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Genetic diversity of host-pathogen and breeding for resistance to *Venturia inaequalis* Cooke (Wint.): a review

Diversitatea genetică gazdă-parazit și ameliorarea pentru rezistența la rapănul mărului – *Venturia inaequalis* Cooke (Wint.): sinteză

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ABSTRACT

Malus x domestica Borkh. is one of the most important fruit tree species grown in temperate climates, ranking second in fruit production and cultivated area after bananas, worldwide. Despite the apple industry's success, the apple scab disease poses a serious problem for commercial orchards around the world. Apple scab is caused by the ascomycete fungus *Venturia inaequalis*, which affects both leaves and fruits of most apple trees, leading to significant economic losses, except for the cultivars that show monogenic or complex resistance. Due to the eight physiological races of the pathogen identified to date and their ability to proliferate in various ecological conditions, the disease still remains difficult to control in apple orchards. Numerous, expensive and phenological stage-specific treatments need to be applied during the vegetative period in order to keep this disease under control. In this context, the use of scab-resistant apple cultivars becomes a reliable and promising alternative for cost-effective and profitable orchard management. Therefore, this review focuses on current trends and details regarding the genetic determination of apple scab resistance (both vertical and horizontal) and their association to achieve complex resistance. The review also describes the genes involved and the phenomenon of apple scab resistance breakdown. Special attention is given to Marker-Assisted Selection (MAS) and specific markers for scab resistance, which are frequently used in current apple breeding programs.

Keywords: *Malus x domestica*, MAS, scab resistance genes, vertical resistance, horizontal resistance

REZUMAT

Malus x domestica Borkh. este una dintre cele mai importante specii de pomi fructiferi cultivate în climat temperat, ocupând la nivel mondial, locul al doilea în producția și suprafața cultivată cu fructe, după banane. În ciuda succesului industriei mărului, rapănul reprezintă o problemă serioasă pentru livezile comerciale din întreaga lume. Rapănul mărului este cauzat de ciuperca *Venturia inaequalis*, care afectează atât frunzele, cât și fructele majorității soiurilor de măr. Acest lucru ducând la pierderi economice semnificative, excepție făcând soiurile care prezintă rezistență monogenică sau complexă. Din cauza celor opt rase fiziologice ale patogenului, identificate până în prezent și capacității lor de a prolifera în diverse condiții ecologice, boala rămâne încă dificil de controlat în livezile de măr. Sunt necesare numeroase tratamente scumpe și specifice stadiului fenologic în care se află pomul, pentru a ține sub control această boală în timpul perioadei vegetative. În acest context, utilizarea soiurilor de măr rezistente la rapân devine o alternativă fiabilă și promițătoare pentru o gestionare rentabilă și profitabilă a livezii. Prin urmare, în acest review, s-a acordat atenție tendințelor actuale și detaliilor privind determinarea genetică a rezistenței (atât verticală cât și orizontală) la rapănul mărului, dar și asocierea lor pentru obținerea rezistenței complexe. De asemenea, s-a realizat descrierea genelor și fenomenul de degradare a rezistenței mărului la rapăn. O atenție deosebită a fost acordată și selecției asistate de markeri (MAS) și markerilor specifici pentru rezistența la rapăn, care sunt frecvent utilizați în programele actuale de ameliorare a mărului.

Cuvinte cheie: *Malus x domestica*, MAS, gene de rezistență la rapăn, rezistență verticală, rezistență orizontală

INTRODUCTION

The apple is one of the most valuable fruit crops worldwide, playing a major role in agriculture due to its economic, nutritional, and cultural significance. This fruit species is cultivated on all continents and is appreciated for its versatility, from fresh consumption to use in various culinary preparations and the food industry. Moreover, the apple is considered a symbol of health, thanks to its high content of vitamins, fiber, and antioxidants, significantly contributing to human nutrition (Spengler, 2019). In Romania, apple cultivation ranks second in importance after plum cultivation. Globally, apples are grown on approximately 4.65 million hectares, yielding a total production of 95.6 million tons, according to FAO STAT data from 2024 (FAO STAT, 2024). According to the most recent statistics, Romania recorded a production of approximately 543 thousand tons, obtained from a cultivated area of 54 thousand hectares (FAO STAT, 2024).

The domesticated apple, also known as *Malus x domestica* Borkh. belongs to the *Malus* genus, Rosaceae family and the *Meloideae* subfamily, being considered a functional diploid (2n = 34) (Ryugo, 1988; Volk et al., 2021). The *Malus* genus itself consists of both wild and commercial apple genotypes, but *Malus x domestica* is the most important fruit crop in temperate regions cultivated worldwide (Zelmene et al., 2022). The large distribution of apples is due to their ecological plasticity, which allows them to adapt to various environmental conditions, even with high ecological amplitude. On the other hand, this species is very attractive both for farmers and consumers because the shelf life and storage duration of fresh fruits are very long in comparison to other fruits, up to six months (Akyereko et al., 2023). This fact is supported by the famous proverb “An apple a day keeps the doctor away” (Raskin and Ripoll, 2004).

During the 20th century, apples, like other fruit species, were subjected to intense breeding programs to improve the existing assortment by introducing foreign cultivars among the local ones or creating new cultivars with increased resistance against pests, diseases and abiotic stresses associated with high sensory qualities of the fruits. Currently, major breeding works are focusing on the breeding of new apple cultivars with long-term resistance against major and specific diseases. In this context, combining different resistance genes in the same genotype is a reliable way to achieve durable resistance (Höfer et al., 2021).

The currently existing scientific literature in the field suggests that, along with abiotic stress, apple trees are affected by a wide variety of pathogens as well, such as fungi, bacteria, viruses, and mycoplasmas. It has been reported that apple species can be affected by more than 70 fungal diseases, which severely depreciate both the tree and fruits (Belete and Boyraz, 2017) if no control measures are taken. For this reason, in orchards where severe fungal diseases such as scab are present, rigorous and intensive tree protection management is needed (Rancāne et al., 2023). Thus, in some cases, around 20 and 30 fungicide-based treatments must be applied annually to avoid severe infections with this destructive fungus (Sokolova and Moročko-Bičevska, 2022). Ineffective scab treatments can cause scab epidemics at harvest time, with severe scab lesions on both fruits and leaves and lead to early defoliation of the apple trees in autumn (Antal et al., 2024). Ecological management practices, which are environmentally friendly, are increasingly desired in the current context of EU requirements to drastically reduce the use of pesticides for crop protection. Unfortunately, these approaches are associated with a yield reduction of an average of 48% compared to the one obtained through conventional management (Belete and Boyraz, 2017). One of the promising strategies that could solve these problems partially is the selection and use of scab-resistant cultivars in the fruit production industry. In the global assortment, there are some newly-introduced apple cultivars which, besides their high degree of resistance against this disease, stand out by their fruit quality, such as: Akane, Ariwa, Crimson Crisp, Dalinred, Generos, Goldrush, Grenadier, Primula, Red Topaz, Regunde, Relinda, Virginia Crab, etc. (Jönsson, 2007; Höfer et al., 2021; Strickland, 2023; Crassweller, 2023). Therefore, breeding approaches in developing new cultivars, with long-lasting resistance and high fruit quality, are indispensable not only for nature-friendly but also for an economically feasible apple cultivation.

In this regard, all the work that is being carried out to obtain new and valuable apple genotypes requires first vast amounts of knowledge of the pathogen. Thus, the current paper aims to review (i) the biology and symptomatology of the pathogen, (ii) the defense mechanisms of apple species against scab, and (iii) the new selection methods through molecular markers to support future breeding programs.

PATHOGEN TAXONOMY AND SYMPTOMATOLOGY

Venturia inaequalis belongs to the *Fungi* kingdom, subphylum *Ascomycota*, order *Dothideales*, which consists of evolved fungi.

The genus *Venturia* includes the species *Venturia inaequalis* (specific to apple species) and *Venturia pyrina* (specific to pear species). The fungus is a facultative parasite and presents two phases, one parasitic and one saprophytic, each playing a role in the evolution of the disease. The first phase, the parasitic one, corresponds to the development of the imperfect (asexual) form known as *Fusicladium dendriticum*, and the second phase, saprophytic, with the development of the perfect form - *Venturia inaequalis* (Baicu and Șesan, 1996) evolutionary cycle of the fungus begins with the parasitic phase, from the moment the primary infection is realized (Mesarich, 2011).

Apple scab (brown spotting) is known globally by farmers and researchers where apples are cultivated. In general, it occurs in established orchards from wet climate areas, but it can also take place during fruit storage if the appropriate treatments were not applied before fruits are introduced to storage conditions. The disease was first described in the summer phase by a Swedish botanist (Fries, 1819). The winter phase of the disease was illustrated by Cooke (1866). In years with rainy springs and summers, the fruit production of an apple orchard can decrease by 30% or up to 98% if no plant protection measures are applied against *Venturia inaequalis* (Şuta and Floru, 1974). Scab can affect aerial organs of the apple tree: leaves, flowers, branches, and fruits (Figure 1).

The infected leaves fall before the healthy ones during the summer (Aćimović et al., 2016). In the case of flowers, scabs mainly attack the sepals (Ward, 2012). On the surface of the fruits, as well as on other organs, grey-olive spots appear, in front of which the tissue is corking and cracking. Most of the time, the cracks on the surface of the fruits represent entry holes for the spores of the *Monilinia fructigena* fungus, but also for other fungi that can destroy the fruit pulp (Sestraș, 2003). The fungus causes an exfoliation of the tree bark, and under the infected tissues, the cork is formed, which separates the healthy part from the affected one (Oliver, 2024).

PHYSIOLOGICAL RACES OF THE *VENTURIA INAEQUALIS* FUNGUS

The first research on the races of *Venturia inaequalis* fungus began very early, although most of the investigations undertaken in the field were focused on studying morphological differences and the mode of infection. The first three races of *Venturia inaequalis* were characterized by Williams and Brown (1968), races 4 and 5 by Williams and Kuc (1969), race 6 by Parisi et al., (1993) race 7 by Roberts and Crute (1994) and the last one, race 8, by Bus et al. (2005a). In the end, eight physiological races of scab were cataloged based on differences in pathogenicity depending on apple cultivars (the pathogen *Venturia inaequalis* - Table 1). Due to the ability of some *Venturia* isolates to develop on two different hosts and

Figure 1. Scab symptoms on leaves and fruits

the difficulty in classifying them into distinct races, a new terminology was introduced, reconsidering the differential hosts used in previous studies. It was proposed to use differential hosts that carry only one *R* (resistance) gene. This nomenclature system is compatible with international standards and was updated at the time of the discovery of a new gene called *R* (Gopaljee et al., 2009).

In addition to the previously identified eight races, Bus et al. (2011) introduced a new race, namely race 0, which demonstrates its inability to sporulate on apple cultivars containing major scab resistance genes. Theoretically, *Venturia inaequalis* could possess as many races as there are key scab resistance genes (Khajuria et al., 2023). Moreover, Masny (2017) reported the discovery of races 9, 10, 13, and 14, which emerged from a mixed inoculum of *Venturia inaequalis* collected from various apple orchards in Poland. This suggests a high potential to recombine its genetic material, which led to a high variability of scab races capable of overcoming resistance in different cultivar stems (Masny, 2017).

SCAB RESISTANCE IN APPLES

Resistance mechanisms, types and genes

While the evolution of scab races is increasingly pronounced, it is necessary to know the types of apple resistance, the resistance genes and implicitly the way of combining them to obtain a durable type of resistance. Improving apple resistance to scab became one of the main objectives of different apple breeding programs in many countries such as: Canada (McClure et al., 2018), France (Lasserre-Zuber et al., 2018), Spain (Pereira-Lorenzo et al., 2018), Czech Republic (Zelmene et al., 2022), Switzerland, (Bühlmann-Schütz et al., 2023), UK (Stewart et al., 2023), Kazakhstan (Madenova et al., 2024) including Romania (Sturzeanu et al., 2020; Militaru et al., 2020; Bivolariu et al., 2021; Militaru et al., 2022; Sestraș and Sestraș, 2023; Bivolariu et al., 2023). The use of high-quality apple cultivars with durable resistance can help to face the challenges caused by scab (Švara, 2022).

Table 1. Physiological races of the fungus *Venturia inaequalis* (Gopaljee et al., 2009)

The name of the race	Typical symptoms for each race	The origin of physiological races	References
Race 1	Lesions that do not sporulate on Dolgo, R 12740-7A (Russian cultivar) and Geneva	USA	Williams and Brown, 1968
Race 2	Lesions that sporulate on Dolgo, Geneva, and progeny of R 12740-7A		
Race 3	Lesions that sporulate on Geneva and lesions that do not sporulate on Dolgo and R 12740-7A		
Race 4	Lesions that do not sporulate on Dolgo, Geneva and lesions that sporulate on the progeny of R 12740-7A, on which the isolates from Race 2 do not sporulate	Purdue (USA)	Williams and Kuc, 1969
Race 5	Lesions that sporulate on cultivars containing the *Vm* R gene	USA	
Race 6	Lesions that sporulate on *Vf* hybrids but cannot infect *Malus Floribunda* 821, which contains the *Vfh* R gene	Ahrensburg, Germania	Parisi et al., 1993
Race 7	It can infect cultivars that contain the *Vf* and *Vfh* R genes, but cannot infect Golden Delicious, which contains the *Vg* gene	England	Roberts and Crute, 1994
Race 8	It can infect Golden Delicious, Royal Gala, and cultivars that contain the *Vh8* R gene	New Zealand	Bus et al., 2005b

Improving apple resistance to scab is achieved through modern methods such as genetic modifications (GM), specifically the creation of cisgenic and intergenic apple lines (Krens et al., 2015). GMs (Genetically Modified organisms) are used to accelerate the breeding process, given that traditional breeding requires a longer time. The first reports of obtaining cis-genic lines were made in 2011 by Vanblaere et al. (2011). Another significant result in the field was reported by Würdig et al. (2015), who used 22 transgenic lines for the apple cultivars Brookfield, Baigent, Mitchgala, Navajo, and Pinova. Of these, 16 lines showed resistance to race 1 of the fungus *Venturia inaequalis*. The analysis of the transgenic lines showed that *Rvi6* (scab resistance gene) mRNA (messenger RNA) expression levels were similar to those in several traditionally bred *Rvi6*-containing cultivars and identified four transgenic lines with a single T-DNA (Transfer DNA) insertion as suitable candidates for producing cis-genic lines.

Genetic determinism of apple resistance to scab is presented in Figure 2. The resistance of the apple to the scab is of two kinds: inherited (constitutive) into a natural host (vertical or horizontal) or induced (won) by genetic engineering (transgenesis) or NGT (New Genomic Techniques).

Vertical resistance (monogenic)

Vertical resistance has a monogenic determinism (a single major gene) or oligogenic determinism (which involves a small number of genes, usually 2-3 genes). In the case of oligogenic determinism, the major contribution to the manifestation of resistance is provided by one of the genes, which is called the major gene. In both cases, the major genes can be dominant or recessive. On one hand, if the resistance is monogenic, in the crossing between a resistant cultivar (heterozygote) and a sensitive variety, the resistance will be inherited by half of the progeny according to the Mendelian ratio.

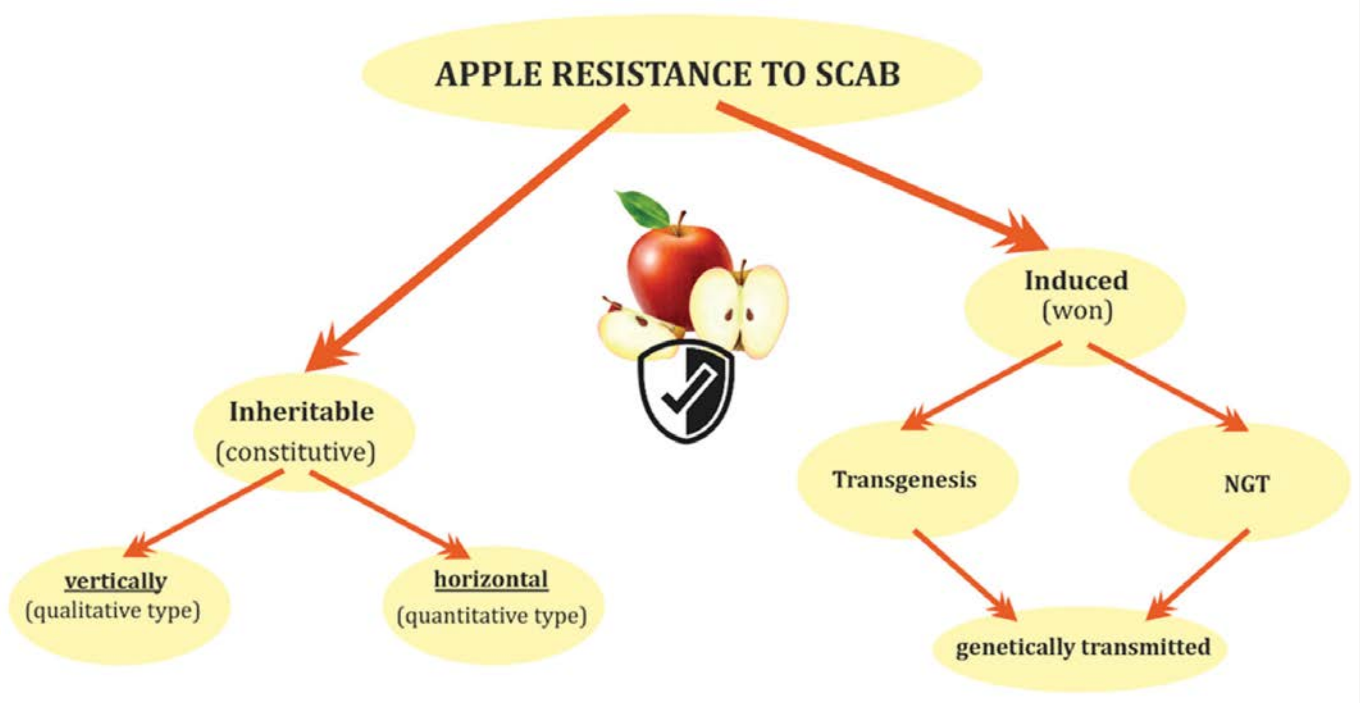

Figure 2. Genetic determinism and protection of apple trees against *Venturia inaequalis* (modified according to Zagrai (2002))

The main advantage of this type of resistance is its ease of transfer and selection through conventional breeding methods. However, the drawback lies in its relative stability, as it is race-specific. The stability of vertical resistance is determined by the robustness of the genes responsible for it.

Thus, the stronger the gene, respectively the oligogenes, the more durable the protection will be (Zagrai, 2002). This means that the barriers of vertical resistance can be easily overcome if the variability of the pathogen is very high, respectively if the gene is not strong enough.

The qualitative resistance against apple scab, also known as gene-for-gene (GfG – Papp et al., 2020) is given by one or just a few dominant genes with a major effect, like those *Rvi* (monogenic or oligogenic control) that confer specific race resistance which is complete or at least almost complete when the pathogen carries a corresponding *Avr* (avirulence) gene (Švara, 2022). By definition, an *Avr* gene is a type of gene whose product negatively affects the ability of a pathogen to cause diseases (Khajuria et al., 2023). It is worth mentioning that qualitative resistance, when it is encountered it often expresses a hypersensitivity response (Zagrai, 2002). Nowadays, the improvement of cultivars for scab resistance mainly involves the integration of qualitative resistance genes like those of *Rvi*, as they come from different species of *Malus*.

The monogenic sources of apple resistance to scab

More than 20 major genes (Table 2) that confer resistance to scab have been discovered in the apple's genome that come either from different cultivars or wild species of *Malus* (Khajuria et al., 2018). These discovered genes show a variable phenotypic reaction and a variation in the duration at which the resistance reaction was exhibited when the plant was infected by the fungus *Venturia inaequalis*. Depending on the surface area affected by the pathogen and the nature of the symptoms found in different cultivars, the classification of responses could be made in classes: 0, 1, 2, 3a (syn. Class M), 3b (syn. Class 3), and 4 (Gopaljee et al., 2009). Classes between 0 and 3 are known to have resistant responses, and class 4 contains those that have a susceptible response.

Table 2. The resistance genes of apple to scab, respectively, the *Avr* gene corresponding to each *Rvi* gene (Khajuria et al., 2018)

No	Differential host/ source ascension	Old name	New name	Resistance locus (genomic location in LG)	Resistance reaction	Corresponding avirulence gene
1	Royal Gala	-	-		Susceptible	0
2	Golden Delicious	*Vg*	*Rvi1*	12 (Distal end)	Necrosis	*AvrRvi1*
3	TSR34T15	*Vh2*	*Rvi2*	2 (Distal end)	Stellate necrosis	*AvrRvi2*
4	Geneva[a]	*Vh3.1*[b]	*Rvi3*	4	Stellate necrosis	*AvrRvi3*
5	TSR33T239	*Vh4=Vx=Vr1*	*Rvi4*	2 (Distal end)	Hypersensitive response	*AvrRvi4*
6	9-AR2T19	*Vm*	*Rvi5*	17 (Distal end)	Hypersensitive response	*AvrRvi5*
7	Priscilla	*Vf*	*Rvi6*	1 (Distal end)	Chlorosis	*AvrRvi6*
8	*Malus × floribunda* 821[a]	*Vfh*	*Rvi7*	8	Hypersensitive response	*AvrRvi7*
9	4B5	*Vh8*	*Rvi8*	2 (Distal end)	Stellate necrosis	*AvrRvi8*
10	K2	*Vdg*	*Rvi9*	2	Stellate necrosis	*AvrRvi9*
11	A723–6[a]	*Va*	*Rvi10*	1	Hypersensitive response	*AvrRvi10*
12	A722–7	*Vbj*	*Rvi11*	2 (Distal end)	Stellate necrosis/chlorosis	*AvrRvi11*
13	Hansen’s baccata #2	*Vb*	*Rvi12*	12 (Distal end)	Chlorosis	*AvrRvi12*
14	Durello di Forli	*Vd*	*Rvi13*	10 (Proximal end)	Stellate necrosis	*AvrRvi13*
15	Dülmener Rosenapfel[a]	*Vdr1*	*Rvi14*	6 (Proximal end)	Chlorosis	*AvrRvi14*
16	GMAL 2473	*Vr2*	*Rvi15*	2 (Proximal end)	Hypersensitive response	*AvrRvi15*
17	MIS op 93.051 G07[a]	*Vmis*	*Rvi16*	3	Hypersensitive response	*AvrRvi16*
18	Antonovka APF22[a]	*Va 1*	*Rvi17*	1	Chlorosis	*AvrRvi17*
19	1980-015-025[a]	*V25*	*Rvi18*	11	Hypersensitive response	*AvrRvi18*
20	Honeycrisp[a]	-	*Rvi19*	1	All classes of resistance reactions	*AvrRvi19*
21	Honeycrisp[a]	-	*Rvi20*	15	All classes of resistance reactions	*AvrRvi20*

a – a temporary differential host until the monogenic nature of the resistance gene is confirmed or a monogenic cultivar is generated

b – *Vh3.4* may be the true *Rvi3* but not confirmed

c – The avirulence gene has been identified

LG – linkage group

Mature leaves show an ontogenic resistance, due to which the development of the pathogen is inhibited after the cuticle penetration and the appearance of specific disease symptoms is delayed. The thickness of the leaf cuticle of apple cultivars, as well as the thickness of the wax layer on its surface, plays an important role in this type of resistance by the fact that mycelial hyphae cannot penetrate through the thickened cuticle (Cociu et al., 1999; Leca et al., 2020).

According to Höfer et al. (2021), although the apple's resistance to scab is conferred by 20 resistance genes, some of them (*Rvi1*, *Rvi3*, *Rvi8*, and *Rvi10*) can no longer be used in breeding programs since the infection races of scab exceed the resistance provided by these genes. Other genes, such as *Rvi2*, *Rvi4*, *Rvi6*, *Rvi7*, *Rvi9*, and *Rvi13*, are only useful when used in combination with other resistance genes. Thus, the only genes that offer optimal resistance are *Rvi5*, *Rvi11*, *Rvi12*, *Rvi14*, and *Rvi15*. This is further confirmed by the VINQUEST project (Patocchi et al., 2020). Four of these come from wild species, which have small-sized fruits with low commercial value. Therefore, the genetic bases for improving resistance to scab are quite limited. In this context, expanding the genetic base by introducing additional resistance genes into a single genotype is necessary and of global interest, especially since the quality of the fruits is directly influenced by the health of the tree (Höfer et al., 2021).

Horizontal resistance (polygenic)

Horizontal resistance is not associated with a particular resistance mechanism but only refers to the number of genes involved in resistance (Yousaf, 2022). These genes are called quantitative trait loci (QTLs) as reported by Lindhout (2002). Horizontal resistance (polygenic) is formed from the simultaneous action of several minor genes (polygenes), and for this reason, it is also called quantitative resistance (Khan and Korban, 2022; Mookiah et al., 2021; Zagrai, 2002). In the case of this type of resistance, the effects of the genes are cumulative, with each gene acting individually but vertically. The major disadvantage of polygenic resistance is because of its only partly transmission to progeny, losing effectiveness over time. However, horizontal resistance involves mechanisms that are outside the variability of the pathogen, being thus more stable and durable than the vertical one (Karlström et al., 2019).

The spectrum of polygenic resistance genes of apple to scab will be rendered according to Khajuria et al. (2018) (Table 3).

Association of vertical with horizontal resistance

An association between vertical and horizontal resistance is the most desirable trait in a new cultivar because it confers more effectiveness and durability. Ignoring the horizontal resistance in breeding works by replacing it with the vertical one can have negative consequences when vertical resistance is overcome by new races of the pathogen, and the given cultivars are completely devoid of other forms of resistance. Incorporating horizontal resistance into a new genotype that possesses vertical resistance is difficult to achieve by classical selection due to the dominance of vertical resistance over horizontal resistance (Zagrai, 2002). However, in this case, backcrosses are needed to incorporate horizontal resistance into the genotype, sometimes even five, according to research conducted by Luo et al. (2020).

Other reports suggest that the most effective strategy for improving durable resistance to scab is the association of a genotype with monogenic (vertical) resistance of the maximum number of horizontal resistance polygenes (Braniște and Andreieș, 1990). Similar results were revealed by Lespinasse (1992), claiming that Institut National de la Recherche Agronomique (INRA) Angers, in France, crosses of the type *Vf x Vf*, *Vf x poly*, and poly x poly were made, while in Germany crosses of the type *Vf x Vr*, *Vf x Va*, *Vr x Va* were made, obtaining a resistance considered more durable.

Improving the resistance of genotypes that already have horizontal-type resistance is possible by incorporating vertical resistance genes from other genotypes. In this case, the most suitable method is also the backcross, using as a recurrent parent the variety whose resistance is desired to be strengthened, and as a donor parent the variety with vertical resistance (Zagrai, 2002).

Table 3. QTLs associated with the resistance against *Venturia inaequalis* in apple (Khajuria et al., 2018)

S. no.	Location	No. of QTLs	Identified from the cross	Remarks
1	LG1	1	Cross progeny of Prima x Fiesta Discovery x TN10-8 progeny	Located close to the *Rvi6* (*Vf*)
2	LG2	1	Discovery x TN10-8 progeny	Broad spectrum Co localized with *Rvi15* (*Vr2*) and *Rvi8* (*Vh8*)
3	LG5		Discovery x TN10-8 progeny	Isolated specific (two isolates)
4	LG6	1	Progeny of the cross, Gala and Dulmener Rosenapfel	Co-localised with *Rvi14*
5	LG7	1	Cross of Fiesta and Discovery	-
6	LG10	1	Cross of Fiesta and Discovery	-
7	LG11	1	Cross of Fiesta and Discovery Progeny of the cross, Gala and Dulmener Rosenapfel	Broad-spectrum QTL Same as detected by (Durel et al. (2003) and Liebhard et al. (2002)
8	LG12	1 1	Cross of Fiesta and Discovery Discovery x TN10-8 progeny	Isolate specific
9	LG13	1	Discovery x TN10-8 progeny	Isolate specific
10	LG15	1 1	Cross of Fiesta and Discovery Discovery x TN10-8 progeny	Isolate specific
11	LG17	1	Cross of Fiesta and Discovery Discovery x TN10-8 progeny Cross of Prima and Discovery Progeny of the cross, Gala and Dulmener Rosennapfel	Highly scab-resistant Broad spectrum Same as detected by Durel et al. (2003) and Liebhard et al. 2002)

LG- linkage group; QTLs - quantitative trait loci

New genomic techniques

NGT (New Genomic Techniques) represents a set of modern genetic engineering tools and technologies that have a significant impact on the development and improvement of apple cultivars (Jacobson et al., 2023). These techniques include advanced gene editing methods and other modern genomic interventions that enable precise and rapid changes to the genomes of organisms.

For example, CRISPR-Cas9 is a precise gene editing method that allows the addition, deletion or modification of specific DNA sequences (Jacobson et al., 2023). This method has been used to modify genes involved in ripening and pectin degradation processes, which can lead to crisper and longer-lasting apples.

The ARCTIC apple cultivar was developed by Okanagan Speciality Fruits in Canada using this method. The "Arctic" apple has been genetically modified to suppress the expression of the polyphenol oxidase (PPO) gene responsible for browning. This modification does not affect the taste, texture or safety of the apple, but only prevents the enzymatic reaction that causes browning when the fruit is cut or damaged (Waltz, 2015).

The researchers also used CRISPR-Cas9 to edit the MdDIPM-1 gene in the genome of *Malus domestica*, a gene involved in susceptibility to fire blight (*Erwinia amylovora*). By knocking out this gene, the apple becomes resistant to the pathogen (Pompili et al., 2020).

Another example of disease resistance obtained through NGT is the overexpression of the Lc gene from *Zea mays* (corn) that led to improved resistance to both apple scab and fire blight (Flachowsky et al., 2010). However, the transgenic plants (Holsteiner Cox) had an altered tree form in commercially unacceptable ways (Jacobson et al., 2023).

Although NGT methods are of great interest mainly due to the short time required, for now, the progress achieved in resistance to apple scab is limited. Regarding the *Vf* gene, it is possible that the researchers' interest in its transfer is not very high due to its defeat in many areas of the globe.

Scab resistance degradation as a phenomenon

Research work in apple breeding shows that the most frequently used gene that confers resistance to scab, namely *Rvi6* (*Vf*), was defeated by races 6 and 7 of the pathogen *Venturia inaequalis*. This might be explained by the presence of a new physiological race of scab in certain geographical areas, or a degradation of the genes that confer resistance to certain diseases. For example, in Romania, the cultivars that have been proved to be resistant due to the presence of the resistance gene *Rvi6* (Florina, Aura, Bistritean, Salva – Figure 3) a fact also proven through MAS (Marker-Assisted Selection), showed typical symptoms of scab on leaves (Roșu-Mareș et al., 2022). Another example has been revealed in Poland, in different apple orchards containing cultivars carrying the *Rvi6* gene (Topaz, Freedom, Ariwa, Rajka, etc.) that showed apple scab symptoms in various percentages along three years of study (Masny, 2017).

As a result of the extensive investigations related to the different resistance genes in apple, it is known now that *Rvi6* gene is the only gene that exerts selective pressure on pathogen populations and the populations virulent to *Rvi6* cultivars can interbreed with the avirulent populations when they occur in the same orchard (Michalecka et al., 2018). This represents valuable information in understanding the unique genetic and virulence patterns that occur in scab populations that infect *Rvi6* apple cultivars. In terms of losing the resistance to scab is not necessarily a degradation of an apple resistance gene but more often a change of the virulence of *Venturia inaequalis* population (Passey, 2019).

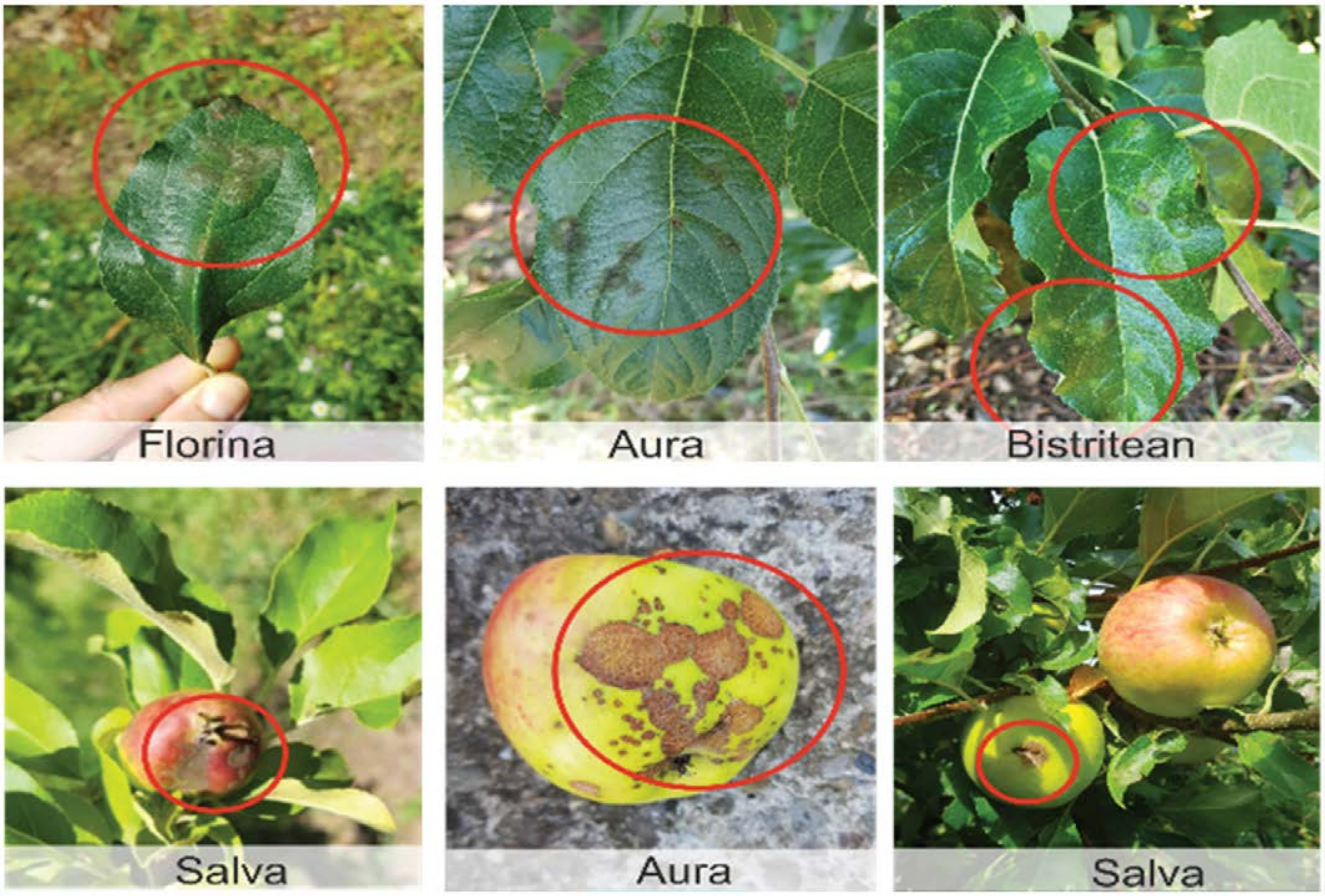

Figure 3. The symptoms of scab on leaves and fruits in cultivars that contain the *Vf* gene

THE USE OF MAS IN APPLE BREEDING

Marker Assisted Selection (MAS) is a process in which a molecular marker is used for the indirect selection of a gene that defines a trait of interest. MAS combines conventional breeding techniques with those of molecular biology (Lema, 2018). Modern breeding and selection methods are continuously developing, and MAS has become one of the most commonly used techniques owing to multiple advantages. For example, hybrid plants resulting from artificial hybridizations can be tested by molecular methods from the seedling stage, maintenance costs for hybrid plants decrease as it is no longer necessary to grow them for several years to check the response to a certain disease, and it reduces the land area occupied by hybrid plants because only plants resistant to a certain disease are kept in the field of hybrids (Cobb et al., 2019). Therefore, the applicability and efficiency of this method emerge from the above. Another advantage of MAS was confirmed by Guzu et al. (2022), within an investigation indicating that the new selection method and implicitly specific molecular markers may help to clarify the presence of genes that offer resistance to scab in the genome of some cultivars that were initially selected by empirical methods.

Molecular marking has the advantage of not being limited by the phenotypic expression of traits at the level of the entire plant, thus allowing the quantification of genetic variation directly at the DNA level (Hasan et al., 2021). Throughout the research, a series of techniques have been developed to highlight genetic variation; thus, a large number of molecular markers have appeared. Molecular markers that are closely linked with important genes, in an agronomic sense, are used as molecular tools for MAS in plant breeding. Through molecular markers, it becomes possible to monitor the performance of an indirect selection of characters of interest using the close link (linkage) between the gene or genes that control the desired trait and a molecular marker (Hasan et al., 2021). In Table 4, the advantages and disadvantages of the most commonly used molecular markers are presented.

Molecular markers are often used in apple species due to their high level of heterozygosity and pronounced polymorphism (Yao, 2022). Isoenzymatic techniques can be used as methods of identifying cultivars. Markers can have a special utility in making selections from the early phase of hybrids, which simplifies and significantly reduces costs. Most of the morphological and physiological characteristics, especially those important for apple breeding, can be identified at the DNA level, such as scab resistance given by the *Vf* type gene, the anthocyanin color of the fruits, columnar growth and flowering time (Jain et al., 2022). The most important applications of molecular markers in plant breeding are represented by marker-assisted selection, backcross acceleration, genetic differences between different populations and diversity detection (Leonte, 2011).

In apple species, molecular markers are used on the one hand to identify genes that provide scab resistance in progenies, and on the other hand to identify the sources of resistance, all of these through MAS (Zahid et al., 2022).

In apple species, molecular markers are used to identify genes that provide scab resistance in progenies and the sources of resistance (Zahid et al., 2022). The latest research in the field conducted by Švara et al., (2024) provides an overview and count of the markers used for identifying the commonly genes in apple that confer resistance to apple scab. According to this study, a total of 83 molecular markers are utilized, as follows: 5 (gene *Rvi1*); 5 (gene *Rvi2*); 6 (gene *Rvi3*); 13 (gene *Rvi4*); 11 (gene *Rvi5*); 14 (gene *Rvi6*); 1 (gene *Rvi7*); 1 (gene *Rvi8*); 1 (gene *Rvi10*); 7 (gene *Rvi11*); 3 (gene *Rvi12*); 2 (gene *Rvi13*); 3 (gene *Rvi14*); 5 (gene *Rvi16*); 1 (gene *Rvi17*); 5 (gene *Rvi18*).

In order to achieve good results within the selection process, it is crucial to choose the proper molecular markers. In the past, the early used molecular markers were about 10 cM from the actual gene of interest; nowadays, they are much closer, about 1cM from the gene (De Mori and Cipriani, 2023). The first markers (Tartarini et al., 1999; Patocchi et al., 2005) were RAPD (Random Amplified Polymorphic DNA) markers linked to *Rvi6* and

Rvi11. Afterwards, AFLP markers (Amplified Fragment Length Polymorphism) were mapped (Xu and Korban, 2000), linked to different loci and SSR (Simple Sequence Repeat) markers (Bianco et al., 2014, 2016) were used to genotype apple hybrid populations. Several SSR makers were found to be tightly linked to *Rvi* loci. Nowadays, there are different SNPs (single-nucleotide polymorphisms) that are used. The MAS process is eased by the use of SNP microarrays; these markers were discovered in biparental populations and have been linked to a series of scab-resistant loci. It has been suggested that the resistance to scab could be enhanced by the pyramiding of effective *Rvi* genes and QRLs (Quantitative Resistance Loci). QRLs are quantitative resistance loci with functional alleles that could be involved in quantitative resistance against *Venturia inaequalis*. However, recent studies showed that even effective *Rvi* genes can be overcome in such pyramiding of *Rvi* and QRLs (Švara et al., 2024).

Pyramiding scab resistance genes against a spectrum of *Venturia inaequalis* strains has been demonstrated in experimental hybridizations of seedlings from a cross between Regia and Ariwa that carry different combinations of *Rvi2* and *Rvi4* (Baumgartner et al., 2015). QRLs can enhance the durability of vertical resistance controlled by several *Rvi* genes. The screening of local *Venturia inaequalis* races could be applied to already known QRLs and would help against breakdown of resistance in a new cultivar which has incorporated pyramided resistance.

Environmental factors play a vital role in shaping the effectiveness of scab resistance achieved through marker-assisted selection (MAS), and this aspect should be carefully considered in breeding strategies (Jiang, 2015). While MAS facilitates the identification and introgression of resistance loci such as major genes (e.g., *Rvi6*) or quantitative resistance loci (QRLs), the actual expression of resistance in the plant can be strongly influenced by environmental variables. (Khan et al., 2024). Elements like temperature, relative humidity, precipitation patterns, and the intensity of pathogen pressure can affect both the activation of resistance genes and the virulence of *Venturia inaequalis*.

For example, in conditions of high humidity and extended leaf wetness, plants carrying resistance markers may still show partial susceptibility, as environmental stress can weaken their defense mechanisms (Okoro et al., 2024). Additionally, gene–environment interactions (G×E) may impact the consistency of resistance performance across different geographical locations and growing seasons. Including a dedicated section on these environmental interactions would offer a more holistic perspective on the challenges and opportunities of MAS, helping breeders design cultivars with stable, long-term resistance under variable and evolving climate conditions.

Table 4. The advantages and disadvantages of the most commonly used molecular markers for QTL analysis (Collard et al., 2005)

Molecular marker	Codominant - C Dominant - D	Advantage	Disadvantage
RFLP (Restriction fragment Length Polymorphism)	C	Reproducibility	Time-consuming, complex method, requires large amounts of DNA
RAPD (Random Amplified Polymorphic DNA)	D	A quick and simple method, cheap, requires small amounts of DNA	Non-reproducible method
SSR (Simple Sequence Repeat)	C	Simple method, repeatability	Time-consuming, complex method, requires the use of polyacrylamide gels
AFLP (Amplified Fragment Length Polymorphism)	D	High polymorphism	It requires large amounts of DNA, complex method

CONCLUSION

In the present review, we have emphasized the importance of *Venturia inaequalis* as a devastating plant pathogen which causes considerable economic losses in apple orchards. Therefore, it has never been more important to find alternatives by creating new promising candidates for future orchards to enhance cultivar resilience. Using high-quality, durably resistant cultivars offers a significant solution to scab challenges faced by growers. Currently, breeding programs primarily focus on integrating qualitative resistance genes (*Rvi* type) from various *Malus* sp. into newly developed commercial cultivars. The mechanisms involved in the breakdown of *R* gene-mediated resistance by the pathogen should be explored further. Choosing appropriate markers and overcoming the potential breakdown of resistance by evolving pathogens requires ongoing research and development. Understanding defense response pathways of apple and characterizing key genes involved in resistance would be very useful in engineering scab-resistant apple. Combining or pyramiding different resistance and defense connected genes into a single variety might offer effective a durable resistance. However, conventional breeding techniques might take years to create potential candidates against this disease. Therefore, transgenic approaches should also be considered to identify the key genes related to defense or resistance. Cisgenic and intragenic apple lines have been tested already and displayed a high level of resistance against apple scab, but marker-free lines need to be created to increase the acceptability of the transgenic or cisgenic apple cultivars amongst the customers.

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