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Development of a hybrid apple population: MAS-based testing for *Vf* gene detection and preliminary field evaluation of its scab resistance

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Abstract: The apple scab, caused by *Venturia inaequalis*, is one of the most devastating diseases of apples because it drastically affects the aerial parts of the tree. Apple breeding programs frequently aim to develop cultivars exhibiting resistance to scab. Thus, controlled pollinations were performed on apple trees in 2021. The experiment was organised into a hybridisation scheme involving six apple cultivars. The scheme consists of: five female genitors ('Auriu de Bistrița', 'Jonathan', 'Golden Delicious', 'Starkrimson', and 'Idared') and one male genitor ('Florina' – donor for the *Vf*). To quickly identify the *Vf* gene in the early development stage of progenies, three pairs of primers were used: AM19, AL07, and VFC. The hybrid combinations with the highest percentage of progenies inheriting the *Vf* resistance gene were: 'Starkrimson' × 'Florina' (59.2%), followed by 'Jonathan' × 'Florina' (57.1%). The hybrids that inherited the *Vf* resistance gene have been transferred to the field and are being further examined for their resistance against scab infections in the field, in the proximity of a natural source of the inoculum infection pressure.

Keywords: marker-assisted selection; molecular markers; monogenic resistance; *Rvi6* gene; *Venturia inaequalis*

The domesticated apple, scientifically known as *Malus* × *domestica* Borkh., is part of the *Malus* genus, within the Rosaceae family and the Meloideae subfamily. It is regarded as a functional diploid ($2n = 34$) (Volk et al. 2021). Recent apple breeding programs utilising controlled hybridisation are focused on: achieving strong resistance to specific diseases, especially apple scab; improving the performance and yield of apple trees; enhancing the sensory attributes of apples to increase consumer demand; boosting resistance to abiotic stressors that intensively affect fruit trees due to climate change (Höfer et al. 2021). One of the most destructive fungal diseases of apple trees is apple scab, caused by the fungus *Venturia inaequalis*. Originally from Europe, the

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disease was first described in its summer stage by a Swedish botanist (Fries 1819). It was reported in the United States in 1834, in Austria in 1862, and in England in 1945. In Romania, the disease is present in all fruit-growing regions where the apple is cultivated. In neglected orchards, during years with rainy springs and summers, losses can range from approximately 30% to as high as 98% (Șuta, Floru 1974). It damages the aerial parts of the trees, often leading to significant economic losses in most commercial cultivars. The damage caused by *Venturia inaequalis* is primarily reflected in significant yield losses, qualitative depreciation of fruits both in the orchard and during storage, weakening of the infected trees, and, not least, increased production costs resulting from the higher number of chemical treatments required (Belete, Boyraz 2017). Therefore, orchards facing severe fungal issues such as scab require rigorous and intensive management strategies (Rancāne et al. 2023). In some instances, preventing severe infections from these harmful fungi may involve administering 20 to 30 fungicide treatments annually (Sokolova, Moročko-Bičevska 2022). The use of resistant cultivars could lead to lower costs of production for growers and also help to promote a cleaner environment (Kaymak et al. 2013). Improving apple resistance to scab has become one of the main objectives of various apple breeding programs in many countries, including Canada (McClure et al. 2018), France (Lasserre-Zuber et al. 2018), Spain (Pereira-Lorenzo et al. 2018), the Czech Republic (Zelmene et al. 2022), Switzerland (Bühlmann-Schütz et al. 2022), the United Kingdom (Stewart et al. 2023), Kazakhstan (Madenova et al. 2024), and Romania (Sturzeanu et al. 2020; Bivolariu et al. 2021; Militaru et al. 2022), particularly in the region of Transylvania. The genetic determinism of apple scab resistance is of two types: inherited (vertical or horizontal resistance) and induced (acquired) through genetic engineering (transgenesis) or New Genomic Techniques (NGTs). Through the VINQUEST project, conducted over a ten-year period, in-depth studies were carried out on apple resistance to scab. Among the major resistance genes of high interest is the *Vf* gene. (Patocchi et al. 2020). With the introduction of the new nomenclature by Bus et al. (2009), the *Vf* gene was renamed *Rvi6*. The *Vf* gene is derived from the small-fruited species *Malus floribunda* Siebold clone 821 and is found in most commercial cultivars considered resistant to scab (Gao, Van de Weg 2006). This gene is the most commonly used source of scab resistance in apple breeding programs (Militaru et al. 2020; Soriano et al. 2009). Calenge et al. (2004) state that this gene confers resistance to six out of the eight races developed so far by the pathogen *Venturia inaequalis*. Furthermore, apple cultivars with the *Vf* gene have remained scab-free for over 80 years across different regions (Sheikh et al. 2017). Unfortunately, the majority of scab-resistant cultivars released so far contain only the *Rvi6* (*Vf*) gene, whose effectiveness is now uncertain due to the widespread presence of *avrRvi6* isolates across Europe and their detection in the U.S. as well (Patocchi et al. 2020). Scab in apple, caused by *Venturia inaequalis,* is governed by a gene-for-gene interaction between avirulence genes (*avr*) in the pathogen and resistance genes (*R*) in the host plant (Bastiaanse et al. 2016). The *avrRvi6* gene in *Venturia inaequalis* is recognised by the *Rvi6* resistance gene (formerly known as *Vf*) in apple, triggering a defence response and conferring resistance to infection. However, some pathogen races may lose or mutate *avrRvi6*, allowing them to evade recognition and overcome resistance, which highlights the importance of understanding this mechanism in resistance breeding. Considering that the cultivars carrying the *Vf* gene still exhibit a certain degree of resistance, the use of this gene in apple breeding remains valuable. It is still of interest to use *Vf* monogenic resistance in areas where the presence of *avrRvi6* isolates has not been identified. Other studies suggest that promising results regarding apple scab resistance can be achieved through the use of durable resistance derived from crosses such as *Vf* × *Vf*, *Vf* × *polygenic*, and *polygenic* × *polygenic*, as well as *Vf* × *Vr*, *Vf* × *Va*, and *Vr* × *Va*. (Lespinasse 1992). In apple, molecular markers are used both for identifying resistance genes in the offspring and for detecting resistance sources, primarily through marker-assisted selection (MAS) techniques (Zahid et al. 2022). The most recent research in the field, conducted by Švara et al. in 2024, provides an overview of the molecular markers used to identify common apple genes that confer resistance to apple scab. According to this study, a total of 83 molecular markers are used, 14 of which are specific to the *Vf* gene.

Detection of molecular markers associated with the *Vf* (*Rvi6*) gene is valuable for MAS (King et al. 1999) and has been successfully used in apple breeding.

The aim of this study was to assess the effectiveness of a controlled hybridisation process by confirming the transmission of the *Vf* resistance gene in the obtained hybrids through molecular analyses and by monitoring their response to natural scab infection during the first year of cultivation.

MATERIAL AND METHODS

The experiment was conducted at the Fruit Research & Development Station Bistrița (FRDS Bistrița), located in Bistrița-Năsăud County, in the north-eastern part of Transylvania, Romania. The regional climate is classified as moderately temperate-continental, characterised by warm summers, cold winters, and an uneven distribution of precipitation throughout the year, frequently exceeding the national average (Minoiu, Bilegan 1990).

Controlled pollinations were carried out in 2021 within a 20-year-old apple orchard. The trees were grafted onto the vigorous rootstock MM106. All selected cultivars are well adapted to the climatic and pedological conditions specific to the Bistrița growing region. Consequently, the biological material used in this study consisted of six apple cultivars and their respective F_1 hybrids obtained through controlled cross-pollination. A brief characterisation of each parental genotype is provided in Table 1 and illustrated in Figure 1.

The main traits intended to be transferred to the hybrids from the selected genitors are presented below for each genotype, as follow: ‘Florina’ – the presence of the *Vf* and *Vg* gene, resistance to fruit handling, resistant on fruits to scab; ‘Auriu de Bistrița’ – high fruit characteristics, harvesting period, tolerant to scab; ‘Jonathan’ – harvesting period, taste qualities, susceptible to scab; ‘Golden Delicious’ – appreciated among consumers, the fruit’s taste qualities; ‘Starkrimson’ – fruit quality appreciated for some consumers; ‘Idared’ – very good storage capacity in the warehouse.

According to the specialised literature, the cultivar ‘Florina’ (male genitor) possesses the *Vf* gene (and also the *Vg* gene) (Bénaouf, Parisi 2000). It has a heterozygous genotype (*Vfvf*), so it has a probability of transmitting its resistance to progenies with a rate of 50%. In contrast, the female genitors do not have the *Vf* gene: ‘Auriu de Bistrița’ (Guzu et al. 2021), ‘Jonathan’ (Afunian et al. 2004), ‘Golden Delicious’ (Sestraș 2004), ‘Starkrimson’ (Pătrașcu et al. 2006), and ‘Idared’ (Vejl et al. 2003), all have a homozygous recessive genotype (*vfvf*).

Table 1. Main pomological characteristics of the apple cultivars used as genitors in controlled crosses

Cultivar	Genitors	Blooming period	Ripening period	Fruit weight (g)	Flesh characteristics	Skin characteristics
Florina	612-1 × Jonathan	early	mid to late	150–200	creamy-yellow, juicy	yellow background colour with red overcolour in stripes
Auriu de Bistrița	Golden Delicious × Parmain d’Or	late	mid-season	170–200	yellow, firm, juicy, sweet, mildly acidic	golden-yellow background with pink overcolour
Jonathan	Esopus Spitzenburg × unknown cultivar	medium	mid to late	120–140	yellowish-white, moderately firm, aromatic	light red over a yellow background
Golden Delicious	Grimes Golden × Golden Reinette	late	late	140–180	yellow, fine-textured, juicy, sweet	yellow background, occasionally with light russeting
Starkrimson	mutation of Starking	medium	late	180–225	greenish-white, firm	purplish-red skin
Idared	Jonathan × Wagener	early	semi-late	170–200	yellowish-white, crisp, acidic	greenish-yellow background with red overcolour

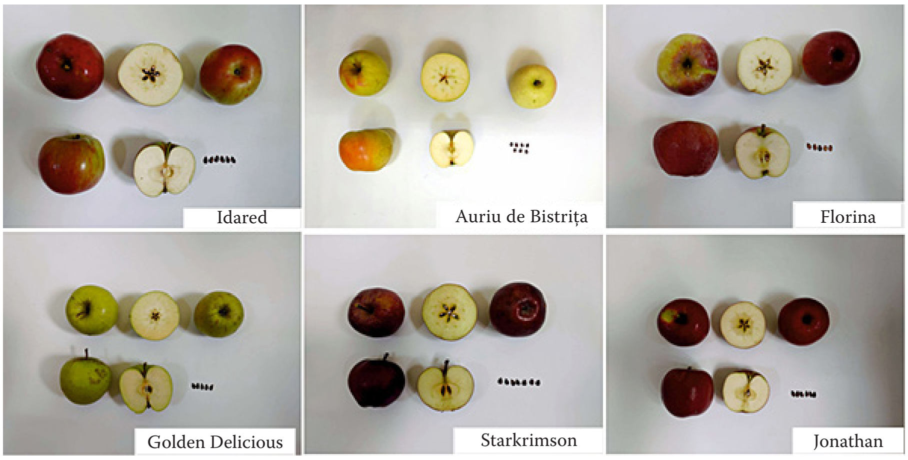

Figure 1. Aspects of fruit shape, skin colour, and flesh characteristics in the selected genitors

The classical hybridisation steps were followed for artificial pollination in apples (Cordea 2014) and are detailed below. Following the selection of the parental genotypes based on the previously described traits, the second stage involved the selection of trees in the orchard and the identification of inflorescences within the tree canopy. Selected trees were healthy and expressed the phenotypic traits of the cultivar accurately. Inflorescences of the paternal parent (the one from which the pollen was collected) were at the advanced floral bud stage. The third stage consisted of isolating the maternal parent to prevent uncontrolled pollination, given that apple is an allogamous species and requires cross-pollination to produce fruit. The fourth stage involved pollen collection from the flowers of the paternal genotype, which was carried out using sterile tweezers. After anthers removing the pollen grains, were stored at 20–22 °C and 70–80% relative humidity to promote the maturation process. The fifth stage involved artificial pollination, performed at the time when the stigma of the maternal parent showed maximum receptivity as indicated by the appearance of a clear, sticky secretion. The sixth stage involved monitoring fruit set, conducted in two steps: the first control at 8–10 days after artificial pollination, and the second one at 12–15 days after the first check. The seventh and final stage consisted of harvesting of hybrid fruits at their technological maturity. Seed stratification was then carried out at physiological maturity to ensure the seeds passed through the post-maturation stage. Following seed germination, the resulting hybrids were maintained in a greenhouse. Due to physiological reasons, some of the hybrids did not survive, and only the viable and properly developed hybrids were included in the molecular testing process. During the seedling stage, the hybrids were selected, retaining only those that were well-developed and healthy. For the correct identification of the hybrids, each hybrid combination created was assigned a unique identification code consisting of 6–7 digits (Cociu, Oprea 1989). The controlled hybridisations in the experiment are organised as follows in Table 2.

The interpretation of the results was quantified as follows: the number of hybrid seeds obtained, germination rate, hybrids that inherited the *Vf* gene, and the monitoring of the behaviour of the hybrids in the field trial related to scab infection.

The hybrids were tested using molecular methods to identify the presence/absence of the *Vf* (*Rvi6*) gene, at a very early stage of development. DNA isolation was performed from young leaves ground in liquid nitrogen using the Invisorb Spin Plant Mini Kit – Invitek Molecular extraction kit (INVITEK

Table 2. The hybrid combinations created in the apple species

Code scheme	Female genitor	Male genitor
21.01.	Auriu de Bistrița	Florina
21.02.	Jonathan	
21.03.	Golden Delicious	
21.04.	Starkrimson	
21.05.	Idared	

Table 3. Primer pairs used in the experiment for identifying the presence of the *Vf* gene in a hybrid apple population

Primer name	Primer type	Fragment size (bp)	Cycling parameters for amplification	References
AL07	Co	570 (*Vf*) 820 (*vf*)	initial denaturation: 95 °C 1 min 35 cycles × (denaturation: 94 °C 1 min, annealing: 60 °C 1 min, extension: 72 °C 2 min) final extension: 72 °C 10 min	Khajuria et al. (2014) Tartarini et al. (1999)
AM19	D	526 (*Vf*)		
VFC	Co	286 (*Vf*) 484 (*vf*) 646 (*vf*)	initial denaturation: 94 °C 4 min 35 cycles × (denaturation 94 °C 1 min, annealing: 58 °C 1 min, extension 72 °C 1 min) final extension 72 °C 7 min	Afunian et al. (2004)

Co – codominant; D – dominant; *Vf* – dominant gene; *vf* – recessive gene

Diagnostics, Germany), following the protocol provided by the manufacturer. The quantification of the extracted DNA was carried out using the NANODROP 2000c Spectrophotometer (Thermo Scientific, USA), measuring the optical density of the samples at absorbance rates of A260 and A280 nm. PCR reactions were carried out in 25 µL mix using the amplification kit My *Taq* Red Mix following the protocol provided by the manufacturer. After the amplification stages, a 10 µL aliquot of the amplified products was run onto a 1.5% agarose gel in 1× TAE buffer for 50 minutes. The 100 bp DNA Ladder RTU served as a size marker. The bands were visualised using RedSafe Nucleic Acid Staining Solution and analysed with the Quantity One 1-D Analysis Software system (version 4.6.8, 2009) under UV light.

To identify the *Vf* gene, three pairs of specific primers were used: AM19, AL07 and VFC (Table 3).

The statistical determination of the results (germination rate, as well as the hybrids that inherited the *Vf* gene was conducted using the XLSTAT – Addinsoft software (Addinsoft 2022), which runs on the Microsoft Office Excel platform. For variance analysis, the ANOVA test (Fisher 1925) was used, followed by the Duncan test at a significance level of $P < 0.0001$.

RESULTS AND DISCUSSION

Resistant hybrid selections. Following the analysis of the results regarding the quantification of hybrid seed number and the hybrid plants obtained through germination, the corresponding values for each hybrid combination are presented in Table 4.

After germination, healthy hybrid plants suitable for transplanting were selected. Thus, from the 802 hybrid seeds, 368 hybrids (46.3%) were transplanted after germination (Figure 2).

The hybrid combination with the highest percentage of germinated hybrids seeds was 'Auriu de Bistrița' × 'Florina' followed by the hybrid combination 'Jonathan' × 'Florina. On the opposite was the hybrid combination 'Golden Delicious' × 'Florina'. Other studies (Pasqualetto et al. 2019) have reported similar results in obtaining hybrid seeds from combinations that included some of the cultivars used in the present study. The differences among the hybrid combinations were statistically

Tabel 4. Number of seeds and resulting hybrid plants obtained from five apple hybrid combinations

Hybrid combination code	Hybrid combination	No. of seeds	Hybrid plants from germination	Germination rate (%)
21.01.	Auriu de Bistrița × Florina	162	145	89.5
21.02.	Jonathan × Florina	19	14	73.7
21.03.	Golden Delicious × Florina	203	45	22.2
21.04.	Starkrimson × Florina	322	133	41.3
21.05.	Idared × Florina	96	31	32.3
Total		802	368	45.9

Figure 2. Aspects regarding the development of the obtained apple hybrids

Table 5. Statistical interpretation of the germination rate for the obtained hybrid combinations

Hybrid combination	Germinated seeds (mean ± SE)
Auriu de Bistrița × Florina	89.500 ± 1.322^{a}
Jonathan × Florina	73.700 ± 5.271^{b}
Starkrimson × Florina	41.300 ± 3.411^{c}
Idared × Florina	32.300 ± 2.163^{d}
Golden Delicious × Florina	22.200 ± 5.040^{e}
$P > F$ (model)	< 0.0001

$^{a–e}$significant difference between the means with different letters (Duncan) within the error limits of $P < 0.0001$; P – probability; F – F-test (ANOVA)

significant, which were grouped into five distinct significance classes, with each combination falling into a separate class (Table 5).

The results obtained from the molecular analyses varied depending on the hybrid combination. In the case of the codominant primer AL07, amplification bands were obtained at 570 bp (*Vf*) and 820 bp (*vf*) (Figure 3A). For the dominant primer AM19, amplification bands were observed at 526 bp (*Vf*) (Figure 3B), while for the second codominant primer VFC, amplification bands were observed at 286 bp (*Vf*) as well as 484 and 646 bp (*vf*).

As shown in Table 6, it can be observed that in the combination 'Auriu de Bistrița' × 'Florina', a total of 95 hybrids were selected and tested. Among these, 38 inherited the resistance gene *Vf*, indicating that they are heterozygous (*Vfvf*), as evidenced by the amplification bands for both the recessive gene (*vf*) and the dominant gene (*Vf*). The remaining 57 hybrids exhibit a homozygous recessive genotype (*vfvf*), with amplification in the gel observed only for the recessive allele.

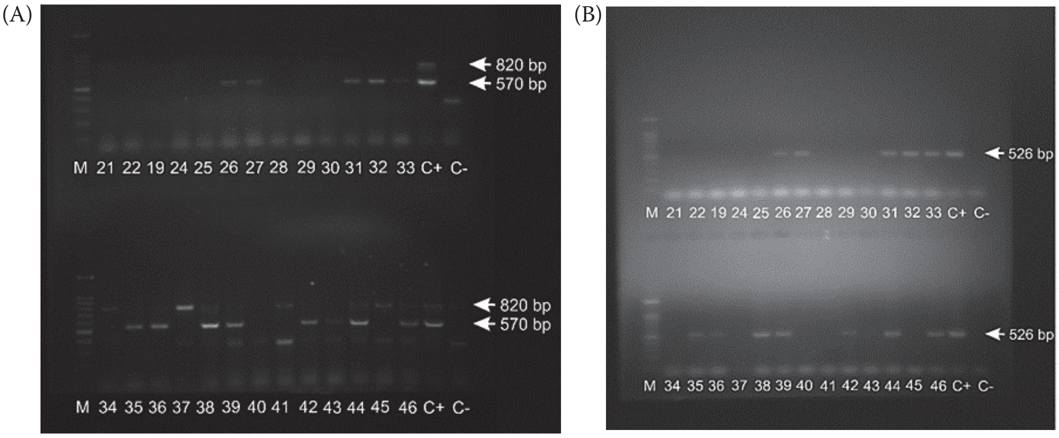

Figure 3. Agarose gel electrophoresis of some DNA products using two of the markers employed to identify the presence of the *Rvi6* (*Vf*) gene: (A) AL07 codominant primer; (B) AM19 dominant primer

M – molecular weight marker (DNA ladder); C+ – positive control; C– – negative control

Table 6. Distribution of the *Vf* resistance gene among hybrids from selected parental combinations

Hybrid combination code	Hybrid combination	Total apple hybrids tested	Hybrids inheriting the *Vf* gene	Hybrids not inheriting the *Vf* gene
21.01	Auriu de Bistrița × Florina	95	38	57
21.02	Jonathan × Florina	7	4	3
21.03	Golden Delicious × Florina	14	3	11
21.04	Starkrimson × Florina	49	29	20
21.05	Idared × Florina	31	13	18

A total of 7 hybrids from the combination 'Jonathan' × 'Florina' were selected for molecular analysis to identify the *Vf* gene. Among them, four were found to have the heterozygous genotype (*Vfvf*) as they inherited the dominant *Vf* gene. The remaining three hybrids did not inherit the dominant gene, resulting in a homozygous recessive genotype (*vfvf*).

From the hybrid combination 'Golden Delicious' × 'Florina', a total of 14 hybrids were selected for testing using the MAS method. Among them, only three inherited the resistance gene *Vf*, being heterozygous, while the remaining 11 carried only the recessive allele and were therefore excluded from further analysis.

A total of 49 descendant plants from the combination 'Starkrimson' × 'Florina' were selected and tested to determine the inheritance of the gene of interest (*Vf*) that provides resistance to apple scab. Among them, more than half (29) exhibited the heterozygous genotype (*Vfvf*) because they inherited the *Vf* gene. The other 20 did not inherit the resistance gene, were indentified as homozygous recessive (*vfvf*).

The last hybrid combination in the established scheme is 'Idared' × 'Florina'. A total of 31 hybrids were tested molecularly for the identification of the *Vf* gene. Among them, 13 inherited the resistance gene, making their genotype heterozygous (*Vfvf*), while the remaining 18 did not inherit the gene, thus presenting a homozygous recessive genotype (*vfvf*).

The inheritance percentages of the apple scab resistance gene (Table 7) show that the highest proportion was observed in the 'Starkrimson' × 'Florina' combination (59.2%), followed closely by 'Jonathan' × 'Florina' (57.1%). The lowest percentage of hybrids carrying the *Vf* gene was recorded in the 'Golden Delicious' × 'Florina' combination (21.4%)

Statistical analysis revealed significant differences among the hybrid combinations, which were grouped into five distinct significance classes. The combinations 'Starkrimson' × 'Florina' and 'Jonathan' × 'Florina' showed similar proportions of hybrids inheriting the *Vf* gene, with no significant difference between them. In contrast, the 'Golden Delicious' × 'Florina' combination differed significantly from all other combinations ('Starkrimson' × 'Florina', 'Jonathan' × 'Florina', 'Idared' × 'Florina', and 'Auriu de Bistrița' × 'Florina'), as indicated by the statistical results.

The observed deviation from expected Mendelian segregation ratios in the progeny of various hybrid combinations can be attributed to multiple, potentially interacting factors. One of the primary explanations is the limited number of plants used in the experiment, which inherently reduces statistical power and increases the influence of sampling error. Mendelian fashion is probabilistic and becomes more accurate as population size increases. In small populations, random genetic drift can significantly distort observed ratios, making it difficult to detect clear inheritance patterns. The deviation from expected segregation

Table 7. Transmission of the *Vf* gene to the offspring in the five hybrid combinations

Hybrid combination	Hybrids with *Vf* gene
Starkrimson × Florina	59 200 ± 3 064^{a}
Jonathan × Florina	57 100 ± 2 594^{a}
Idared × Florina	41 900 ± 3 568^{b}
Auriu de Bistrița × Florina	40 000^{c} ± 2 000^{bc}
Golden Delicious × Florina	21 400 ± 2 358^{d}
$P > F$ (model)	< 0.0001

$^{a–d}$significant difference between the means with different letters (Duncan) within the error limits of P < 0.0001; *P* – probability; *F* – *F*-test (ANOVA) ratios may also be influenced by complex genetic factors, including multiple genes with additive or epistatic effects. In addition, modifier genes, which do not directly control the trait, can alter its expression, potentially masking or enhancing the observed phenotypes.

Behaviour of the descendant plants in the hybrid field (1st year). Following the identification of hybrid plants carrying the *Vf* gene through the MAS method, a hybrid field was established to continue the selection process, with particular focus on evaluating phenotypic resistance to apple scab infection. The hybrid plants were planted in an area with high natural infection pressure of *Venturia inaequalis*, specifically in a field located near an old apple orchards containing cultivars known for to be susceptibility to apple scab ('Golden Delicious', 'Idared' and 'Jonathan'). Moreover, no fungicide treatments were applied in these orchards, which allowed for the clear expression of scab symptoms on the foliage. As a result, a constant and active source of pathogenic inoculum was ensured throughout the entire growing season. Although all hybrids transferred to the field in the spring of 2023 carried the *Vf* gene as confirmed by molecular analyses, some of them exhibited symptoms of apple scab after one year of growth. This may suggest that the *avrRvi6* isolates are present in this area. However, under the same field conditions, the hybrids exhibited varying responses to apple scab infection. According to preliminary results, hybrids from the combinations involving the female genitors 'Idared', 'Starkrimson' and 'Auriu de Bistrița' showed typical symptoms of apple scab, while those involving the female genitor 'Golden Delicious' and 'Jonathan' did not show symptoms (Figure 4). Thus, in the combinations 'Jonathan' × 'Florina' and 'Golden Delicious' × 'Florina', none of the hybrids exhibited symptoms of apple scab on the leaves.

The differences in the response to apple scab infection among the hybrids carrying the *Vf* gene can be attributed to the complex interaction between this gene and the genetic background of the parental lines While monogenic resistance, such as that conferred by the *Rvi6* (*Vf*) gene from 'Florina', follows clear inheritance patterns, other genetic backgrounds may involve quantitative resistance loci and minor effect genes interacting epistatically. Therefore, the presence or absence of tolerance in offspring may depend on the interaction of multiple genes inherited from both parents. This complexity may help explain why offspring from a susceptible parent like 'Jonathan' do not exhibit a higher susceptibility rate compared to other crosses. Although 'Jonathan' lacks major resistance genes, some of its progeny might inherit tolerance. In addition, transgressive segregation could occur, where offspring display phenotypes outside the range of the parents due to novel gene combinations. On the other hand the differences in the response to apple scab infection among the hybrids carrying the *Vf* gene can be attributed to the possible presence of virulent strains of *Venturia inaequalis*. This outcome highlights the importance of phenotypic evaluation alongside molecular screening in the selection for durable resistance. Conversely, the highest leaf infection rate was observed in the combination 'Idared' × 'Florina', where typical disease symptoms were noted in 76.9% of the hybrids, followed by the hybrid combination 'Starkrimson' × 'Florina' (45.8%) and 'Auriu de Bistrița' × 'Florina' (13.5%). Out of the total hybrids planted in the selection field,

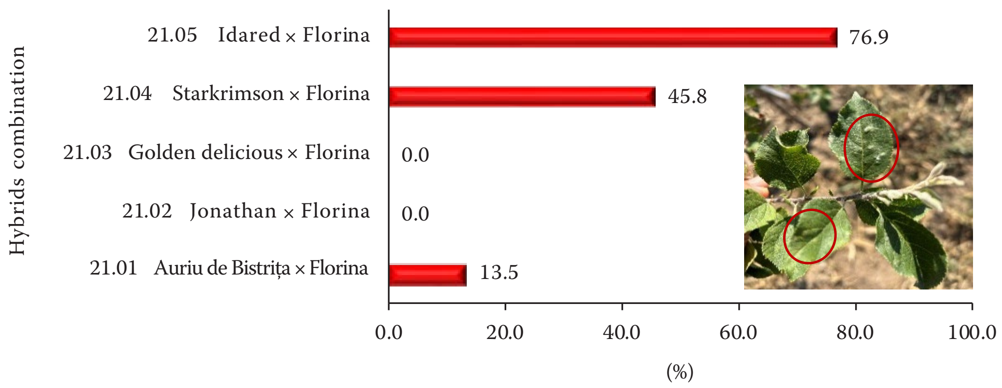

Figure 4. Results regarding apple hybrids infected with scab in the first year in the experimental field

32.1% were infected with apple scab. It is important to note that the hybrid plants in the field were not treated for fungal diseases.

According to recent studies in the field, 'Florina', used as the male parent in the present study, exhibits apple scab symptoms only on the leaves, and these occur less frequently than in other *Vf* cultivars. (Roșu Mareș et al. 2022). This study demonstrates that symptoms of apple scab also appear in the descendants of 'Florina' cv. Apart from the two combinations 'Jonathan' × 'Florina' and 'Golden delicious' × 'Florina', which showed no symptoms of scab, combination 'Auriu de Bistrița' × 'Florina' comes in third place, with only 13.5% of the hybrids exhibiting symptoms of scab. This can be explained by the fact that the female genitor ('Auriu de Bistrița'), is known as tolerant to apple scab infections (Guzu et al. 2021), and hence the possibility to partly transmit it to progenies.

CONCLUSION

The MAS method has proven to be an essential tool in the selection of apple hybrids, significantly reducing the duration of the breeding process. The hybrids obtained through the hybridisation scheme showed different percentages of inheritance of the *Vf* gene, generally following Mendelian segregation patterns. However, under field conditions, the progeny carrying the *Vf* gene exhibited variable levels of resistance to apple scab. Thus, in the first year after being transferred to the field, approximately 30% of the hybrids with the *Vf* gene were affected by scab, suggesting that the resistance provided by this gene is not enough to fully protect the plants. Based on these results, it is recommended that apple breeding programs in Romania be focused on developing complex resistances to apple scab.

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