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Fire blight *(Erwinia amylovora)* susceptibility of old Hungarian apple cultivars

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Summary: The aim of the Hungarian apple breeding program started in the Department of Fruit Science was to find resistant apple cultivars against major diseases (scab, powdery mildew, fire blight). The outbreak of fire blight (*Erwinia amylovora*) in 1996 motivated us to search new resistant sources principally from old traditional apple cultivars. First of all, cultivars have been gathered since 1997 from Carpathia (Visk) and evaluated between 2001 and 2003. In this recent study evaluation of resistance of old Hungarian genotypes to fire blight collected from the English National Fruit Collection (Brogdale, Faversham) is presented.

13 old Hungarian apple cultivars in 2002, and 38 genotypes in 2005 have been evaluated. We used 'Idared' and 'Jonathan M41' as susceptible controls and 'Liberty' and 'Remo' as resistant ones. Shoots of two-year-old potted plants were inoculated with a mixture of virulent *E. amylovora* isolates (Ea2, Ea60, Ea67) at a concentration of 5 x 10^8 cells/ml. Resistance of apple cultivars was evaluated weekly, four times after inoculation by disease severity of symptoms. Numbers of bacterial colonies in 1 cm length shoot were determined in the fourth week after infection.

8 cultivars in 2002 and 9 cultivars in 2005 displayed notable resistance to fire blight based on one-year data. Based on the coincident data of both years, out of the cultivars collected also from Carpathia 'Pónyik', and 'Sikulai' were found to be resistant and gene sources additional old Hungarian valuable apple genotypes could be selected: 'Szabadkai szercsika' and 'Tordai piros kálvil'. The cultivar 'Szemes alma' originated from Visk has proved to be recurrently resistant.

The number of bacterial cells in shoots of the investigated cultivars correlated with the severity of symptoms. With this method, which was introduced by us earlier, we can screen cultivars displaying weak visible symptoms, which cannot be proposed as a source of resistance because of their latent infection.

Key words: *Malus x domestica*, old cultivars, fire blight, shoot susceptibility, apple

Introduction

In the breeding program of the Department of Fruit Science, resistance for several diseases (scab, powdery mildew, fire blight) and high quality have been aimed to be incorporated into apple cultivars. Initial results are published already (*Tóth* et al., 2004). Fire blight (*Erwinia amylovora* (Burrill) Winslow et al.) appeared in Hungary in 1996, since then sources of resistance have been spotted and utilised as being available in old, traditional local apple cultivars. The collection and testing of apple accessions started in 1997 (*Tóth* et al., 2005a). Since 2001, regular greenhouse tests are made with artificial inoculation of the bacterium *Erwinia amylovora* on local cultivars collected in the Sub-Carpathian region (community of Visk) (*Kása* et al., 2002). The results of several years revealed three cultivars being precious sources of resistance ('Ponyik', 'Szemes alma' és 'Sikulai') (*Tóth* et al., 2005b).

From the Hungarian national collections of cultivars, some local cultivars disappeared already but they have been maintained, fortunately, in the British National Collection as old Hungarian cultivars. Going back to those and other introduced genotypes, a systematic search for resistance – first of all to fire blight – ensued. The present account deals with the results of greenhouse tests performed beween 2002 and 2005.

Material and method

Plant material

Old Hungarian cultivars involved in examination (*Table 1*) were imported from the National Fruit Collection, Brogdale (Faversham, Kent, England). As susceptible control, 'Idared' and 'Jonathan M40' (*van der Zwet & Beer*, 1995), as well as 'Liberty' and 'Remo' scab resistant cultivars proved resistant by *Fischer & Richter* (1996) were used. For examining susceptibility of shoots, the material was propagated by shield-grafting to M9 rootstock, and four test plants per each cultivar were forced in containers in a greenhouse at the end of winter.

Inoculum and inoculation

The bacterium was first isolated in Hungary by *Hevesi* (1996) after the infection appeared in the field. For the present infections, we used a mixture of three bacterium strains of different origin selected from our gene bank by virulence test (Ea2, Ea60 and Ea67), which represented the probably different *E. amylovora* populations of the Carpathian basin. Short term conservation can be carried out by keeping

them in 1% preservation liquid at -18 °C, while long term conservation is by lyophilization. The concentration of the suspension used for infections was – 5×10^8 c/u ml^{-1}.

Our examinations to determine resistance to fire blight were carried out in of the Department of Fruit Science (laboratory, greenhouse). In *in vitro* examinations, 25–30 cm shoots of grafts were inoculated using hypodermic needles with a dose of approx. 100 ìl/pinhole, for evaluating shoot susceptibility. The bacterium suspension was placed in the third leaf-axil counted from the apex, then the plants were covered with a transparent plastic foil to ensure high humidity optimal for bacterium development. The necrotized shoot part was measured after four weeks.

Evaluation

Evaluation was realized on the basis of three different methods:

(*1*) Necrotization of the shoot was expressed as the percentage of its total length (*Le Lezec and Paulin*, 1984; *Le Lezec* et al., 1987) weekly after inoculation. Resistance classes were defined as follows: MR (moderately resistant): 0–30%; MS (moderately susceptible): 31–70%; S (susceptible): 71–100%.

(*2*) The severity of fire blight symptoms (disease rating) was rated according to a scale of 0–5 (index of infection) (0 = symptomless, 5 = completely infected). The disease rating DR_s on shoots is expressed by the formula: $DR_s = \Sigma f_i * n_i/n$, where f_i = scale value (index of infection); n_i = frequency referring to the infection index; n = number of plant parts examined within the respective cultivar. Resistance classes (on the basis of DR) were as follows: MR: 0–2; MS: 2,1–3,5; S: 3,6–5 (*Hevesi* et al., 2000).

(*3*) Determination of bacterial cell numbers inside the shoot derived from a 1 cm length of stem (3 cm below the inoculation site) was carried out by counting colony numbers on Nutrient agar plates. Resistance classes were calculated as follows: MR: $\leq10^6$ cfu/1 cm shoot; MS: 10^6–10^7 cfu/1 cm shoot; S: $10^{7>}$ cfu/1 cm shoot.

Statistical data obtained for susceptibility to the bacterium *Erwinia amylovora* were evaluated using cluster-analysis.

Results

Susceptibility of shoots was checked by three different methods. The severity of disease symptoms has been judged on a numerical scale presented in *Table 1*. The indices of susceptibility could be processed statistically, and the dendrogram of data reveals the relation of cultivars belonging to individual groups of susceptibility *(Figure 1)*. Intensity of multiplication of the bacterium *Erwinia amylovora* on the different hosts is presented in *Figure 2*.

During the first year, 2002, the tests of inoculation comprised the standard cultivars ('Idared', 'Jonathan M41', 'Liberty' and 'Remo') and thirteen of the revived old Hungarian cultivars. The preliminary results called our attention to the cultivars 'Szabadkai szercsika', 'Damjanich' and 'Tordai piros kálvil' being remarkably better than the rest according to the symptoms on the shoots. However, 'Szabadkai szercsika' did not show visible symptoms on the shoots at all, the multiplication of the bacterium indicated a moderately susceptible character.

In 2005, tests included beyond the four standard cultivars 39 old genotypes. Out of the three methods (indices of susceptibility, degree of sickness, multiplication of bacteria) at least two produced proofs of remarkable resistance in nine cultivars: 'Citrom alma', 'Fekete tányéralma', 'Hamvas alma', 'Herczeg Batthyány alma', 'Kéresi muskotály', 'Orbai alma', 'Ponyik', 'Sikulai', 'Szabadkai szercsika'. Repeated tests are needed to confirm the value of those

Table 1 Disease rating of shoots after inoculation by the bacterium *Erwinia amylovora*

Genotypes	Disease rating (0–5)	
	2002	2005
Bánffy Pál		4.13
Bereczki Máté		2.75
Budai Ignác		4.83
Búzával érő		4.25
Cigány alma		2.50
Citrom alma		0.80
Csíkos óriás halasi		2.94
Damjanich	1	
Dániel féle renet		2.40
Daru sóvári		2.38
Desseffy Arisztid		5.00
Entz rozmaring	1	
Édes escoar		4.20
Fekete tányéralma	2.25	2.20
Gomba Károly		4.80
Gyógyi piros		3.55
Hamvas alma		0.18
Harang alma	2	2.14
Hejőcsabai sárga		5.00
Herceg Batthányi alma		0.38
Hosszúfalusi	2.25	5.00
Jászvadóka		3.78
Kéresi muskotály		0.73
Marosszéki piros páris		2.80
Máté Dénes		3.45
Miskolci kormos		4.00
Nemes szercsika		4.07
Orbai alma		1.67
Ponyik	1.0	0.00
Pusztai sárga	1.6	4.20
Sikulai alma	2	1.00
Simonfy piros	1.7	
Szabadkai szercsika	0	0.75
Szászpap alma	3.25	3.88
Széchenyi renet	2.25	4.20
Szemes alma	0.2	1.38
Tordai piros kálvil	1.25	0.40
Tükör alma		2.67
Vajki alma	2	3.00
Idared	5.0	4.31
Jonathan M41	4.5	3.00
Liberty	1.2	2.67
Remo	3.6	1.85

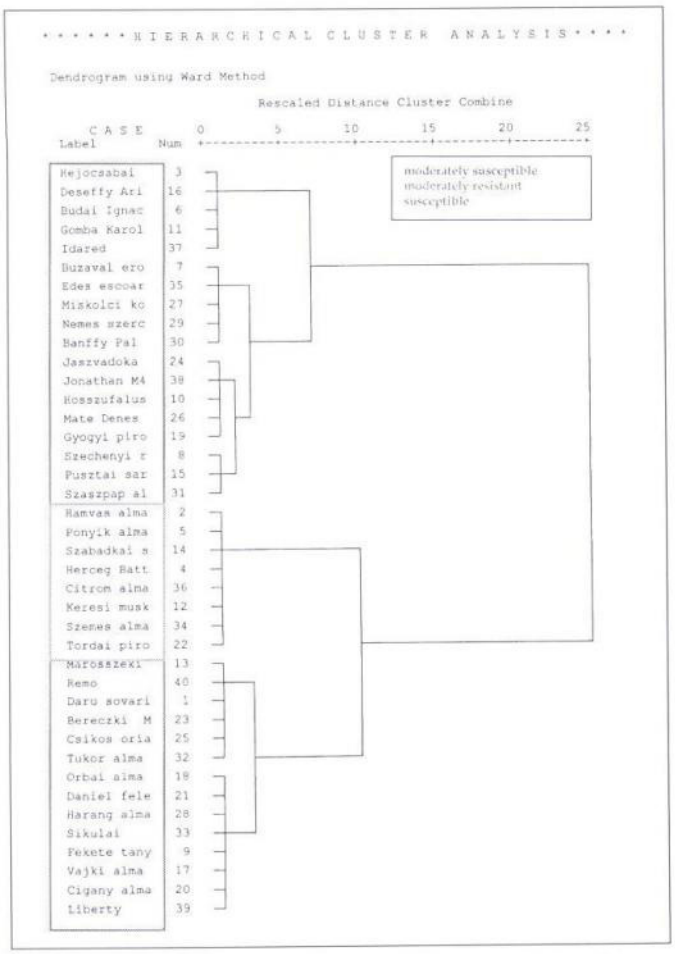

Figure 1 Dendogram indicating the susceptibility index of infected shoots

cultivars as useful sources of resistance. Some of those nine cultivars proved in both years as being resistant according to the performance of their shoots.

Statistically processed data of one or two years, the susceptibility indices presented in the dendrogram (*Table 1*) expressed hopeful signs of resistance. Coincident data of two years diagnosed the cultivars 'Pónyik' and 'Sikulai' of Sub-Carpathian origin as precious sources of resistance, in addition to two other genotypes: 'Szabadkai szercsika' and 'Tordai piros kálvil', moreover, the Sub-Carpathian 'Szemes alma' also.

Data expressing the intensity of sickness (*Table 1*) confirmed essentially those obtained by tests of the shoot symptoms. The counts of the bacterial cells (*Figure 2*) called our attention to the low numbers first of all in 'Hamvas alma', 'Kéresi muskotály' and 'Pónyik', nevertheless, the data of two successive years presented in some cases high cell numbers in cultivars earlier recognised by other tests as being relatively resistant.

The integrated results of the three methods of testing assigned the resistant cultivars into three groups (*Table 2*), where the resistance of individual cultivars is also comparable with the standard cultivars.

Conclusions

A widely recognised opinion states that the greatest moderation of damages could be achieved most by the adoption of less susceptible fruit cultivars (e.g. *Le Lezec* et al., 1987, *Richter & Fischer*, 2000, *Holb*, 2005). 'Remo' and 'Liberty' are resistant cultivars utilised as checks as well as sources of resistance in cross breeding programs as proved also by *Fischer & Richter* 1996, *Richter & Fischer* (2000), and *Lespinasse & Paulin* (1990). 'Remo' showed in our tests moderate resistance to bacterial strains prevailing in Hungary, whereas some genotypes of Hungarian origin exhibited much better resistance.

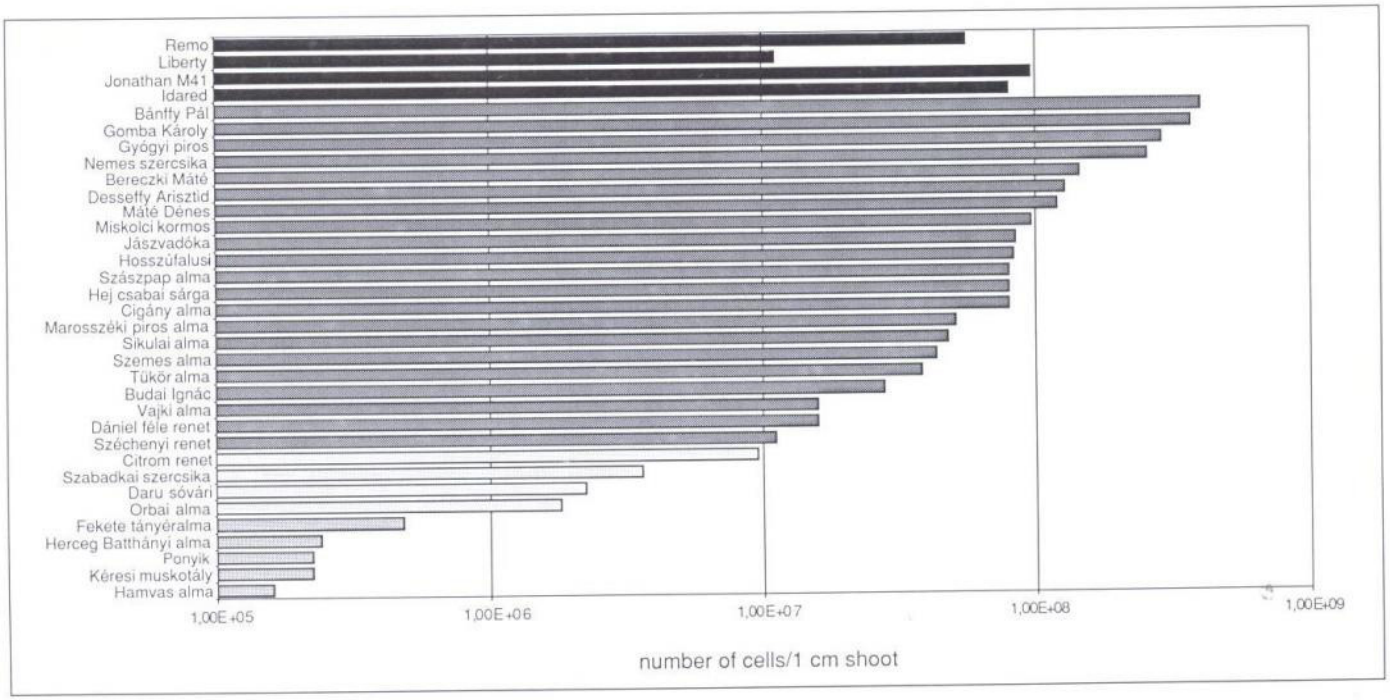

Figure 2 Bacterium cell number of shoots after artificial infection by *Erwinia amylovora* (2002 and 2005)

Table 2 Fire blight susceptibility of Hungarian apple genotypes imported from the English Fruit Collection, on the basis of evaluation of shoot infection data

Genotypes	Necrotization of the shoots		Disease rating		Bacterium cell numbers	
	2002	2005	2002	2005	2002	2005
Pónyik	MR	MR	MR	MR	MR	MR
Tordai piros kálvil	MR	MR	MR	MR	MR	
Szemes alma	MR	MR	MR	MR	MR	MS
Sikulai alma	MR	MR	MR	MR	MR	MS
Szabadkai szercsika	MR	MR	MR	MR	MS	MS
Vajki alma	MR	MS	MR	MS	MS	MS
Hamvas alma		MR		MR		MR
Kéresi muskotály		MR		MR		MR
Herceg Batthányi alma		MR		MR		MR
Damjanich	MR		MR		MR	
Citrom alma		MR		MR		MS
Entz rozmaring	MR		MR		MS	
Fekete tányéralma	MS	MS	MS	MS	MR	MR
Harang alma	MR	MS	MR	MS	S	
Orbai alma		MS		MR		MR
Cigány alma		MS		MS		MS
Dániel féle renet		MS		MS		MS
Daru sóvári		MS		MS		MS
Csíkos óriás halasi		MS		MS		
Bereczki Máté		MS		MS		S
Marosszéki piros páris		MS		MS		
Gyógyi piros		S		MS		S
Hosszúfalusi	MS	S	MS	S	S	S
Pusztai sárga	MS	S	MR	S	MS	
Széchenyi renet	MR	S	MS	S	MS	MS
Szászpap alma	MS	S	MS	S	MS	
Máté Dénes		S		MS		S
Tükör alma		MS		S		
Simonffy piros	S		MS			
Jászvadóka		S		S		MS
Budai Ignác		S		S		MS
Búzával érő		S		S		
Miskolci kormos		S		S		S
Nemes szercsika		S		S		S
Desseffy Arisztid		S		S		S
Bánffy Pál		S		S		S
Édes escoar		S		S		S
Gomba Károly		S		S		S
Hejőcsabai sárga		S		S		
Idared	S	S	S	S	S	S
Jonathan M41	S	MS	S	MS	S	S
Liberty	MR	MS	MR	MS	MR	MS
Remo	MS	MS	MS	MR	MS	MS

MR = moderately resistant, MS = moderately susceptible, S = susceptible
*: originated from Carpathia

The Sub-Carpathian and old Hungarian cultivars recognised as sources of resistance are a novelties. The cultivars of moderate resistance, as 'Szemes alma', 'Pónyik' and 'Sikulai', moreover 'Szabadkai szercsika' and 'Tordai piros kálvil' are clearly recognised as useful sources of resistance to *Erwinia amylovora* in breeeding programs. Some of them exhibit good fruit quality and fairly good resistance to fungal diseases (*Tóth* et al., 2005c) under environmentally conscient growing conditions (e.g. home gardens or ecological growing techniques), consequently, they could be recommended for the purpose of replanting or for orchards.

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