

Historical apple cultivars that display high level of resistance to fire blight

Kása K., G. Tóth M. and Hevesi M.

BUESPA, Faculty of Horticultural Science, Department of Fruit Science
1118 Budapest, Villányi út 29-43., Hungary

Summary: Following the first outbreak of fire blight caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al. in Hungary, we have started research with the aim to screen domestic gene sources, mostly historical Hungarian apple cultivars, for disease resistance as part of an apple breeding program for multiple resistance. The present study was conducted with the aim to choose the most tolerant historical apple cultivars among 25 selected cultivars by screening their behavior towards fire blight under controlled conditions. Six cultivars were shown to be the most disease tolerant: 'Batul', 'London pepin', 'Nemes Sívári Alma', 'Sikulai', 'Szemes alma', 'Vilmos renet'. We evaluated these cultivars by investigating both morphological- characteristics under original environmental circumstances and fruit quality. The cultivars had a remarkable degree of fire blight resistance compared to the control cultivars. These were not competitive with the commercially grown 'Jonathan M40' during cultivar tests but on the basis of certain characteristics they could serve as genetic sources for breeding new varieties.

Key words: bacterial multiplication, cultivar characteristics, disease severity, *Erwinia amylovora*, soluble solids, Sub-Carpathia, titratable acid content

Introduction

Apple is one of the most important fruit crops in Hungary. International and national experience shows, that the main apple cultivars are susceptible to the bacterium *Erwinia amylovora*. So far there is no chemical available that confers complete protection against the bacterium and the use of antibiotics has resulted in development of resistant strains (Psallidas & Tsiantos 2000, Jones & Schnabel 2000) and the other hand streptomycin will be discontinued in the European market. An important feature of integrated pest management, the use of highly disease-resistant cultivars seems to be the only way for solving this pathological problem. Genetic resistance is more often identified in primitive species or in historical cultivars with mediocre appearance and quality (Lespinasse & Aldwinckle 2000).

Following the appearance of fire blight caused by the bacterium *E. amylovora* (Burrill) Winslow et al. in Hungary (Hevesi, 1996) we have started research with the aim to find new gene sources of disease resistance with improved horticultural qualities for breeding new apple cultivars (G. Tóth et al. 1994). Since 1997 several collecting trips have been organized to Sub-Carpathia (Visk, Ukraine) on the North-East of the Hungarian border for collecting old local cultivars in order to conserve them for posterity. In this area, which was previously a part of Hungary, numerous extensive orchards can be found in the mountains. By these fieldtrips more than 20 local apple cultivars have been collected in order to conserve them for posterity and, following disease resistance evaluations, make them available for breeders and geneticists (G. Tóth et al., 2004).

Previously – in 2001, 2002 and 2003 – twenty five apple cultivars were screened for their behavior towards fire blight under controlled conditions in our laboratory (Kása et al., 2002). In this study, the six apple cultivars, which were resistant to fire blight, are described and compared to susceptible and resistant controls. The selected resistant cultivars were the following: 'Batul', 'London pepin', 'Nemes Sívári Alma', 'Sikulai', 'Szemes alma', 'Vilmos renet'. Cultivar 'Jonathan M40' and 'Idared' were used as susceptible controls, while 'Liberty' and 'Remo' were used as resistant controls. An additional aim of our study was to evaluate these resistant cultivars by their quality-related morphological-, principal physical- and chemical characteristics.

Material and method

Fire blight resistance

Test plants

Experiments in 2001 were conducted with the cultivars 'Batul', 'Sikulai', 'Vilmos renet', 'Idared' and 'Remo'. In 2002 we evaluated the cultivars 'Batul', 'Nemes Sívári Alma', 'Sikulai', 'Szemes alma', 'Vilmos renet', 'Idared', 'Jonathan M40', 'Liberty' and 'Remo'. In 2003 'London pepin' (syn. London pippin), 'Nemes Sívári Alma' (syn. Sovari nobil), 'Szemes alma', 'Idared', 'Jonathan M40', 'Liberty' and 'Remo' were evaluated.

Test plants were grafted on M9 rootstocks with 8 or 10 replicates and potted in the spring. Cultivars 'Idared' and 'Jonathan M40' were used as susceptible controls, while

cultivars 'Liberty' and 'Remo' were chosen as resistant controls (van der Zwet, 1995; Fischer, 2000 Richter & Fischer 2003, Fischer & Fischer 2003).

Bacterial strains

Strains of *E. amylovora* are from the collection of BUESPA, Faculty of Horticultural Science, Department of Fruit Science, and were isolated from apple during different seasons and from different sites of Hungary (Table 1). The isolates were cultivated on King's B medium and stored in 1% glycerol at -18°C or lyophilized for long term storage.

Table 1 Hungarian *Erwinia amylovora* (apple) strains used in mixture for shoot inoculation

Strain	Origin	Date
Ea2	Nyárlőrinc	1996
Ea60	Érd	2000
Ea67	Monostorpályi	2000

Virulence test

Immature (fire blight susceptible) pear fruits were needle-inoculated with bacterial suspension (5×10^8 cell/ml). Control fruits were treated with sterile distilled water. Fruits were maintained in plastic boxes lined on the bottom with moist paper towels. Isolates that produced extensive water-soaking accompanied by bacterial ooze within 3 days were considered to be virulent (Figure 1).

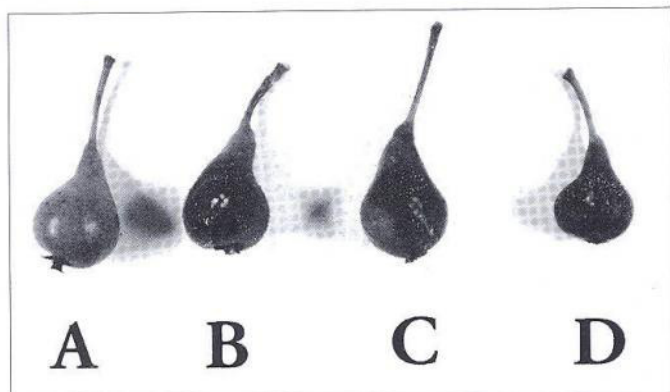


Figure 1 Virulence test of selected *Erwinia amylovora* isolates (Budapest, 2001) (A) Control; (B) Ea2; (C) Ea60; (D) Ea67.

Inoculation

The experiment was done in our 'Erwinia amylovora Laboratory' which is supplied with safety devices preventing the bacteria to spread outside. Inoculation was carried out on vigorously growing shoots of 25–35 cm in length by a hypodermic syringe (Lespinasse & Paulin, 1990; Maroofi & Mostavafi, 1996).

Inocula of 5×10^8 cells/ml concentration were made from an overnight culture grown in King's B medium. A mixture of three highly virulent Hungarian *E. amylovora* isolates was used (Aldwinckle et al. 1984, Lespinasse & Paulin 1990).

After inoculation the shoot tips were covered with plastic to provide high humidity during the incubation period.

Methods of evaluation

Speed of disease development: Necrotization of the shoot was expressed as the percentage of its total length (Le Lezec & Paulin, 1984, Le Lezec et al., 1987) weekly after inoculation. Resistance classes were defined by modifying the proposal of Gardner et al. (1980). MR—moderately resistant: 0–30 %; MS—moderately susceptible: 31–70 %; S—susceptible: 71–100%.

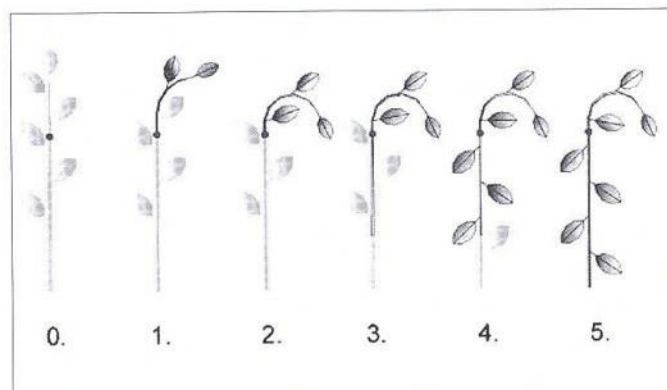


Figure 2 Fire blight disease scale (dot: inoculation site) (Illustration: Stéhlí, Zoltán)

Disease severity (disease rating): Data were evaluated by calculation of DR (Disease rating), described by Bertrand and Gottwald (1978) and modified by Hevesi et al. (2000).

$$\text{Disease rating} = \frac{\sum [(N1 \times 1) + (N2 \times 2) \dots (N5 \times 5)]}{\sum N}$$

where N1 – 5: Number of plants in each disease category. (Disease categories are shown in Figure 2)

Resistance classes (on the basis of DR): MR: 0–2; MS: 2,1–3,5; S: 3,6–5.

Bacterial multiplication: Numbers of bacterial colonies on agar plates were counted from samples derived from a 1 cm length of stem 3 cm below the inoculation site. Resistance classes on the basis of bacterial cell numbers were as follows: MR: $0-10^6$ cells/1 cm shoot; MS: 10^6-10^7 cells/1 cm shoot; S: 10^7-10^8 cells/1 cm shoot.

Cultivar characteristics

The selected trees were evaluated at blossoming time and in the middle of summer in 2003. Morphological characteristics (such as blossoming vigor, leaf characteristics etc.) and pathological behavior (such as that to *Podospheera leucotricha*, *Venturia inaequalis* etc.) were evaluated.

Fruit samples were collected over a period of the vegetative season. The following cultivars were sampled: 'Batul', 'London pepin', 'Nemes Sívári Alma', 'Sikulai', 'Szemes alma', 'Vilmos renet'. 'Jonathan M 40' was the control cultivar. A 15-fruit sample was selected at random and returned to the laboratory from each cultivar.

Fruit size was determined by measuring the diameter of the fruit to the nearest millimeter at the equator. Weight was determined by grams. The shape was evaluated on each fruit according to the characteristics of the cultivar: oblate, globular, conic, cylindro, cone-frustum type, bell-shaped. A Magness-Taylor hand penetrometer was used to measure firmness. Measurements were done on the blush and the non-blush sides with skin removed. The average of the two maximum force measurements was used as the firmness value for each fruit. Blush surface was determined visually and recorded as percentage of the total surface.

Juice content was calculated on a juice-to-weight basis (ml/g), which was used for further analyses. Soluble solids were determined from a sample of well-mixed juice using a hand refractometer with a range of 0% to 45%. Titratable acid content was determined by titrating 10 ml well-mixed juice with 0.1M NaOH to the phenolphthalein end point (pH=8.8). Titratable acid content was expressed as percentage of malic acid.

For statistical analysis, the average value of the fruits was used as one sample.

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 3). Following evaluation of disease severity bacterial cell numbers were counted on historical cultivars and two control (resistant, susceptible) cultivars under laboratory circumstances (Figure 4.).

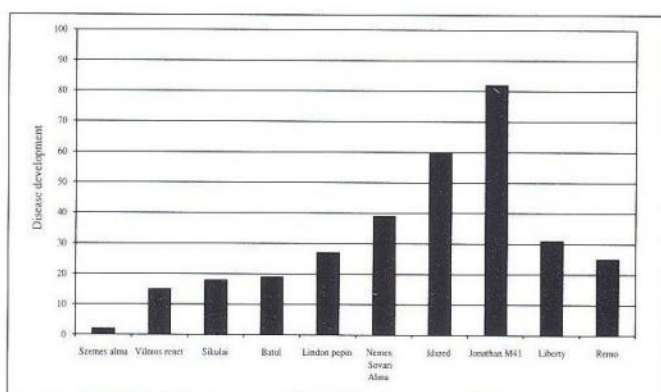


Figure 3 Fire blight susceptibility of apple shoots on the 28th days after inoculation (Disease development, %)

Fire blight symptoms have occurred on almost all of the inoculated shoots of susceptible control cultivars. After four days of incubation, shoots first appeared water soaked, then they wilted rapidly, later turned brown to black, displaying the very typical 'shepherd's crook' symptom. As the infection has spread down the shoot axis, leaves also became affected, first showing dark streaks in the midveins, and then they wilted and turned brown. These infections often

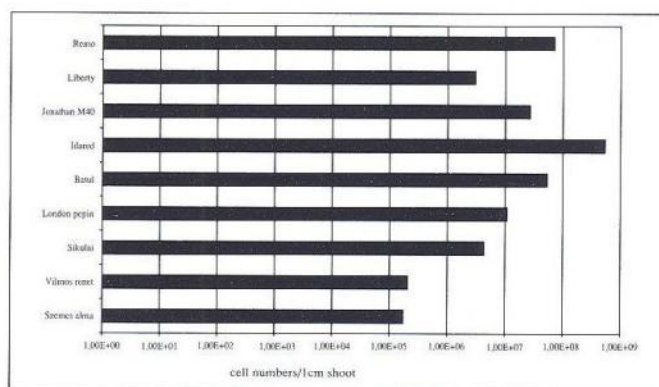


Figure 4 Fire blight susceptibility of apple shoots on the 28th days after inoculation (bacterial multiplication)

produced dripping ooze drops resulting in the formation of long, thin strands.

Behavior of (susceptible and resistant) control cultivars in response to *E. amylovora* inoculation agreed with literature data.

The cultivar 'Batul', 'London pepin', 'Nemes Sovári Alma', and 'Vilmos renet' were moderately resistant, whereas 'Sikulai', and 'Szemes alma' were the most resistant (Table 2.). Disease symptoms were not observed on cultivar 'Szemes alma' but host tissues contained considerably high numbers of *E. amylovora* cells. This illustrates that symptom severity does not necessarily correlate with bacterial multiplication in the host. Therefore the revision of bacterial cell number in the plant tissue is especially important during evaluation of cultivars or breeding material for fire blight resistance in order to exclude latent infections.

Based on our results those cultivars that display a very high or moderate resistance could be used as sources of fire blight resistance in breeding programs and also grown in organic orchards, where chemical plant protection is very limited or not at all applied.

Cultivar characteristics

The blooming time of the selected cultivars was recorded from the end of April to the beginning of May. 'Vilmos renet' was the earliest on the basis of full blooming period. We measured the longest shoot development period at cultivars 'Sikulai' and 'Vilmos renet'. The cultivars mostly have medium fruit dropping characteristics. Fruits ripened from the end of September to the beginning of October. (Table 3)

The largest fruits size developed on 'Vilmos renet' and 'Batul'. 'Sikulai' and 'London pepin' had the highest juice content. The measured values of the fruit quality are coincident with our earlier experiences (G. Tóth et al., 2004). The highest titratable acid content was determined at 'Batul', and the lowest was measured at 'Nemes Sovári Alma'. The highest firmness was measured at 'Nemes Sovári Alma', and the lowest firmness at 'Szemes alma'. 'Batul', and 'Sikulai' had considerably high soluble solid contents. The blush surface of 'Sikulai', and 'Vilmos renet' had an outstanding cover. (Table 4)

Table 2 Fire blight susceptibility of apple cultivars by shoot infection (2001–2003)

Cultivar	Speed of disease development			Disease severity			Bacterial multiplication		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
Batul	MR	MR	–	MR	MR	–	MS	MR	–
London pepin	–	–	MR	–	–	MR	–	–	MR
Nemes									
Sóvári Alma	–	MR	MR	–	MR	MR	–	MR	MR
Sikulai	MR	MR	–	MR	MR	–	MR	MR	–
Szemes alma	–	MR	MR	–	MR	MR	–	MR	MR
Vilmos renet	MR	MS	MR	MR	MS	MR	MS	MS	–
Jonathan M40	–	S	S	–	S	S	–	S	S
Idared	S	S	S	S	S	S	S	S	S
Liberty	–	MR	MR	–	MR	MR	–	MR	–
Remo	MR	MS	MR	MR	MS	MR	MS	MS	–

(MR–moderately resistant, MS–moderately susceptible, S–susceptible)

Table 3 Growing and morphological characteristics of the trees (2003)

Cultivar	Density of flowers	Fruit set (0–3)	Canker (0–3)	Apple scab (0–3)	Powdery mildew (0–3)	Growth (dwarf, medium, vigorous)	Canopy (upright, spreading, dooping)	Leaf size (1–3)	Thickness of shoot (1–3)
Batul	3	2	2	2	0	m.	s.	2	1
London pepin	1	2	2	2	0	m.	s.	2	
Nemes									
Sóvári Alma	3	2	0	1	0	m.	s.	2	2
Sikulai	2	2	2	1	0	d.	s.	3	2
Szemes alma	2	2	0	2	0	v.	d.	2	2
Vilmos renet	3	2	0	1	0	d.	s.–d.	3	2

These historical apple cultivars are not competitive beside the commercially grown cultivars, but on the basis of certain pomological characteristics and the resistance to fire blight, they could serve as genetic sources for breeding new varieties.

Finally, we summarize the characteristics of these historical cultivars by the description of *G. Tóth* (2001) and *Balikó* (2000):

'Batul': It was discovered in an area of the Maros River in Transylvania, Romania. It has been described as very attractive, of quite large size, crisp, sweet, low acid, very juicy with pleasant aroma. Fruits have short stems. It has a good sugar/acid balance and is juicy with white flesh. Trees are productive, upright and globular type and crop regularly every year but have biennial bearing. Fruits are medium in size, symmetrical, globular oblate shaped. The surface is clean, glossy yellow with an attractive red blush.

Table 4 Internal characteristics of the fruits (2003)

Cultivar	Juice content (%)	Soluble solid content (%)	Titrateable acid content %	Firmness (kg/cm ²)
Batul	–	14.3	0.9	8.3
London pepin	50	12.4	0.6	6.9
Nemes Sóvári Alma	–	12.7	0.4	8.9
Sikulai	52	14.3	0.6	8.7
Szemes alma	45.8	13.5	0.7	5.1
Vilmos renet	–	11.7	0.7	7.4
Jonathan M40	53.8	13.9	0.4	4.2

Sampling data: 29. Sept.

'London pepin': An old English cultivar. In Sub-Carpathia, generally grown in lowlands, where it matures in the end of October. Fruits are medium to large in size, symmetrical, globular oblate shaped, ribbed at the calyx. Fruits have long stems. The surface is clean, slightly greasy; the background turns lemon yellow by maturing. Fruits have a brownish-red blush which is dependent on adequate sun exposure. The flesh is yellow, and hard with a spicy flavor. Trees are of medium vigor with spreading branches, productive, open wide type.

'Nemes Sóvári Alma': An old Hungarian apple cultivar which was discovered in the upper part of the Tisza river. Harvest is recommended from the end of September. Fruits are medium in size, oblate to conic in shape. The surface is thin, greasy and bloomy. Fruits have dark red stripes on a yellow background. Its flesh is white and hard. Trees are vigorous, productive, globular type and crop regularly every year.

'Sikulai': An old Hungarian apple cultivar which was discovered in the area of the Fehér-Körös river and matures in the middle of September. Fruits are globular oblate shaped. Fruit size is dependent on tree age. The surface is clean and stripped, blushed with dark wine red. Fruits have short stems. Trees are of medium vigor and upright type.

'Szemes alma': A chance seedling discovered in Sub-Carpathia. It ripens from the end of September. Fruits are medium in size, globular. The surface turns to yellow and has a bright red blush on the sunny side. Fruit appearance is very poor, covered with scabs. The surface is clean, glossy with a bright red blush. The flesh is white, hard, and juicy by ripening. Trees are vigorous, open wide type with hanging branches.

‘Vilmos renet’: It ripens in the first decade of October. Fruits are large in size, globular oblate shaped. Fruits have an attractive appearance. The surface is clean and stripped blushed with dark red. Stems have a medium length. The flesh is white and moderately hard. The tree is of medium vigor with hanging branches, productive, open wide type.

Acknowledgements

This research was supported in part by grants from the Hungarian Scientific Research Fund (OTKA T 035120), and a Hungarian joint NKFP project.

References

- Aldwinckle, H. S. & Schwager, S. I. (1984):** Evaluation of fire blight resistance of apple cultivars and breeding new resistant cultivars. *Acta Hort.* 151: 259.
- Balikó E. (2000):** Történelmi almafajták felkutatása Kárpátalján. Diploma thesis, Budapest.
- Bertrand, F. P. & Gottwald, R. T. (1978):** Evaluation fungicides for Pecan disease control. p. 179–181. In: Zehr E. I. (ed), *Methods for Evaluating Plant Fungicides, Nematicides and Bactericides*. Amer. Phytopath. Soc. and Soc. of Nematologists. St. Paul, Minnesota.
- Fischer, C. (2000):** Multiple resistant apple cultivars and consequences for apple breeding in the future. *Acta Hort.* 538: 229–234.
- Fischer, M. & Fischer C. (2003):** 75 years of classical Pillnitz fruit breeding – Aims, results. *Eucarpia Symposium on Fruit Breeding and Genetics*, 2003 1–5 September, Angers–France. Abstract: 112.
- G. Tóth M. (2001):** Gyümölcsészet. PRIMOM, Nyíregyháza.
- G. Tóth M., Rozsnyay Zs. & Quang D. X. (1994):** Apple breeding for disease resistance in Hungary. In: Schmidt, H. and Kellerhals (eds.): *Progress in temperature fruit breeding*, p: 27–30. Kluwer Academic, Dordrecht Netherlands.
- G. Tóth M., Szani Zs., Balikó E., & Kása K. (2004):** Traditional old apple cultivars as a new gene source for apple breeding. *Acta Hort.* (in print.)
- Gardner, R. G., Cummins, J. N., & Aldwinckle, H. S. (1980):** Inheritance of fire blight resistance in *Malus* in relation to rootstock breeding. *J. Amer. Soc. Hort. Sci.* 105 (6): 912–916.
- Hevesi M. (1996):** Appearance of fire blight in Hungary. *Növényvédelem.* 32 (5): 225–228.
- Hevesi M., Papp J., Jámboor-Benczúr E., Kaszáné Csizsár K., Pozsgai I., Gazdag Gy. & Balla I. (2000):** Testing the virulence of some Hungarian *Erwinia amylovora* strains on *in vitro* cultures apple rootstocks. *Int. J. of Hort. Sci.* 6 (4): 52–56.
- Jones, A. and Schnabel, E. (2000):** The development of streptomycin-resistant strains of *Erwinia amylovora*. p. 235–253. In: Vanneste J. L. (ed.): *Fire blight. The disease and its causative agent, Erwinia amylovora*. CABI Publishing, Oxon, UK.
- Kása K., G. Tóth M., Göndör M. & Hevesi M. 2002.** Evaluation of fire blight resistance of apple cultivars. *Beitrage zur Züchtungsforschung Bundensanstalt für Züchtungsforschung an Kulturpflanzen* 8 (3): 71–74.
- Le Lezec, M. & Paulin, J. P. (1984):** Shoot susceptibility to fire blight of some apple cultivars. *Acta Hort.* 151: 277–283.
- Le Lezec, M., Paulin, J. P. & Lecomte, P. (1987):** Shoot and blossom susceptibility to fire blight of apple cultivars. *Acta Hort.* 217: 311–315.
- Lespinasse, Y. & Paulin, J. P. (1990):** Pathogenicity of strains of *Erwinia amylovora* to some apple cultivars in the greenhouse. *Acta Hort.* 273 : 319–326.
- Lespinasse, Y. & Aldwinckle, H. S. (2000):** Breeding for resistance to fire blight. p. 253–275. In: Vanneste, J. L. (ed.) *Fire blight. The disease and its causative agent, Erwinia amylovora*. CABI Publishing, Oxon.
- Maroofi, A. & Mostavafi, M. (1996):** Evaluation of the resistance of apple, pear and quince varieties to fire blight. *Acta Hort.* 411: 395–399.
- Psallidas, P. G. & Tsiantos, J (2000):** Chemical control of fire blight p. 199–235. In: Vanneste, J. L. (ed.) *Fire blight. The disease and its causative agent, Erwinia amylovora*. CABI Publishing, Oxon.
- Richter, K. & Fischer, C. (2003):** Stability of fire blight resistance. *Eucarpia Symposium on Fruit Breeding and Genetics*, Angers. Abstract: 39.
- van der Zwet T. & Beer S. V. (1995):** Fire blight—Its nature , prevention, and control: A practical guide to integrated disease management. U. S. Department of Agriculture, Agriculture Information Bulletin No. 631: 97.