cultivar, multi-resistant selection, peroxidase activity

Introduction

Control of fire blight caused by *E. amylovora* (Burrill) Winslow et al. is a serious challenge in apple and pear orchards to farmers involved in fruit production. This devastating disease has appeared in Hungary in the spring of 1996 (*Hevesi,* 1996) and has been continuously spreading all over the country. The need for highly disease-resistant cultivars is more pressing than ever (*Psallidas & Tsiantos* 2000).

A new Hungarian apple breeding program in the Department of Fruit Science started at the beginning of the nineties is to widen the Hungarian apple assortment based on good quality and multiple resistance with excellent productivity and ecological capability to local growing areas (*G. Tóth* et al., 1994). The primary aim considering resistance is to reach a durable resistance against Hungarian types of apple scab caused by *Venturia inaequalis*, combined with resistance to powdery mildew (*Podosphera leucotricha*) and fire blight (*E. amylovora).* From the progenies of crosses in 1992, MR-03 was selected as a multiple resistant selection with high fruit quality (*G. Tóth,* 2003)

As the other part of this apple breeding program we have initiated research with the aim to screen domestic gene sources, mostly Hungarian historical apple cultivars (selected in Sub-Carpathia) for disease resistance (*G. Tóth* et al., 2004).

At present, numerous studies deal with abiotic and biotic stress research. The components examined most often are stress enzymes such as peroxidases (*Stefanovits-Bányai* et al., 1998/a, *Stefanovits-Bányai* et al, 1998/b, *Sárdi* et al., 2000, *Velich* et al., 2000). Several studies have been conducted on the involvement of antioxidant enzymes in plant-bacteria interactions (*Brisset* et al., 2000, *Keck* et al., 1999). Peroxidase polymorphism is also used in the identification of apple and pear cultivars (*Vinterhalter* et al., 1986, *Barnes,* 1993, *Manganaris & Alston,* 1993, *Fachinello* et al., 2000).

We want to know whether exist correlation between degree of resistance of apple cultivars to fire blight and changes of peroxidase activity associated with the disease development induced by inoculation of *E. amylovora.* The role of peroxidase as a possible basis of resistance to bacterium was investigated in apple shoot parts and leaves.

Material and method

Fire blight resistance

Experiments have been carried out with the cultivars MR-03, 'Szemes alma', 'Jonathan M40', and 'Remo'. Test plants were grafted on M9 rootstocks with 8 or 10 replicates and potted in the spring of 2001, 2002 and 2003. Cultivar 'Jonathan M40' was used as susceptible control, while cultivar 'Remo' were chosen as resistant control (*van der Zwet,* 1995; *Fischer,* 2000; *Richter & Fischer* 2003, *Fischer & Fischer* 2003).

Standard Hungarian *Erwinia amylovora* strains (isolated from apple) were used in the present experiment. Method of virulence test, shoot inoculation and evaluation of data

(speed of disease development and disease severity) are described in the paper of *Kása* et al. (2004), printed in the present journal.

Peroxidase activity

Plant samples

Shoot sample was taken from a 5 cm length of stem 2 cm above and below the inoculation site. Leaf sample was taken from area adjacent to the inoculation site. Sampling was done one week after inoculation by the bacterium.

Sample preparation for determination of peroxidase activity

Approximately 500 milligram sample was homogenized in 2 ml ice cold extraction buffer (20 mM Tris-HCl buffer pH 7,8 containing 10% glycerol, 10% Triton X-100, 5% PEG 4000, 1% NaCl). The crude extract was centrifuged at 1500 g and 4 °C for 15 minutes. Supernatants were used for further analysis (*Stefanovits-Bányai* et al., 1998).

Determination of peroxidase activity

Peroxidase (POD) enzyme activities were measured by a spectophotometric method (*Shannon* et al., 1966).

Results and discussion

Fire blight resistance

Total shoot length and length of the diseased part was measured and the percentage of blighted shoot length was determined (*Figure 1*). Disease severity on two cultivars and two control (resistant, susceptible) cultivars was evaluated. According to the above mentioned data apple cultivars were characterized in *Table 1*.

Typical fire blight symptoms have occurred on susceptible 'Jonathan M40' cultivar which agreed with literature data. The resistant control 'Remo' showed minimal or no visual symptoms. The cultivar MR-03 and 'Szemes alma' seemed moderately resistant because disease symptoms were not observed.

Based on our results, those cultivars that display moderate resistance could be used as sources of fire blight resistance.

Preliminary results of peroxidase activity

The changes of peroxidase (POD) enzyme activity in stems and leaves of different apple cultivars are shown in *Figure 2* and *Figure 3*. The basis of comparison is 'Jonathan M40' was found as a very susceptible and 'Remo' as a resistant cultivar to fire blight. Following the controls we present the results of MR-03 and 'Szemes alma'.

Comparing the samples from uninfected stems of each cultivar, the enzyme activity in 'Jonathan M40' was lower then in 'Remo'. The activity of MR-03 was found to be

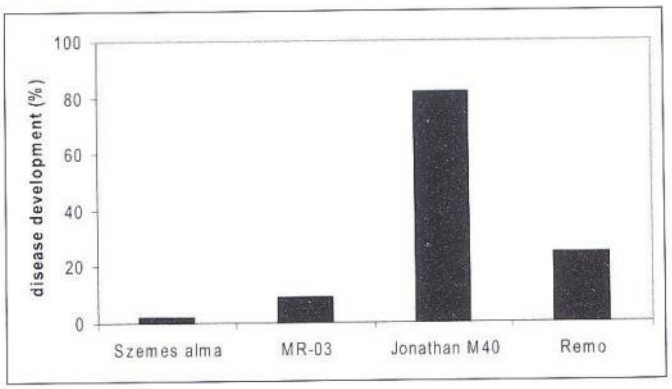

Figure 1 Fire blight susceptibility of apple cultivars on 28th days after inoculation (Disease development, %)

Table 1 Fire blight susceptibility of apple cultivars (2001–2003)

Cultivar	Disease development			Disease severity		
	2001	2002	2003	2001	2002	2003
Szemes alma	–	MR	MR	–	MR	MR
MR–03	–	MR	MR	–	MR	MR
Jonathan M40	–	S	S	–	S	S
Remo	MR	MS	MR	MR	MS	MR

intermediate position in comparison to values observed in susceptible and resistant cultivars, while the enzyme activity of 'Szemes alma' was lower then that of susceptible one. Following bacterial infection POD activity increased, which agrees with literature data on other plant species (*Sárdi* et al., 2000, *Velich* et al., 2000). By the evaluation of resistant cultivars, MR-03 had the highest enzyme activity. The POD activity of 'Szemes alma' was lower then that of 'Remo' but higher than that of 'Jonathan M40'. The enzyme activity of infected MR-03 was two times higher than that of uninfected MR-03, while the activity in 'Szemes alma' was one and a half times higher than that of its control sample.

The POD activity measured in uninfected leaves was lower than in stems in each cultivar. Uninfected 'Remo' had a higher POD activity than 'Jonathan M40'. We found the largest difference between 'Jonathan M40' and 'Remo'. The values of MR-03 and 'Szemes alma' were between 'Jonathan M40' and 'Remo'. After bacterial infection only the resistant cultivars showed remarkable increases in enzyme activity in MR-03 displaying the highest values. As with the results obtained in stems, the POD activity of MR-03 was two times higher in infected samples than in uninfected samples.

Summarizing these results it can be concluded that the effect of *E. amylovora* infection caused typical biochemical changes in apple cultivars concerning peroxidase activity. Based on the results obtained with resistant cultivars MR-03 was shown to be the most remarkable. The measured enzyme activities reflect the differences of apple cultivars in susceptibility to fire blight. The increased POD enzyme activity induced by *E. amylovora* infection could play a role in defense mechanisms in these cultivars. We conclude that this hybrid displays an enhanced resistance to fire blight. Further research is needed to elucidate additional biochemical changes associated with fire blight resistance.

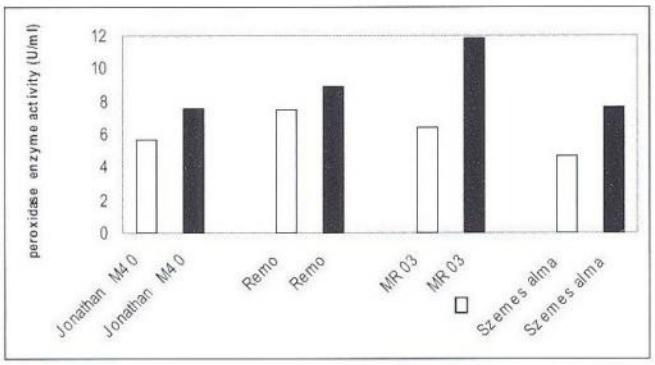

Figure 2 Peroxidase enzyme activity (U/ml) in apple stem on 7th days after inoculation by *Erwinia amylovora* (Ea2, Ea60, Ea67) (white column: uninoculated, black column: inoculated)

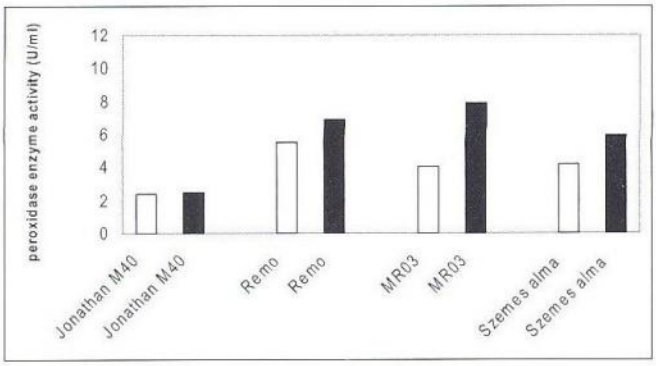

Figure 3 Peroxidase enzyme activity (U/ml) in apple leaf on 7th days after inoculation by *Erwinia amylovora* (Ea2, Ea60, Ea67) (white column: uninoculated, black column: inoculated)

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