# Assessment of resistance to apple scab (*Venturia inaequalis*) of apple genetic resources, and breeding applications at the Institute of Horticulture, LRCAF

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**Abstract.** A qualitative and quantitative resistance to apple scab (*Venturia inaequalis*) is characteristic to *Malus* sp. plants, therefore identification of resistance genes and efficient introduction of the genes to apple cultivars is crucial for apple breeding. Current horticultural research incorporates application of genetic and biotechnological approaches for identification of new sources of resistance to apple scab (Gessler et al., 2006; Kellerhals et al., 2009). In addition, the importance of pyramidization of resistance genes is emphasized in breeding programmes due to the genetic variability of the scab pathogen and the risk of loss of resistance in widely grown apple cultivars.

Monogenic *Vf* resistance remains effective protection against apple scab in part of the Northern Europe. Previously, three apple cultivars 'Skaistis', 'Rudenis' and 'Aldas' with *Vf* resistance were released from the Institute of Horticulture, LRCAF. Another two apple accessions featuring *Vf* resistance traits were developed during last decade (**Fig. 1**). Further breeding activities included pyramidization of monogenic resistance by combination of *Va, Vf, Vm* genes. PCR based molecular markers were used to identify genotypes carrying complex monogenic resistance traits during early development stage of seedling obtained from crosses involving cultivars such as 'Orlovim', 'Reglindis', 'Rubinola' and hybrids No. 24087 ('Redfree' × 'Sylvia'), No. 31888 and No. 31885 ('Freedom' × 'Murray'), No. 34330 ('Noris' × 'Antonovka'), No. 34191 ('Priam' × 'SR0523') (**Fig. 2**).

Apple trees have been grown in the Northern Europe for centuries, and a number of traditional cultivars carrying genes for qualitative and quantitative resistance are spread in the region. However, pedigree of many of the traditional cultivars is unknown, and information on genetic background of resistance of the cultivars is scarce. A number of accessions of the traditional cultivars and cultivars derived from crosses with the traditional cultivars are deposited at the collection of genetic resources of the Institute of Horticulture, LRCAF. A study on genetic polymorphism and morphological traits of 37 cultivars revealed 14 genotypes as potential sources for apple scab resistance breeding (Sikorskaite et al., 2012; **Fig. 3**). Further, a biotechnological method of screening for apple scab resistance at embryonic stage using apple seed cotyledons was employed to resolve a complexity of genetic background of resistance of the identified genotypes (**Fig. 4**).

## Fig. 1. Breeding of apple cultivars with resistance to apple scab.

**No. 24053 (**'Redfree' x 'Sylvia')
Resistance: apple scab (*Vf*, polygenic), apple blotch.

**No. 24087 (**'Redfree' x 'Sylvia')
Resistance: apple scab (*Vf,* polygenic), apple blotch.

Apple breeding program, established in 1952, was oriented towards development of winter apples featuring winter-hardiness, high productivity and fruit quality. Since 1978, donors of monogenic *Vf* and *Vm* resistance to apple scab were introduced into apple breeding program. During 1985-2012 period, 103 hybrids resistant to apple scab were developed, using immune cultivars as one or both parental plants. The program led to release of winter cultivars 'Aldas' [Sasnauskas et al., 2007] and 'Skaistis' [Sasnauskas et al., 2006], autumn cultivar 'Rudenis' [Sasnauskas et al., 2006], and development of summer apple hybrids No. 24053, No. 24087. Another six hybrids, carrying *Vf* and *Vm* genes, were selected as donors for pyramidization of scab resistance genes.

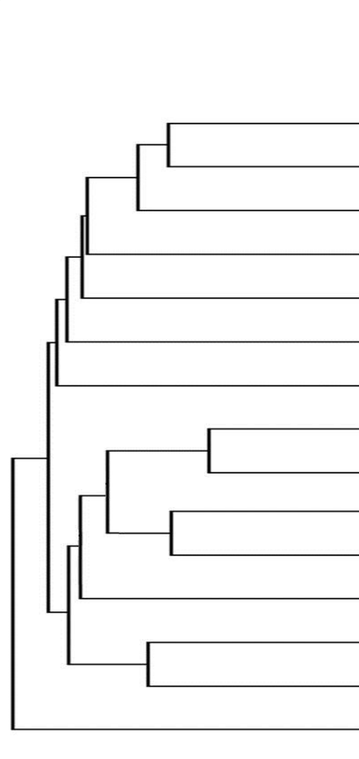
## Fig. 2. Pyramidization of *Va, Vf, Vm* resistance genes.

From crosses involving cultivars 'Orlovim', 'Reglindis', 'Rubinola' and hybrids No. 24087 ('Redfree' × 'Sylvia'), No. 31888 and No. 31885 ('Freedom' × 'Murray'), No. 34330 ('Noris' × 'Antonovka'), No. 34191 ('Priam' × 'SR0523'), were obtained hybrids carrying complex monogenic resistance traits in heterozygous (*Va, Vf, Vm*) and homozygous state (*Vf, Vm*). PCR based molecular markers [Xu and Korban, 2002; Patocchi et al., 2005] were used to identify inheritance of the resistance genes during early development stage. $\chi^2$ statistical analysis revealed that segregation ratio was significantly different for crosses 'Orlovim' × No. 24087 and No. 31885 × 'Reglindis'.

| Cross combination | Ratio | N | Genes inherited | O | E | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|---|---|
| Orlovim (Vm) x No.24087 (Vf) | 1:1:1:1 | 114 | Vf | 22 | 28.5 | 11.26 | 0.01 |
| | | | Vm | 42 | 28.5 | | |
| | | | Vf Vm | 19 | 28.5 | | |
| | | | 0 | 31 | 28.5 | | |
| Reglindis (Va) x Orlovim (Vm) | 1:1 | 49 | Vm | 25 | 24.5 | 0.02 | 0.89 |
| | | | 0 | 24 | 24.5 | | |
| No.34330 (Va) x Orlovim (Vm) | 1:1 | 165 | Vm | 81 | 82.5 | 0.06 | 0.82 |
| | | | 0 | 84 | 82.5 | | |
| No.31885 (Vf+Vm) x Reglindis (Va) | 1:1:1:1 | 154 | Vf | 45 | 38.5 | 23.30 | 0.00 |
| | | | Vm | 36 | 38.5 | | |
| | | | Vf Vm | 57 | 38.5 | | |
| | | | 0 | 16 | 38.5 | | |
| No.34191 (Vf+Vm) x Rubinola (Vf) | 3:1:3:1 | 107 | Vf | 34 | 40.1 | 1.71 | 0.63 |
| | | | Vm | 15 | 13.4 | | |
| | | | Vf Vm | 42 | 40.1 | | |
| | | | 0 | 16 | 13.4 | | |
| No.31888 (Vf+Vm) x Orlovim (Vm) | 1:3:3:1 | 44 | Vf | 5 | 5.5 | 6.30 | 0.10 |
| | | | Vm | 14 | 16.5 | | |
| | | | Vf Vm | 14 | 16.5 | | |
| | | | 0 | 11 | 5.5 | | |

E – expected genotypic ratio; O – observed genotypic ratio; N – total number of individuals; Segregation ratio corresponds gene sequence in the fourth column; Combinations resulting in heterozygous and homozygous hybrids are shown in green and blue font, respectively; Significantly different $\chi^2$ value ($p<0.05$) is indicated in red font.

## Fig. 3. Genetic relationship and characteristics of resistance to apple scab.

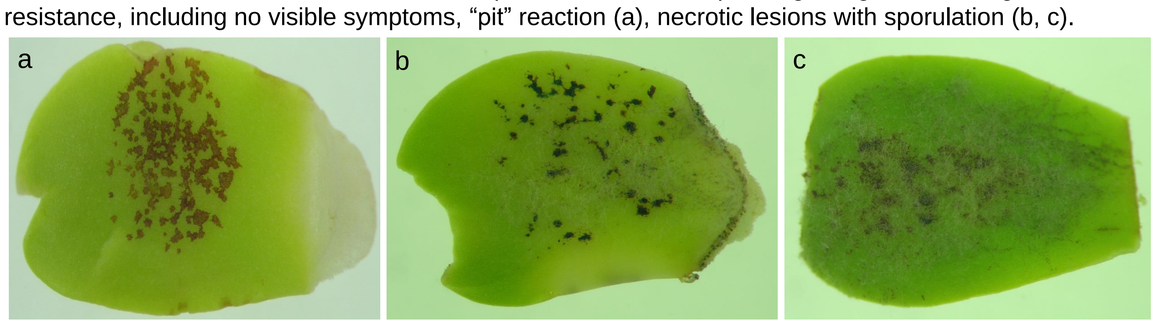


| Cultivar, species | Infection symptoms | | |
|---|---|---|---|
| | class | severity | necrosis |
| Persikinis | 2-3a | 3-7 | |
| P. antaninis (Antonovka) [PT] | 0 | 1 | |
| Pupinis (Bohnapfel) | 2-3a | 2-6 | |
| Biržuvėnų žieminis | 2-3a | 3-5 | |
| Štaris* [PT] | 0 | 1 | |
| Vytis | 2-3a | 2-3 | |
| Panevėžiuko rojinukas | 2-3a | 4-7 | + |
| R. rudeninis dryžuotasis | 2-3a | 2-3 | + |
| Tabokinė | 2 | 2 | ++ |
| Puikis* | 0 | 1 | |
| Montvilinis | 3a | 2-3 | |
| Babtų baltasis | 2-3a | 2-3 | + |
| Raudoniai | 2-3a | 2-3 | |
| Vytėnų pepinas | 2-3b | 2 | + |
| *Malus radient* | | | |

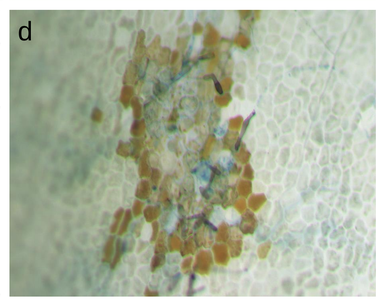
Assessment of apple scab infection characteristic among 37 indigenous and traditional cultivars revealed 14 cultivars with varying degree of resistance (complete resistance (0), chlorosis and/or necrosis symptoms without sporulation (2) or scarce sporulation levels (2-3a). Traditional cultivars and indigenous cultivars released from breeding programs are shown in blue and green, respectively. Star indicates cultivars with known parentage: 'Štaris' – 'Safran Pepin' x 'Antonovka'+'Cox's Pomona'+'Kandil-Kitaika', 'Puikis' – 'Bismarckapfel' open pollination [Tuinyla et al.,1990]. Abreviations in names of cultivars: P. – Paprastasis, R. – Raudonasis. Presence of polygenic resistance trait (PT) is indicated in square brackets. Minimum and maximum values observed during the two year period of evaluation are indicated for severity of infection. Presence of necrotic lesions was evaluated based on a three grade scale: absence of symptoms, moderate abundance (+) and severe damage (++). Dendrogram based on analysis of genetic polymorphism of microsatellite loci is shown on the left. Species *Malus radient* was used to root the tree.

## Fig. 4. Complexity of genetic background of resistance.

Apple seed cotyledons were used to screen for resistance to *V. inaequalis* under *in vitro* conditions [Gelvonauskiene and Stanys, 2000]. The test revealed common types of host-pathogen interactions characteristic to *M. x domestica – V. inaequalis* interaction depending on genetic background of the resistance, including no visible symptoms, "pit" reaction (a), necrotic lesions with sporulation (b, c).




| Cross combination | N | Host-pathogen interaction symptoms | |
|---|---|---|---|
| | | Sporulation, % | Necrotic lesions or "pit", % |
| Lobo x P.antaninis (clone A0409) | 161 | 100 | 18 |
| Lobo x P.antaninis (clone D0205) | 157 | 100 | 54 |
| Lobo x P.antaninis (clone I1) | 154 | 100 | 37 |
| Lobo x Puikis | 127 | 100 | 9 |
| Lobo x Montvilinis | 158 | 100 | 44 |
| Lobo x Orlovim | 142 | 58 | 42 |




Resistance reaction of 'Lobo' × 'Orlovim' (a), 'Lobo' × 'Montvilinis' (b) and 'Lobo' × 'P. antaninis' (clone D0205) (c) cotyledons 12 days post inoculation with *V. inaequalis* conidia. 'Orlovim' has a *Vm* gene and gives a typical 'pit-type' reaction, and was used as a control. Light microscope observations of lesions, characteristic to hybrids of 'Lobo' with 'Orlovim' and 'Monvilinis' cotelydons stained with lactophenol cotton blue (d).

## Conclusions:

Application of donors of *Vf* resistance in the breeding program resulted in development of new apple scab resistant accessions at the Institute. Further, the disease resistance breeding approach was expanded to introduce new traits, such as *Vm* and polygenic resistance, to achieve pyramidization of resistance genes.

Marker-assisted selection was performed to identify genotypes carrying multiple resistance genes into a single cultivar. $\chi^2$ statistical analysis revealed, that survival of seedlings might be effected by inheritance of resistance genes under different genome background for crosses 'Orlovim' × No. 24087 and No. 31885 × 'Reglindis'.

Assessment of apple scab infection characteristics among indigenous and traditional cultivars revealed varying degrees of resistance to apple scab infection. Complete resistance (class 0) was observed in three cultivars carrying polygenic resistance traits ('Paprastasis antaninis') or unknown source of resistance ('Puikis' and 'Štaris'). Partial resistance (class 2) with extensive necrosis symptoms was characteristic to 'Tabokinė'. Ten cultivars exhibited class 2-3a, 3a or 2-3b symptoms and varying levels of necrotic lesions.

Application of *in vitro* test for identification of disease resistant progeny at embryonic stage demonstrated that all cultivars used in analysis were susceptible to apple scab infection, however monogenic trait of necrotic response to the pathogen infection was observed for 'Paprastasis antaninis' (clone D0205) and 'Montvilinis'.

**References.** Gelvonauskiene D. and Stanys V. 2000. Fruit Sci. 207:56-60; Gessler C., et al. 2006. Crit. Rev. Plant Sci. 25: 473-503; Kellerhals M., et al. 2009 Acta Hort. 814: 177-183; Patocchi et al. 2005. Genome 48:630-636; Sasnauskas A., et al. 2007. Acta Hort. 760:507-511; Sasnauskas A., et al. 2006. Fruit Ornam. Plant Res. 14(2):247-255. Sikorskaite S., et al. 2012. Zemdirbyste-Agriculture 99(2):131–138; Tuinyla, V., et al. 1990. Mokslas; Xu and Korban, 2002. Genetics 162: 1995-2006.

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