Fruit Growing Research, Vol. XXXVIII, 2022 DOI 10.33045/fgr.v38.2022.03
https://publications.icdp.ro/index.php

EVALUAREA REZISTENȚEI LA RAPĂN ȘI FĂINARE A GERMOPLASMEI DE MĂR DE LA ICDP PITEȘTI-MĂRĂCINENI

EVALUATION OF SCAB AND POWDERY MILDEW RESISTANCE OF APPLE GERMPLASM COLECTTED AT RIFG PITESTI

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Abstract

The rising incidence of biotic and abiotic stresses makes commercial fruit production increasingly difficult in Europe, especially apple orchards which are affected by fungal diseases, like scab and powdery mildew, caused by *Venturia inaequalis* Cooke Winter and *Podosphaera leucotricha* Ellis & Everh. Salmon. Releasing and planting of apple resistant cultivars is a promising strategy. A set of 527 apple cultivars from the apple gene bank collected at Research Institute for Fruit Growing (RIFG) Pitești was evaluated for the incidence of scab (on leaves and fruits) and powdery mildew (on leaves and top shoots) in three consecutive years (2018, 2019 and 2020). Phenotypic results were compared to data reported at the evaluation made in 2006. Unfortunately, the incidence of both scab and powdery mildew increased significantly in the last years. In 2021, 48 Romanian apple cultivars were analyzed with molecular markers linked to *Rvi2, Rvi4, Rvi5, Rvi6, Rvi8* and *Plw*. The markers linked to *Rvi6* was detected in 31 cultivars. Alleles linked to *Plw* were expressed from three cultivars. The information on resistance / susceptibility of apple genetic resources towards economically important diseases is important for breeding. Using a systematic screening of the apple cultivar collection, several cultivars with high value for resistance breeding and sustainable growing were identified.

Cuvinte cheie: *Malus*, biodiversitate, boli fungice, marker moleculari
Key words: *Malus*, biodiversity, fungal diseases, molecular markers

1. Introduction

Breeding and growing apple disease resistant cultivars is a useful strategy to reduce the use of pesticides and may contribute to a sustainable agriculture economy according the Green Deal Strategy. The evaluation and characterization of available genetic resources of a cultivated species with regard to resistance against diseases represents a great opportunity to identify genotypes and to use biodiversity to counteract existing challenges in the apple fruit production (Höfer et al., 2021).

In Europe, the apple orchards are affected by several fungal pathogens, like *Venturia inaequalis, Podosphaera leucotricha, Alternaria sp.* etc. Of these, apple scab and powdery mildew requests up to 15 fungicide treatments to avoid severe infections. During 20 years, the growing of resistant cultivars was a good strategy, but, unfortunately, the major scab resistance gene *Vf* (*Rvi6*) from *Malus floribunda* clone 821 was overcome by new races (Militaru et al., 2017).

Since 2017 the Research Institute for Fruit Growing (RIFG) Pitesti is taking part in the VINQUEST project which is investigating the genes that are the best suited to breed apple cultivars with durable resistance (http://www.vinquest.ch/monitoring/current_member.htm). The aim of the project is to distribute host plants assigned to specific *Venturia inaequalis* races all cross Europe to collect data in which parts of Europe which races of the fungus are established. Andrea Patocchi et al. (2020) quantified the frequency of *Rvi* breakdowns over the different sites at a specific site each time at least one class 3 score was called. This approach grouped the *Rvi* genes as follows: *Rvi1, Rvi3, Rvi8* and *Rvi10* fell in the category ”often overcome”; *Rvi2, Rvi4, Rvi6, Rvi7, Rvi9* and *Rvi13* in ”sometimes overcome”; *Rvi5, Rvi12* and *Rvi14* in ”rarely overcome”, and *Rvi11* and *Rvi15* in the category ”not overcome”.

Progress in apple breeding depends of a rich diversity of genetic resources. The present paper was aimed at the evaluation of apple genetic resources (*Malus domestica* Borkh.) collected at RIFG Pitesti, Romania for their resistance to apple scab and powdery mildew in order to use them in fruit production,

breeding and research. Phenotypic results collected in 2018, 2019 and 2020 were compared to data available from previous experiments, which were performed in 2006.

2. Material and methods

2.1. Plant material

Five hundred and twenty-seven *Malus* genotypes collected at the Research Institute for Fruit Growing Pitesti, Romania ($44^0$54' N latitude and $24^0$52' E longitude), were used in this study. Trees were grown on MM106 with two replicates per genotype, not fertilized or irrigated. Plant protection was carried according to integrated fruit production. The genotypes were evaluated on fungal disease symptoms during 3 consecutive years (2018, 2019, 2020) at least two experts (curator of genetic resources and phytopathologist). The data were compared with results evaluation registered in 2006.

2.2. Pedo-climatic conditions

The experimental field was located on a flat land, of alluvial type, soil with medium texture, pH around 6. The humus content is relatively low: 1.50% at the surface, 0.90% at 60 cm depth and 0.61% in the Bg horizon. During the study period, from January, 2018 till December, 2020, the annual average temperature was 11.63^{o}C, with 1.43^{o}C higher than the multiannual average temperature registered in 2006. The average annual rainfall was 686.56 mm, with 51.94 mm less than 2006, being very close to the multiannual average of rainfall from the area (678 mm). In 2006, in July and August, the monthly precipitation values exceeded the means for these months by 128% in July (versus 83 mm) and 149% in August (versus 63 mm).

In 2018, the coldest month was March with a minimum temperature of -19.8°C (the average +3.9°C) and the hottest month was August, with the maximum temperature of +33.9°C (the average +22.2°C). The leaves wet period ranged from 130 hours/month in May up to 184 hours/month in August. The rainiest months were May and June with 112.5 and 198 mm and the driest month was October with 2.0 mm/m2 . The total precipitation amount was 746 mm.

Also, in 2019 were oscillatory microclimate conditions. The coldest month was January, with a minimum temperature of -13.5°C (the average -1.2°C) and the hottest month was August with the maximum temperature of 35.1°C (the average 22.8°C). The leaves wet period ranged from 107 hours/month in May to 135 hours/month in July. The rainiest months were July with 197.1 and August with 93.1 mm and the driest month was August with 9.7 mm. Also, the total precipitation amount was 520 mm.

2.3. Phenotyping of scab and powdery mildew

Field evaluation on the occurrence of scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) was conducted once per year, in August. Phenotypic data recorded in 2006 by Branișteare compared with data recorded in the same collection in 2018, 2019 and 2020. Scoring of scab symptoms (on leaves and fruits) and powdery mildew (on leaves and top shoots) was performed according to the assessment scale defined in table 1.

2.4. Molecular analysis

In 2021, phenotypic date for 48 Romanian apple cultivars ('Remus', 'Romus 2', 'Romus 3', 'Romus 4', 'Romus 5', 'Rebra', 'Rustic', 'Nicol', 'Colmar', 'Colonade', 'Rumina', 'Delicios de Voinești', 'Frumos de Voinești', 'Generos', 'Iris', 'Irisem', 'Luca', 'Ciprian', 'Redix', 'Cezar', 'Remar', 'Valery', 'Real', 'Brumar', 'Voinicel', 'Inedit', 'Dacian', 'Voinea', 'Discoprim', 'Pomona', 'Revidar', 'Aura', 'Starkprim', 'Ionaprim', 'Bistritean', 'Dany', 'Salva', 'Alex', 'Doina', 'Estival', 'Productiv de Cluj', 'Precoce de Ardeal', 'Auriu de Cluj', 'Feleac', 'Roșu de Cluj', 'Ancuța', 'Rădășeni', 'Fălticeni') were completed with screening for five scab resistance genes (*Rvi2, Rvi4, Rvi5, Rvi6, Rvi8*) and one powdery mildew resistance gene (*Pl-w*).

DNA was extracted from the milled leaves using the extraction and dilution buffers of the Isolate II Plant DNA Extraction Kit (Bioline) using the recommended standard protocol with Lysis Buffer PA1 based on the well-established CTAB procedure. For the PCR reaction, the kit 2 x MyTaq™ Red Mix was optimized with a specific DNA dye. PCR reactions were performed in a FastGene Ultra Cycler Gradient analyzer model FG-TC01, and amplification programs were optimized for each primer. The reaction mix was made using 2 x MyTaq™ Red Mix in 0.2 ml tubes containing 15 µl final reaction volume, of which 10 µl reaction mix (MyTaq RedMix), 0,1 µl of each primer at a concentration of 100 µmol, 2 µl DNA (20 ng) and 2,9 µl ultrapure water.The amplification reaction for scab was performed using six molecular markers (AL07, AM19, VfC AD13, OPL19, OPB12) and for powdery mildew using EM M01 molecular marker.

PCR product evaluation was performed by 2% high-resolution agarose gel electrophoresis (Cleaver) and 1X TBE buffer, staining with RedSafe Nucleic Acid Staining.

3. Results

Field evaluation

Our phenotypic evaluation shows that, after one decade, the behavior of scab and powdery mildew resistance has major changes. In 2006, among the 527 apple varieties, 416 of them, representing 79%

were evaluated with ”no scab attack on leaves”, and 390 of them, representing 74%, were evaluated with ”no scab attack on fruits”. In 2020, only 66%, respectively 65%, were evaluated with ”no attack on leaves/fruits” (Fig. 1 and 3). Sestraș (2003) shows that, in Central country environment, from 75 apple cultivars verified at the Fruit Research Station Cluj – Napoca, between 1990 - 1996, 31 of them were registered with ”no scab attack”, both on leaves and fruits, including 'Prima', 'Priscilla', 'Sir Prize', 'Liberty', 'Florina', 'Priam', 'Pionier', 'Voinea' cvs.

Higher incidence was observed for powdery mildew. Figure 2 shows the distribution of powdery mildew on leaves and shoots in 2006, 2018, 2019 and 2020. The mean incidence of mildew was significantly higher in 2020 than in 2006.

Evaluation of Romanian cultivars for the presence of marker alleles linked to R genes

***Rvi2 + Rvi8*:** Marker OPL19, used for *Rvi2* and *Rvi8*, amplified two fragments: 433 bp and 1,200 bp in 23 cultivars ('Romus 2', 'Romus 3', 'Romus 4', 'Romus 5', 'Delicios de Voinești', 'Luca', 'Ciprian', 'Redix', 'Cezar', 'Real', 'Remar', 'Voinicel', 'Voinea', 'Discoprim', 'Pomona', 'Aura', 'Starkprim', 'Jonaprim', 'Bistrițean', 'Dany', 'Salva', 'Alex', 'Estival').

***Rvi4*:** To confirm *Rvi4* gene was used AD13 marker which amplified two fragments 950 bp and 1,200 bp only in 12 cultivars: 'Romus 3', 'Romus 5', 'Generos', 'Iris', 'Irisem', 'Redix', 'Cezar', 'Remar', 'Voinicel', 'Aura', 'Estival' , 'Discoprim'.

***Rvi5*:** Marker OPB12 amplified 687 bp in only three cultivars: 'Nicol', 'Generos' and 'Irisem', all phenotypic susceptible to apple scab.

***Rvi6*:** AL07, AM19 and VfC were used as markers for *Rvi6*. Primers for AL07 marker were detected in 31 cultivars and were absent in scab susceptible cultivars like: 'Remus', 'Romus 2', 'Nicol', 'Rumina', 'Delicios de Voinești', 'Frumos de Voinești', 'Generos', 'Irisem', 'Estival', 'Productiv de Cluj', 'Precoce de Ardeal', 'Auriu de Cluj', 'Feleac', 'Roșu de Cluj', 'Ancuța', 'Rădășeni' and 'Fălticeni' (table 2). Primers for AM19 and VfC markers led to amplication of 526 bp, respectively 286, 484 and 646 bp in 31 cultivars like: 'Romus 3', 'Romus 4', 'Romus 5', 'Rebra', 'Rustic', 'Colmar', 'Colonade', 'Iris', 'Luca', 'Ciprian', 'Redix', 'Cezar', 'Valery', 'Real', 'Remar', 'Brumar', 'Voinicel', 'Inedit', 'Dacian', 'Voinea', 'Discoprim', 'Pomona', 'Revidar', 'Aura', Starkprim', 'Jonaprim', 'Bistrițean', 'Dany', 'Salva', 'Alex', 'Doina'.

Pl-w: The 88 bp allele amplified by 'Nicol', 'Generos' and 'Irisem' cvs. Indicates the presence of *Pl-w*.

4. Discussions

The rapid spread of modern, intensive agricultural techniques during the last century could facilitate specialization of pathogen strains on particular cultivars and rapid dissemination of the new strain. Screening of bred Romanian apple cultivars identified some of them with high *R* genes accumulation useful for apple breeding to develop cultivars with durable apple scab and powdery mildew resistance.

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Tables and Figures

Table 1. Assessment scale for apple scab (on leaves and fruits) and powdery mildew (on leaves and top shoots) (Lateur and Populer, 1994)

Scale	Susceptibility	Scab	Powdery mildew
1	no infection	no visible macroscopic symptoms	no visible macroscopic symptoms
2	very low	a few small scab spots are detectable only visible on closer inspection	1 sporulation spot
3	low	visible lesions, very thinly scattered in the tree	up to 25% of leaves/shoots covered with infections symptoms
4	low-medium	x	>25% and <50% of leaves/shoots covered with infections symptoms
5	medium	numerous lesions spread over a large part of the tree/fruits	50% of leaves /shoots covered with infections symptoms
6	medium-high	x	>50% and <75% of leaves/shoots covered with infections symptoms
7	high	severe infection with half of the leaves/fruits by multiple lesions	75% of leaves /shoots covered with infections symptoms
8	high – extremely high	x	>75% and <100% of leaves / shoots covered with infections symptoms
9	extremely high	tree completely affected with (nearly) all the leaves/fruits badly infected by multiple lesions	100% of leaves / shoots covered with infections symptoms

X = intermediary rating

Table 2. Screening of *R* genes for 48 bred Romanian apple cultivars

No.	Cultivar	Average rating on				*R* genes indicated by respective marker alleles
		scab		powdery mildew		
		leaf	fruit	leaf	top shoots	
0	**1**	**2**	**3**	**4**	**5**	**6**
1	Remus	6	4	2	2	-
2	Romus 2	3	4	2	3	*Rvi2*
3	Romus 3	2	2	1	1	*Rvi2, Rvi4, Rvi6, Rvi8*
4	Romus 4	3	3	1	2	*Rvi2, Rvi6, Rvi8*
5	Romus 5	4	3	1	1	*Rvi2, Rvi4, Rvi6, Rvi8*
6	Rebra	4	4	1	1	*Rvi6*
7	Rustic	3	3	1	1	*Rvi6*
8	Nicol	2	1	1	1	*Rvi5, Pl-w*
9	Colmar	1	2	1	1	*Rvi6*
10	Colonade	1	2	2	2	*Rvi6*
11	Rumina	4	1	3	1	-
12	Delicios de Voinești	3	3	2	1	*Rvi2, Rvi8*
13	Frumos de Voinești	3	4	2	2	-
14	Generos	4	5	6	4	*Rvi4, Rvi5, Pl-w*
15	Iris	3	4	2	2	*Rvi4, Rvi6*
16	Irisem	3	3	2	2	*Rvi4, Rvi5, Pl-w*
17	Luca	2	3	3	2	*Rvi2, Rvi6*
18	Ciprian	2	3	1	1	*Rvi2, Rvi6*
19	Redix	4	5	1	1	*Rvi2, Rvi4, Rvi6, Rvi8*
20	Cezar	3	2	2	1	*Rvi2, Rvi4, Rvi6, Rvi8*
21	Remar	3	2	2	2	*Rvi6*
22	Valery	2	2	1	1	*Rvi2, Rvi6, Rvi8*
23	Real	3	4	2	2	*Rvi6*
24	Brumar	3	3	2	2	*Rvi6*
25	Voinicel	2	3	2	1	*Rvi2, Rvi4, Rvi6, Rvi8*
26	Inedit	3	2	1	1	*Rvi6*
27	Dacian	2	2	1	1	*Rvi6*
28	Voinea	3	2	2	2	*Rvi2, Rvi6, Rvi8*

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0	1	2	3	4	5	6
29	Discoprim	2	2	1	1	*Rvi2, Rvi4, Rvi6, Rvi8*
30	Pomona	2	3	2	1	*Rvi2, Rvi6, Rvi8*
31	Revidar	3	2	1	1	*Rvi6*
32	Aura	3	3	2	1	*Rvi2, Rvi4, Rvi6, Rvi8*
33	Starkprim	4	3	2	1	*Rvi2, Rvi6, Rvi8*
34	Jonaprim	3	3	2	1	*Rvi2, Rvi6, Rvi8*
35	Bistrițean	3	3	2	1	*Rvi2, Rvi6, Rvi8*
36	Dany	2	3	2	2	*Rvi2, Rvi6, Rvi8*
37	Salva	2	4	2	2	*Rvi2, Rvi6, Rvi8*
38	Alex	3	3	2	2	*Rvi2, Rvi6, Rvi8*
39	Doina	3	4	2	2	*Rvi6*
40	Estival	4	5	4	3	*Rvi2, Rvi4*
41	Productiv de Cluj	5	4	4	4	-
42	Precoce de Ardeal	4	6	4	4	-
43	Auriu de Cluj	4	5	3	3	-
44	Feleac	4	5	5	5	-
45	Roșu de Cluj	5	5	4	5	-
46	Ancuța	4	4	5	4	-
47	Rădășeni	5	4	5	5	-
48	Fălticeni	4	5	4	4	-

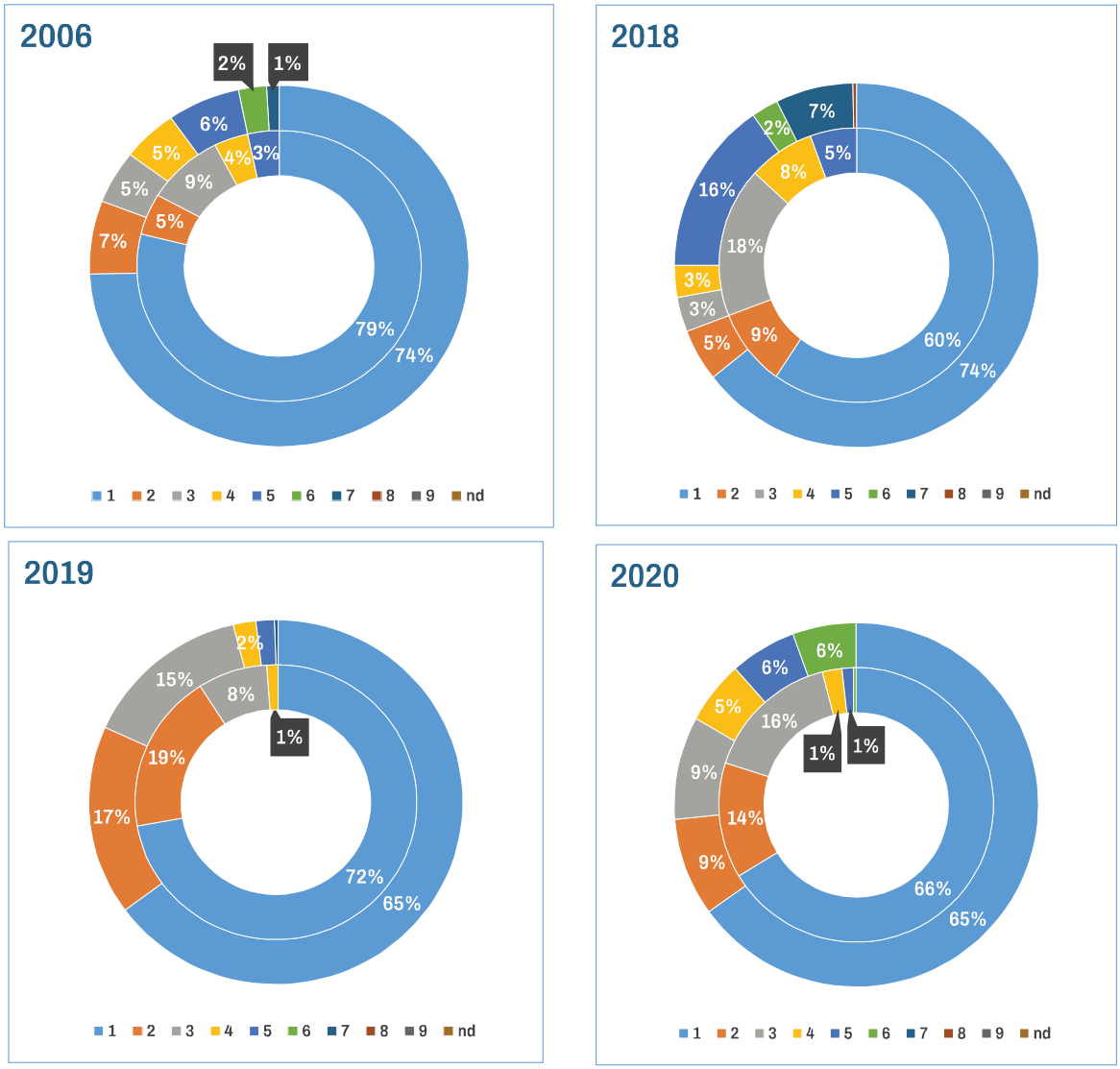

Fig. 1. Proportion of genotypes with scab on leaves (inner circle) and fruits (outer circle)

 DOI 10.33045/fgr.v38.2022.03

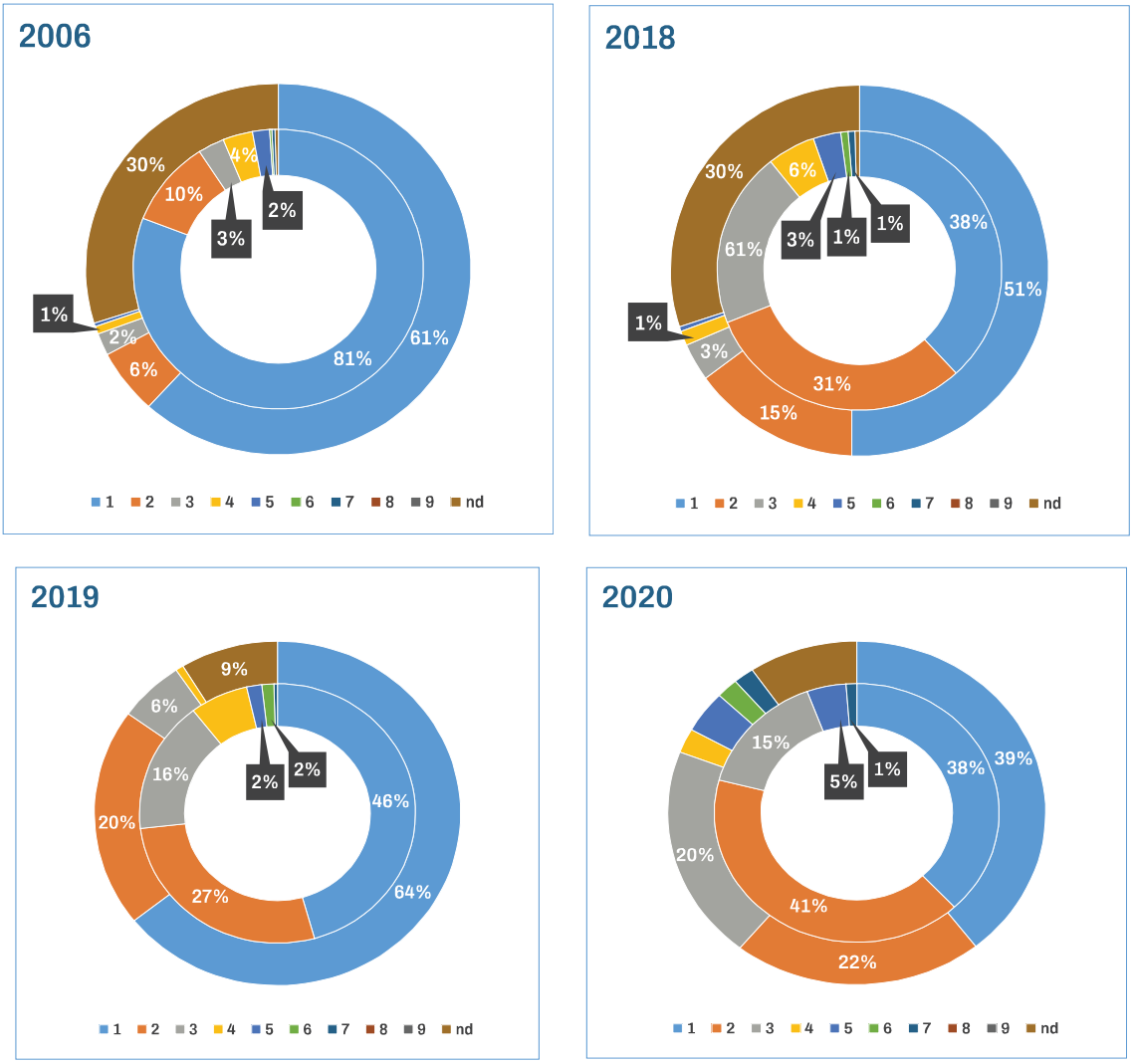

Fig. 2. Proportion of genotypes with powdery mildew on leaves (inner circle) and shoots (outer circle)

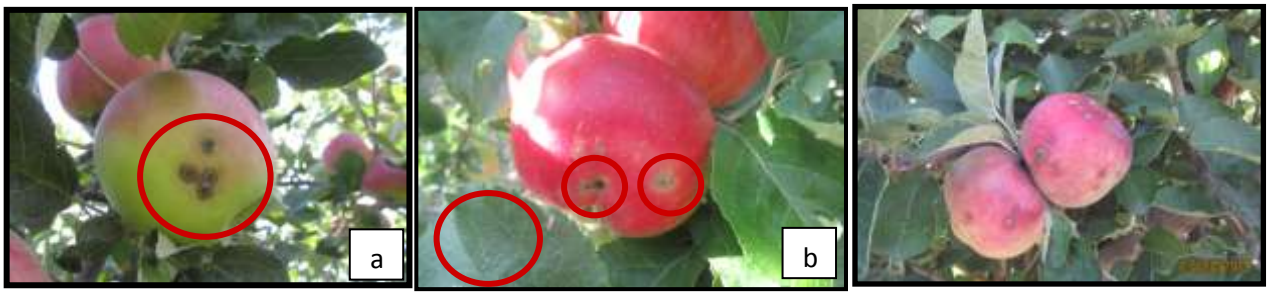

Fig. 3. Apple scab symptoms on 'Romus 3' (a), 'Romus 5' (b) and 'Redix' (c) apples in the *Malus* core collection at RIFG Pitesti, Romania