



An NDR1/HIN1-like gene identified in *Fraxinus excelsior* L. may trigger defence mechanisms in response to virus infection

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Abstract

The defence-associated NDR1/HIN1-like (NHL) gene plays an important role in triggering plant defences in response to biotic stresses. The NHL gene is involved in gene-for-gene-mediated resistance mechanisms in response to attack by bacterial, fungal, and viral pathogens. Our study investigated the variation in 72 virus defence gene candidates annotated in the *F. excelsior* genome. Among all single nucleotide polymorphisms (SNPs) and indels detected in these candidates, one polymorphic site in the NDR1/HIN1-like protein-encoding gene was able to distinguish between common ash samples with different infection and symptomatic statuses. The SNP was a nonsynonymous substitution that would result in an amino acid change. The variation detected here may have an impact on pattern recognition and therefore affect the immune response, indicating tolerance/susceptibility to viruses of common ash. The identified marker may help in successfully identifying individuals with a low level of susceptibility to viral diseases.

Keywords *Fraxinus excelsior* · NDR1/HIN1 · SNP markers · Virus defence · FraxVir · Ash dieback

Background

In most European countries, ash trees account for 1–2% of the forest area or standing volume stock (Chira et al. 2017), with a slightly greater proportion in forests in Central Europe (Queloz et al. 2017). Ash is economically important in Europe, and ash timber is widely used for furniture,

sports equipment, tool handles and other goods (Enderle et al. 2017). Moreover, ash is very important from an ecological perspective. Ash provides critical ecological services, such as wildlife habitats and niches, the stabilization of the water balance and stream banks, and the production of high-quality litter, and is a component of slowing zones for forest fires (Sioen et al. 2017). Many organisms are associated with or even entirely dependent on ash (Heinze et al. 2017; Drenkhan et al. 2017; Bengtsson and Senström 2017). In forestry, ash is also popular because of its robustness, drought tolerance, fast growth, and prolific regeneration (Enderle et al. 2017).

In the last three decades, *Hymenoscyphus fraxineus* has affected the ash species *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl, leading to severe declines (George et al. 2022). Until recently, the cause of ash dieback was primarily attributed to this fungus. However, over the last decade, studies on plant viruses in forest and urban trees have revealed that viruses contribute to loss of vitality and tree damage, leading to tree decline (Büttner et al. 2023). Even though viral diseases are rare, viruses are responsible for great economic losses because the symptoms are very different from those attributed to fungi and bacteria, which means that the losses are more insidious and less conspicuous and therefore unnoticed and untreated (Büttner et al.

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2013). Plant viruses play a central role in the health status of plants since the degree of disease that they cause leads to extensive tissue damage (Nienhaus 1985). On the basis of the assumption that virus-infected trees may be more susceptible to the ash dieback pathogen, the FraxVir subproject was launched in Germany in October 2021 as part of the FraxForFuture project. This supplementary study consists of four closely interlinked subprojects investigating the effects of viral infections on ash trees.

The list of plant viruses known to commonly infect ash comprises species from the *Rhabdoviridae* and *Fimoviridae* families (Gaskin et al. 2021) or of the genus *Idaeovirus* (family *Mayoviridae* order *Martellivirales*) (Navarro et al. 2017; ICTV MSL 2023). *Cytorhabdovirus* is a genus that includes ash-infecting viruses (family *Rhabdoviridae*, subfamily *Betarhabdovirinae*) (Walker et al. 2022). Two closely related cytorhabdoviruses (Fraxinus gammacytorhabdovirus 1, FraGCRV1; Fraxinus gammacytorhabdovirus 2, FraGCRV2) were recently reported to affect different ash species (Bejerman et al. 2023; Rehanek et al. 2024). Symptoms of infection by cytorhabdoviruses are often inconspicuous in ashes (Rehanek et al. 2024). The transmission of this genus occurs via various insects (aphids, grasshoppers, whiteflies) or mechanically (Jackson et al. 2005). In tree and insect cells, cytorhabdoviruses develop cytoplasmic viroplasm, undergo morphogenesis from cytoplasmic membranes, and accumulate in the cytoplasm (Dietzgen 1995). Ash shoestring-associated emaravirus (ASaV) was recently found to be associated with ash shoestring disease in European ash and manna ash (*Fraxinus ornus*) trees, which exhibit chlorotic ringspots, mottle, and leaf deformation, such as curling and shoestring symptoms (Gaskin et al. 2021). *Emaravirus* (order *Bunyavirales*; family *Fimoviridae*) is a genus comprising more than 20 emerging plant viruses with a worldwide distribution and economic impact. These viruses are enveloped, with a segmented, single-stranded, negative-sense RNA genome, and are transmitted by eriophyid mites or mechanical transmission. Initially, privet leaf blotch-associated virus (PrL-BaV, *Idaeovirus ligustri*) was identified by next-generation sequencing (NGS) in privet (*Ligustrum japonicum* L.) affected by a graft-transmissible disease characterised by leaf blotch symptoms (Navarro et al. 2017). In ash trees, the virus was detected in samples displaying a chlorotic line pattern (Rehanek et al. 2024).

The fact that some plants do not succumb to the many existing potential pathogens may be due to the existence of nonhost resistance (Nürnberg and Scheel 2001). Initially, following infection, plant cells use basal defences to recognize pathogen-associated molecular patterns. These elicit responses via mechanisms involving pathogenesis-related, pathogen-nonspecific proteins (Almagro et al.

2009). Many host defence genes are induced upon virus infection. As a counterdefense mechanism, pathogens suppress basal defences using specific virulence proteins, also called effectors (Marwal and Gaur 2020). Effectors are recognized by the second, target-specific level of host defence provided by R proteins. R genes elicit a quick and powerful defence response, which can take the form of a hypersensitive response or extreme resistance (Balint-Kurti 2019). The signalling cascade induced by R genes leads to hypersensitive response and activates a wide range of genes and defence responses. This response prevents virus loading to the phloem in the initially infected leaf and translocation to other parts of the plant, but virus replication and initial cell-to-cell movement are not affected. Later, most of the infected cells die, leading to a visible necrotic local lesion at the site of infection. Extreme resistance, however, suppresses virus multiplication in initially infected cells, and usually, no visible signs of infection can be observed (Barker and Harrison 1984). Only tiny, necrotic pinpoint lesions may be perceived, and no virus is detected in the inoculated tissue (Valkonen et al. 1996). The activation of the hypersensitive response often induces systemic acquired resistance, which often relies on the salicylic acid (SA)-signalling pathway and the production of pathogenesis-related proteins (Balint-Kurti 2019; Liu et al. 2020).

NDR1/HIN1-like (NHL) genes encoding a membrane protein (Century et al. 1997) provide the proper function of a subclass of resistance genes (Aarts et al. 1998) required for nonrace-specific disease resistance. Several members of the NHL family exhibit cell membrane subcellular localization (Varet et al. 2002), and some members reside in the endoplasmic reticulum due to the presence of sarcolipin-like sequences in the protein (Lee et al. 2006). NHL genes have been shown to be involved in maintaining the integrity of the cell wall and plasma membrane connection (Knepfer et al. 2011). NDR1 is considered a signal modulator that is essential for the stimulation of signalling pathways mediated by relevant R proteins (Century et al. 1995; Aarts et al. 1998). Harpin-induced gene 1 (HIN1), which is isolated from tobacco, is highly similar to NDR1, but interestingly, similar to R genes, HIN1 has been reported to be associated with a hypersensitive response (Century et al. 1997; Takahashi et al. 2004). Allelic variation of additional genes involved in virus recognition in addition to R genes or downstream signalling for defence responses may cause genotype-dependent phenotypic changes in the outcome of resistance responses triggered (Valkonen 2015).

SNPs and indels in resistance genes are an important source of polymorphic markers for use in association studies. SNPs offer several advantages, such as high-throughput and cost-effective genotyping (Varshney and Dubey 2009) and the identification of functional/gene-based markers for

association genetics (Rafalski 2002). Direct sequencing of DNA segments amplified by PCR from several individuals is the most direct way to identify SNP polymorphisms. PCR primers are designed for genes of interest and then PCR is performed on a set of highly diverse individuals, after which the PCR products are sequenced. The resulting sequences are aligned and, taking care to distinguish true polymorphisms from sequencing errors, polymorphisms are identified.

Our study aimed to (1) genotype by Sanger sequencing a lower number of *F. excelsior* virus-infected symptomatic and asymptomatic samples with primers designed for 72 virus resistance gene fragments formerly annotated in ash as described in Köbölkuti et al. (2025) and revealing possible nucleotide variations; (2) identify the gene fragments in which relevant SNPs were present that distinguished the two (symptomatic/asymptomatic) groups; and finally, (3) investigate whether the selected gene fragment nucleotide variations found in a lower number of symptomatic/asymptomatic individuals showed a significant correlation with the phenotype in an increased number of samples.

Materials and methods

The complete workflow is shown as a flowchart in Fig. 1.

In Step 1, samples were collected from both offspring and adult trees. In Step 2, 120 of these samples were phenotyped. Step 3 involved identifying 72 candidate genes, for which 72 primer pairs were designed. In Step 4, DNA from eight phenotyped samples was amplified using these primers and then sequenced. On the basis of visual assessment, a fragment of the NDR/HIN1 gene was selected and validated in Step 5 through genotyping of the 115 phenotyped samples from Step 2.

Plant material

Five selected stands (Köbölkuti et al. 2025) were distributed across Germany, of which two seed orchards (special plantings for seed production) in Emmendingen and Schorndorf (Baden-Württemberg), a clone archive in Grabenstätt (Bavaria), and two intensive monitoring plots (IBF) by the FraxForFuture research network (BB 1 - Stegelitz/Melzower Forst in Brandenburg and BY 1 - Monheim/Kaisheim in Bavaria) served as a common working platform for investigations of all participating project partners in the FraxVir project (Fig. 2). As a first step, all adult trees available for sampling at Kaisheim and Melzower Forst were labelled, georeferenced and sampled, and leaf samples from all trees at Emmendingen, Schorndorf and Grabenstätt were collected in 2022. In the second step, 200 seedlings from

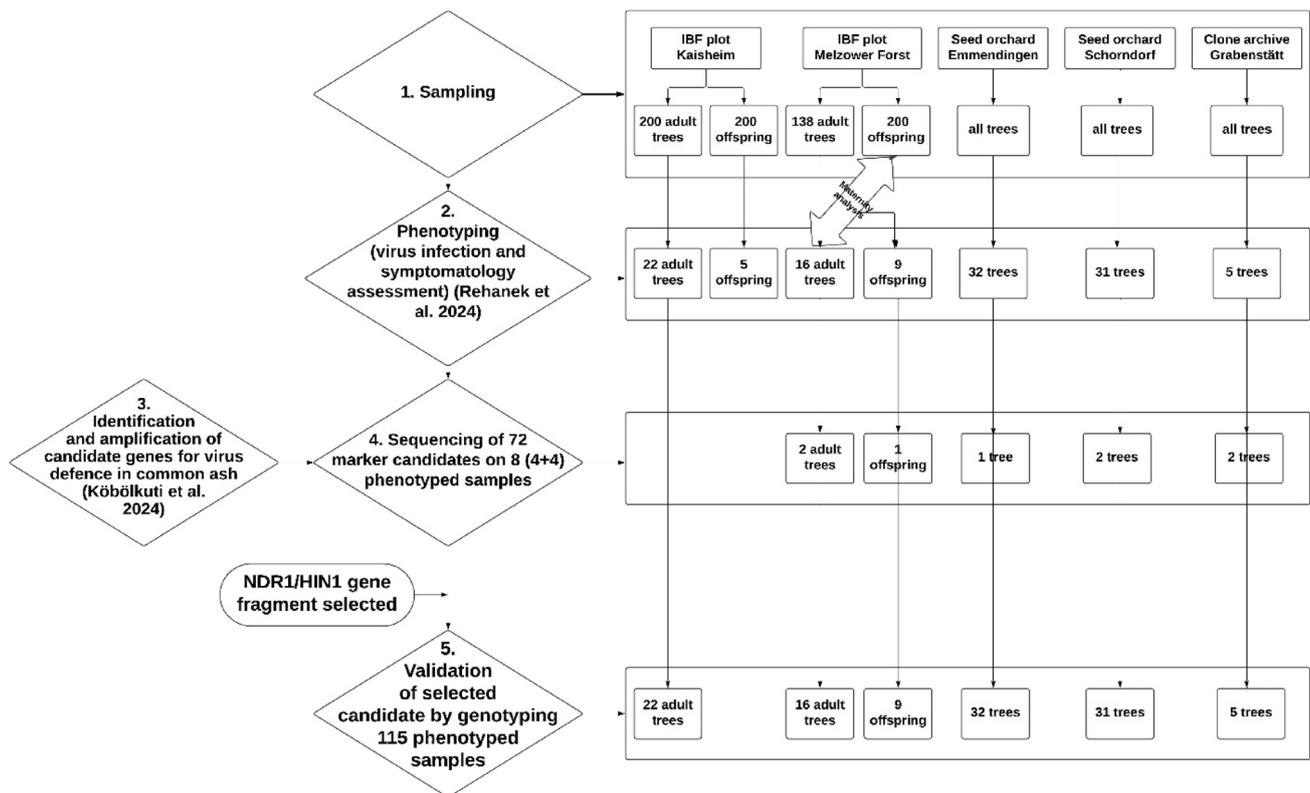


Fig. 1 Steps of marker development for virus defence genes in *Fraxinus excelsior*



Fig. 2 Study areas: location of seed orchards near Schorndorf ($48^{\circ}77'$ N, $9^{\circ}25'$ E), Emmendingen ($48^{\circ}11'$ N, $7^{\circ}87'$ E), clone archive near

Grabenstätt ($47^{\circ}84'$ N, $12^{\circ}51'$ E), IBF plots Melzower Forst ($53^{\circ}19'$ N, $13^{\circ}95'$ E) and Kaisheim ($48^{\circ}80'$ N, $10^{\circ}79'$ E) in Germany.

Source of the maps: Google Earth Landsat/Copernicus Data SIO, NOAA and Cartography Vectors

Kaisheim and Melzower Forst were labelled, georeferenced and sampled in 2023.

This step was followed by a comprehensive survey of the viruses that affect *F. excelsior* (ASaV, PrLBaV, and the cytorhabdoviruses FGCRV1 and FGCRV2) at the five previously mentioned stands, which was carried out as described in Rehanek et al. (2024). As a third step, to select gene fragments with SNPs correlated with virus-infected symptomatic/asymptomatic phenotypes, eight individuals previously investigated for virus infection (all infected, four with and four without symptoms) were chosen (Table S1). Detailed information on the selection strategy followed in this step can be found in Köbölkuti et al. (2025).

Finally, in the fourth step, to test whether the marker candidates selected in step three were relevant to larger sample sizes, we assessed samples from virus infection and symptomatology from 16 mother trees (Table S2, Fig. 3a), and their offspring, as validated by maternity analysis (Fig. 3b) in Melzower Forst; samples from 22 adult trees in Kaisheim; 31 individuals in Schorndorf; 32 trees in Emmendingen; and five young individuals in Grabenstätt (Table S3). In this final step, a total of 115 samples were used for marker selection on the basis of relevant polymorphisms correlated with symptomatic/asymptomatic status.

DNA extraction

For all trees, leaflets were used for DNA extraction. After sampling, the genetic material was preserved at -20 °C until further analyses. DNA extraction was performed using a modified hexadecyltrimethylammonium bromide (CTAB) rapid protocol (Lefort and Douglas 1999).

Genotyping for parentage analysis

In Melzower Forst, all adult trees and offspring with known virus infection statuses were genotyped at microsatellite loci to detect parent–offspring relationships. After DNA extraction, polymerase chain reaction (PCR) was performed to examine 12 microsatellite loci in three different multiplexes (Table 1) (Sollars et al. 2017; Beatty et al. 2015; Noakes et al. 2014; Lefort et al. 1999; Bai et al. 2011; Aggarwal et al. 2011). DNA was separated by high-resolution capillary electrophoresis using a GeXP automated sequencer (Beckman Coulter, Inc., Fullerton, CA, USA) and software-assisted allele scoring.

Statistics for relationship assessments

Since we consider the validation of the detected genealogical relationships highly important and the application of likelihood equations by multiple programs could

outperform single tests in their estimation, we applied distinct approaches to determine whether they converged and detected the same mother–offspring pairs. Altogether, maternity analysis by two software programs was performed. After the samples were genotyped at 12 microsatellite loci, on the basis of the multilocus genotype data, the maternity relationships between the virus-tested adult trees and all the seedlings collected at the monitoring site Melzower Forst were determined using the software packages CERVUS 3.0.7 (Kalinowski et al. 2007) and COLONY 2.0.6.9 (Jones and Wang 2010). In CERVUS, the allocation is based on delta (Δ) (Labuschagne et al. 2015). Therefore, the difference in the likelihood–odds ratio (LOD) score between the two most likely parents is examined to find the actual parents. The critical values of Δ were calculated at strict (95%) and relaxed (80%) confidence intervals during the simulations (Labuschagne et al. 2015). For Melzower Forst, 100,000 offspring and 8% of the sampled potential mothers were simulated. The minimum number of loci was set to 6. The error rate was maintained at 0.01 (Semizer-Cuming et al. 2019). COLONY implements full-pedigree likelihood methods to infer siblings and parents among individuals simultaneously via multilocus genotype data (Jones and Wang 2010). In COLONY, the input parameters were set as follows: For the mating system settings, ‘female and male polygamy’ was selected. For mating system II, ‘with inbreeding’ was enabled, and ‘without clone’ was specified. The species was set to ‘monoecious’ via the same parental dataset for both candidate mothers and fathers. ‘No’ was selected for both sibship prior and the allele frequency update. The number of known and excluded paternal and maternal sibships was set to 0. The probability of a mother being included as a candidate was set to 1, whereas the probability for a father was set to 0.1. The analysis method used was ‘full likelihood’ with medium likelihood precision, and the run length was set to ‘medium’.

Phenotyping the offspring of the mother–offspring pairs

Following maternity analysis, a comprehensive survey was performed in Melzower Forst for virus infection and viral symptoms in offspring that showed a significant maternal link by maternity analysis to mother trees that had been previously tested for virus infection. Since phenotyping is required to find offspring 20–40 cm in size in a rather large swampy area with dense ground vegetation and ash populations affected by ash dieback, the following strategy was used: first, the identification of offspring identified by both CERVUS and COLONY was prioritized. This was followed by a search and phenotyping of those offspring identified separately by the two software programs.

Fig. 3 a, b Virus-infected/no virus detected symptomatic (+) and asymptomatic (-) mother trees (M) were sampled and previously tested in the Melzower Forst IBF plot (a), 200 seedlings were sampled from the same site in 2023; among them, red represents the offspring of some of the mother trees tested (b). The white dots in b represent offspring for which the parents were not tested for virus infection during the study.

Source of the maps: Google Earth
Landsat/Copernicus Data SIO, NOAA

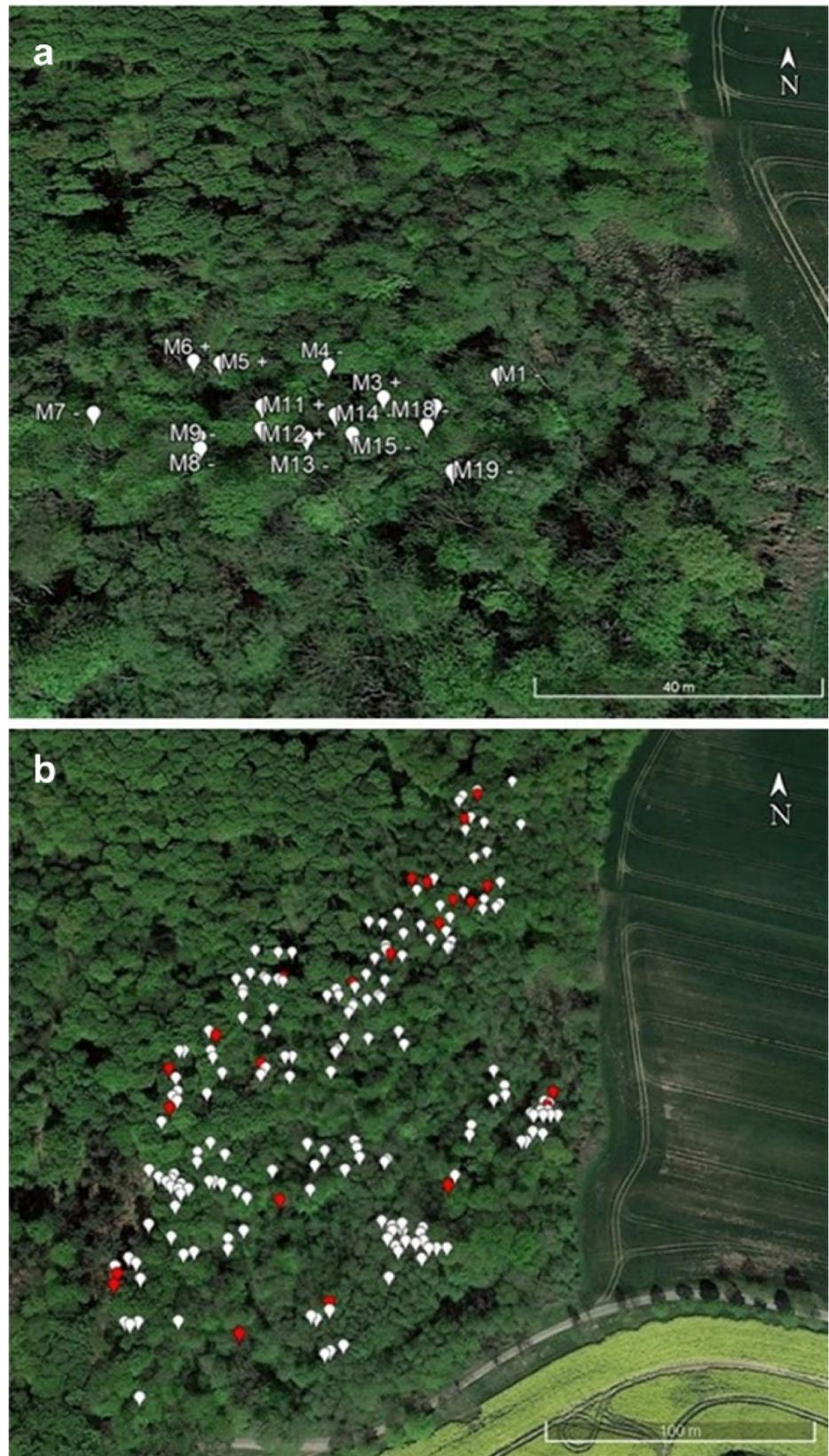


Table 1 Twelve microsatellite markers were used for genetic analysis of ash trees, including multiplex sets and loci in the genome

Multiplex-Sets	Marker	Locus	Literature
1	F27	Con-tig5418_11372_11513	Sollars et al. (2017)
1	F30	Contig344_81629_81375	Sollars et al. (2017)
1	Frex02	Con-tig1795_237744_237898	Sollars et al. (2017)
1	Frex06	Con-tig3809_118941_119122	Sollars et al. (2017)
1	Frex10	Con-tig87_333704_333466	Sollars et al. (2017)
2	F6	M230B	Beatty et al. (2015)
2	F21	Fp18437	Noakes et al. (2014)
2	Fem4	Femsatl 4	Lefort et al. (1999)
2	Fem11	Femsatl 11	Lefort et al. (1999)
3	ASH2429	ASH2429	Bai et al. (2011)
3	Fem19	Femsatl19	Lefort et al. (1999)
3	FR308	FRESTSSR308	Aggarwal et al. (2011)

Genotyping by the SNP marker candidates

The bioinformatic steps of gene annotation, primer design, primer laboratory testing by PCR amplification and gel electrophoresis preceding sequencing are described in detail in Köbölkuti et al. (2025). The products were purified after PCR, and sequencing in both directions was completed at Microsynth SeqLab GmbH (Göttingen, Germany). Editing, visualization, and alignment of the amplified sequences were performed with CLC Genomic Workbench version 22.0 (QIAGEN Bioinformatics) software. Following sequence alignment, genotyping and final validation of all potential SNPs were performed with the use of SeqTrace 0.9.0 (Stucky 2012). The number of polymorphic sites, number of insertions/deletions and characteristics of the SNPs (synonymous or nonsynonymous) were calculated using DNA Sequence Polymorphism v6.10.01 (DNASP) software (Rozas and Rozas 1995) and validated by visual inspection via the CLC Genomic Workbench. To find the first nucleotide position in a codon, candidate DNA sequences were translated to protein sequences by NCBI BLASTX (<https://www.ncbi.nlm.nih.gov/>), after which the number of synonymous and nonsynonymous sites was computed as described by Nei and Gojobori (1986), excluding all cases that involved stop codons.

Table 2 Phenotype file versions (a, b, c) used to detect potential significant correlations between loci in sequences previously selected through visual assessment and phenotype

Phenotype file versions	Infection and symptomatic status of groups	Numerical equivalent of groups in file
a	No symptom	1
	Nonspecific symptom	2
	Specific symptom	3
b	Virus detected, with specific or nonspecific symptom	1
	Virus detected, without any symptom	2
	No virus detected	3
c	Virus detected, with specific symptom	1
	Virus detected, with nonspecific symptom	2
	Virus detected, without symptom	3
	No virus detected	4

The term “nonspecific symptom” refers to inconspicuous local lesions

Sequence and SNP locus selection on the basis of genotyping results, symptomatology, and correlation analysis

After genotyping, eight DNA samples (four from symptomatic and four from asymptomatic ash trees) with 72 marker candidates (the marker genes identified in Köbölkuti et al. 2025) were selected by visual assessment of those sequences in which relevant SNPs distinguished the two sample groups. After sequencing the PCR products of all 115 samples using the selected marker candidates, an association analysis was performed for each SNP variant–symptom combination. This analysis employed the general linear model (GLM) function implemented in TASSEL (Bradbury et al. 2007) as follows: the sequences of the 115 samples were quality checked and filtered by quality, trimmed to equal length, and then aligned, and the position of the relevant SNP loci selected in the previous phase was determined again. The alignment FASTA file was used as an input genotype file for TASSEL software. Indel sites were removed, the FASTA file was converted to a numerical file, and then a PCA based on covariance and limited to three components was performed. The reasons behind the infection and different symptomatic statuses may vary from susceptibility to tolerance or full resistance. In the input numerical phenotype file, as illustrated in Table 2, the infection status and symptoms of the samples were quantified in three (a, b, c) variations, each of which was subjected to association with genotype. The genotype, each version of the phenotype files and PCA results were merged by intersect join. GLM analysis was performed with 1,000 permutations, and all options were set to defaults. The p values of associated markers

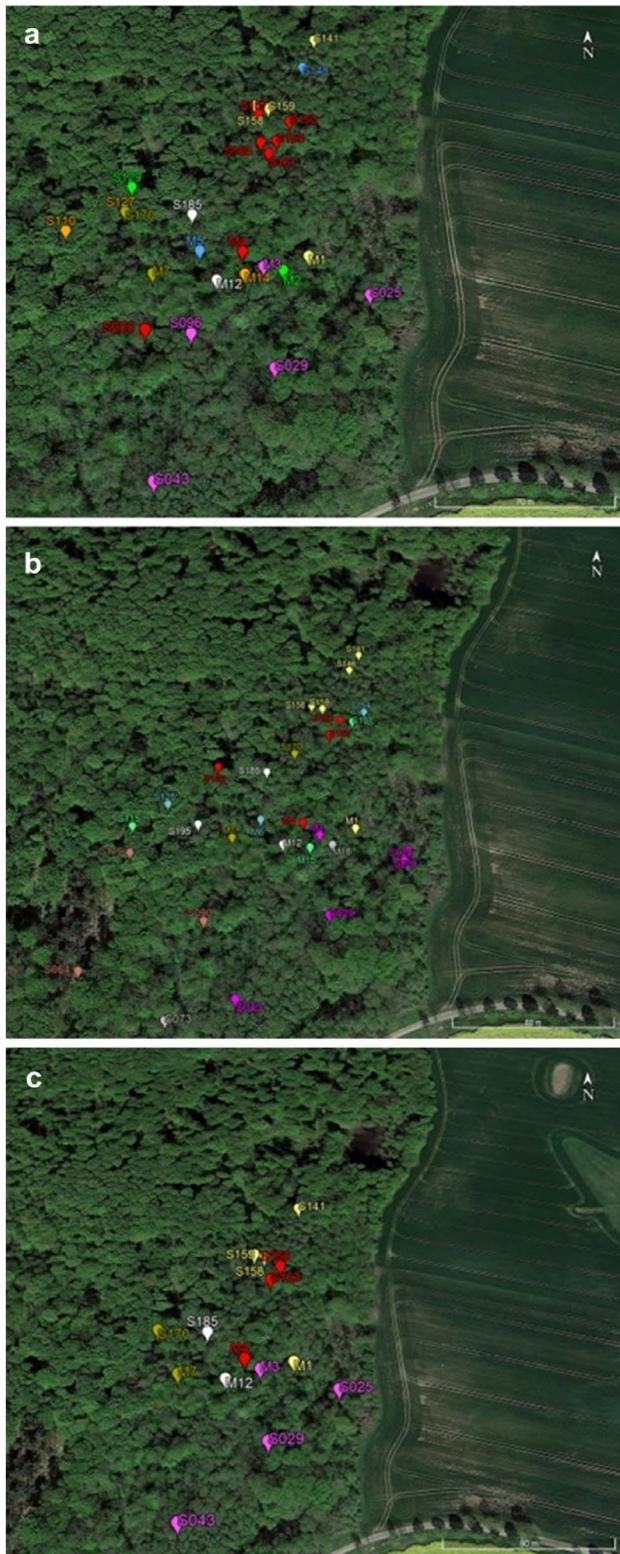


Fig. 4 a, c Candidate mother–offspring pairs identified by maternity analysis with CERVUS (a), COLONY (b) and the joint result (c) with 16 virus-tested adults and 200 seedlings sampled at Melzower Forst IBF plot as inputs. M–mother, S–seedling. The offspring belonging to a given mother tree are marked with the same colour as the mother tree

were tested against significance thresholds at 0.05 (conservative), 0.01 and 0.001 (stringent) significance levels.

Results

Mother–offspring pairs detected in Melzower Forst by genotyping with microsatellite markers

Among the 200 examined offspring, parentage analysis identified 19 pairs using CERVUS (Fig. 4a), and 23 were identified via COLONY (Fig. 4b). However, only eight offspring (Fig. 4c) were consistently assigned to a potential mother by both methods, with 16 virus-tested adult individuals used as inputs. The 12 microsatellite loci yielded altogether 177 alleles in Melzower Forst, from 3 (FR308, ASH2429) to 36 (F6) alleles with a mean value of 14,8.

Assessment of virus infection and symptomatology of the offspring of the mother–offspring pairs.

Among the eight offspring identified jointly by CERVUS and COLONY, only five were found and visually assessed for symptoms at the site. In the next step, we searched for seedlings that were separately identified by the two software programs. In this way, a total of nine seedlings were symptomatologically evaluated and then tested in the laboratory for virus infection (ASaV, PRLBaV, and the cytorhabdoviruses FraGCRV1 and FraGCRV2, respectively), and their DNA samples were selected for further genotyping (Fig. 5).

Genotyping of eight samples with 72 marker candidates

As described in Köbölkuti et al. (2025), using 72 (36 coding + 36 promoter) primer pairs, PCR amplification of DNA from eight leaf samples previously investigated for virus infection (all infected, four with and four without symptoms) was performed. Sequencing of the PCR products was performed for all markers. High-quality sequences were obtained for 66 markers. Six markers (ARLA1C, YSL9/1, pr_AT5G42950, pr_NHL3, pr_GR-RBP2_A, and pr_AGO4/4) produced unclear chromatograms and were excluded from further analyses. Among the remaining 66 markers, 15 (AGO4/2, AGO5, AGO10, AT3G21120, GRP7, GRP7s, GR-RBP2_A, MIPS2, NHL3, PLP1, YSL9/2, WRKY71, pr_CBP20, pr_NIK1, pr_OZF1) produced clear sequences for PCR products from only seven samples each; three markers (AGO4/4, pr_GRP7, pr_YSL9) yielded clear sequences from six samples each; and three markers (WRKY60, TOR, pr_DCL3) from five samples each. In



Fig. 5 Nine of the tested *F. excelsior* adult trees' presumed offspring were sampled in 2023 and found in 2024 on Melzower Forst IBF plot, evaluated for virus infection and symptomatology and selected for genotyping

Fig. 6 The 30-nucleotide fragment of the alignment of the AT3G44220 sequence with a relevant SNP at position 273 amplified from eight DNA samples. The nucleotides coloured in red indicate the nucleotide polymorphisms between symptomatic (+) and asymptomatic (-) samples

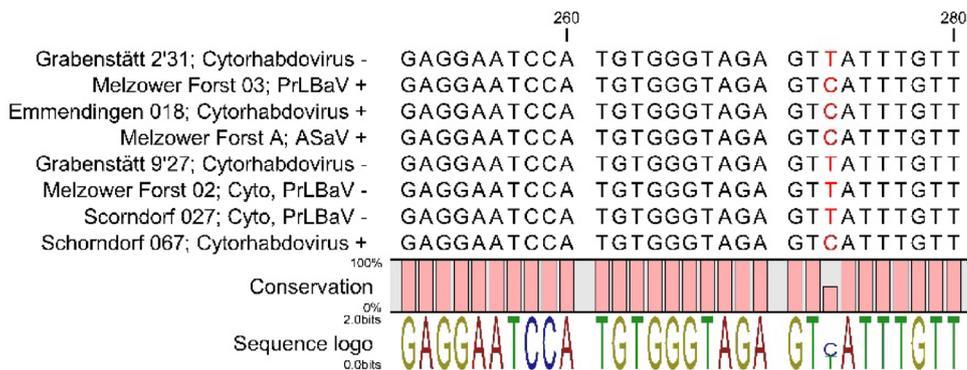


Table 3 Position of the relevant locus, the encoded protein, minimum number of recombination events with the loci where this recombination occurs, and type of mutation calculated by DNA sequence polymorphism v6.10.01 for AT3G44220 selected to be tested on 115 samples

Sequence	Nr. of sites	Min. number of recombination events	Recomb. between sites	Relevant SNPs position in seq	Encoded protein of the gene	Codon position of the first site by NCBI blastx	Nr. of syn-mutation	Nr. of nonsyn mutation
AT3G44220	549	1	273, 399	273	transmembrane protein (R receptor)	2	0	1

total, 498 sequences and 23,808 sites were analysed. Table S4 summarizes the results of the sequence analysis.

As shown in Table S4, the sequence analysis revealed 377 SNPs and 122 indels. However, in the case of only one sequence (AT3G44220), a nucleotide variation that distinguished the symptomatic (+) and asymptomatic (-) groups of samples was detected (Fig. 6). In the second phase of the

analysis for this sequence, the minimum number of recombination events, the site where recombination may occur, the relevant SNP loci in each sequence, the proteins encoded by the gene and the type of mutation (syn/nonsyn) were determined. Table 3 summarizes the results of this analysis.

As shown in Fig. 6, in the AT3G44220 sequence, the nucleotide thymine (T) was found at position 273 in all

virus-infected asymptomatic samples (–), and cytosine (C) was found in all symptomatic samples.

Assessment of virus infection, symptomatology and genotyping of 115 samples with the selected AT3G44220 candidate

For virus infection, 68 of the 115 samples genotyped were infected with only Cytorhabdovirus; four were infected with Cytorhabdovirus and ASaV; eight were infected with Cytorhabdovirus and PrLBaV; one was infected with Cytorhabdovirus, PrLBaV and ASaV; and 33 were not infected with ASaV, PrLBaV, or the cytorhabdoviruses FraGCRV1 or FraGCRV2. In terms of symptomatology, out of the total number of samples, 52 showed nonspecific local lesions, five expressed specific symptoms, whereas 58 had no symptoms at all.

After sequencing, good-quality sequences were obtained for 111 samples. The PCR products of four samples (Melzower Forst 11, Schorndorf 56, Melzower Forst 9 and Schorndorf 83) presented no clear chromatograms and were excluded from further analyses. Except for eight samples, all 30 infected samples without symptoms had thymine T, and all those infected with specific symptoms had cytosine (C) at locus 273 of the AT3G44220 gene fragment. The samples that were exceptions to this pattern included

Melzower Forst seedling 29, Melzower Forst 7, Kaisheim 44, Melzower Forst 6, Melzower Forst 19, Schorndorf 2, Schorndorf 98 and Schorndorf 36. Among all the samples genotyped, 10 were found to be heterozygotes, nine of which lacked any symptoms or only presented nonspecific symptoms. Table S5 presents the virus infection and symptomatic status, as well as the nucleotide variation at locus 273 of the AT3G44220 gene fragment in each of the 115 samples.

For the association analysis input genotype file, the lengths of the sequences adjusted to the shortest sequence were trimmed to 334 nucleotides in length. From all 11 polymorphic loci found in the alignment, the relevant locus position selected previously on eight samples was determined at position 183. Although the GLM detection method using phenotype file “a” revealed that SNPs at position 183 correlated with symptomatology, at the 0.05, 0.01 and 0.001 significance levels, the correlation was not significant (Fig. 7).

Using versions “b” and “c” of the phenotype file for analysis, from all polymorphic loci in sequence AT3G44220, the locus at position 183 was correlated with the phenotype at a P value ≤ 0.05 (Table 4).

P-Values by SNP position for symptom

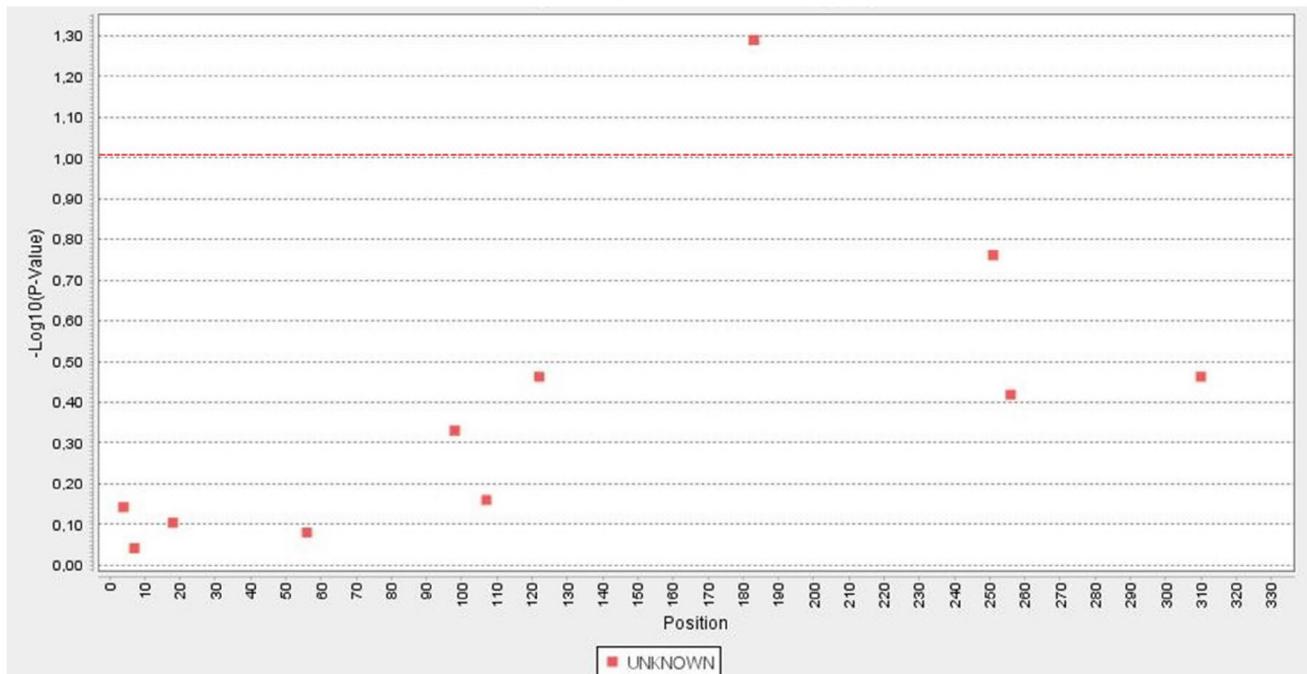


Fig. 7 Detection of loci in the AT3G44220 sequence amplified from 111 samples correlated with the phenotype file version “a”. All loci with a maximum P value = 1 are shown. The correlation of the locus at position 183 above the red line is not significant at the 0.05 (conserva-

tive), 0.01 or 0.001 (stringent) significance level and thus is not considered to be a significant result for the GLM-based method implemented in Tassel by Bradbury et al. (2007) using phenotype file version “a”

Table 4 Locus 183 in the AT3G44220 sequence amplified from 111 samples was correlated with phenotype files “b” and “c”, both of which were significant at $P \leq 0.05$, which was considered to be a significant result for the GLM-based method implemented in Tassel by Bradbury et al. (2007)

Trait	Marker	Position	Phenotype file version	marker_F	p value
Virus caused leaf symptoms	AT3G44220	183	b	4.08199	0.01976
			c	3.90232	0.02334

Discussion

Although advances in the use of molecular markers in the breeding and production of agronomic crops have enabled great progress in the last decade (Hasan et al. 2021), these advances are of little relevance for assessing the potential for marker-aided selection in forest tree populations (Ahmar et al. 2021). There is a lack of literature, particularly on virus management of forest tree species, probably because viruses in forest trees are uncommon (Büttner et al. 2023). Important efforts in forest tree breeding have been carried out mainly to improve traits of economic interest (Maldonado et al. 2020), adapt to climate change (Gray et al. 2016; Cortés et al. 2020; Vacek et al. 2023), or select individuals and populations with genetic resistance to different biotic threats (Mageroy et al. 2023; Sniezko and Liu 2023), with the exception of viruses.

Here, we present data showing that a marker for virus tolerance developed on a defence-associated NDR1/HIN1-like (NHL) gene fragment identified in the *F. excelsior* genome in a previous study (Köbölkuti et al. 2025) may predict the outcome of the tolerance/resistance status of virus-infected common ash individuals. The marker was identified in a small panel of eight virus-infected samples. Although a larger number of genotypes are typically used in most SNP identification and association analyses in forest trees (Ashwath et al. 2023; Liu and Liu 2024), the advantage of low sample size analyses, which permit the identification of only large effect loci, has allowed us to identify this nucleotide variation, which could be validated in a larger number of individuals in this study.

Of the total number (72) of marker candidates, only one of the nucleotide variations found in these sequences amplified from eight samples could predict the virus infection symptomatic status, as expected. There are several reasons why a clear correlation between the nucleotide variation in these gene fragments' polymorphic loci and symptomatology could not be observed. First, our method was not based on capturing genome-wide trait loci with high-density molecular markers but was limited to a few gene fragments identified by comparative genomics in the *F. excelsior*

genome and the detection of nucleotide polymorphisms that might occur in these. It is possible that these few gene fragments cannot capture key genomic points related to polygenic plant disease resistance (Fraser and Van Loon 1986; Caranta and Palloix 1996). Second, specific resistance is controlled mainly at a single genetic locus, although more complex systems are known (Fraser 1992). Consequently, it is conceivable that even a genome-wide analysis would not reveal many loci associated with viral symptomatology. In addition, only a few resistance genes have been proven to be exceptionally durable. The acquisition of virulence can be associated with the loss of general pathogenic fitness, which can be restored by further selection of the virus in resistant hosts (Fraser 1992). Third, our gene fragments were identified in the ash genome by a similarity search starting from sequences annotated in *Arabidopsis*, and although defence mechanisms are conserved at the protein level, it is possible that these sequences are not in interspecies conserved regions in the genome. Some of the genes studied could be pseudogenes, which might lead to incorrect results and false interpretations. Chromosomal duplications or transpositions are important features of multigene families of such large eukaryotic genomes as trees (Kvarnheden et al. 1998). It is also necessary to consider that our samples with different symptomatic statuses were infected with different viruses. Different viruses may contain different virulence determinants and may have different gene-for-gene relationships (Martin et al. 1993; Cesari et al. 2013), with a host having several resistance genes, making it difficult to detect different types of viruses with the same, small number of markers. Another reason might be that other viruses were present in the investigated samples and were not included in the testing. CLRV and ArMV have been reported to affect *Fraxinus* (Cooper 1975; Rebenstorf et al. 2006; Hamacher and Quadt 1991; Rehanek et al. 2023). Furthermore, novel putatively pathogenic plant viruses from the Closteroviridae family and a putative novel trichovirus (Rehanek et al. 2024) were also not considered here.

Finally, the small panel of genotypes used in the first phase of our study may also have influenced the number of polymorphic loci associated with viral symptomatology. During sample size determination for genomic prediction, we followed the strategy of using a first untargeted phase with a smaller training set followed by a targeted step to maximize prediction accuracy. In this way, with a smaller sample panel, some relevant alleles may not have been assessed; however, this approach allowed us to detect a large-effect locus.

The symptoms of infection by cytorhabdoviruses in ashes are often inconspicuous (Rehanek et al. 2024). Almost half of the phenotyped samples in our study, which were all infected with cytorhabdovirus, were assessed as having nonspecific

symptoms. This type of phenotype was difficult to associate with a particular nucleotide variation in the defence associated NDR1/HIN1-like (NHL) gene fragment. These observations indicate that infection, evident from detectable virus multiplication, does not always lead to visible symptoms. The outcome depends on the type or strain of the virus, the external conditions and the genotype of the host (Bos and Bos 1970; Pallas and García 2011). Thus, the severity of pathological symptoms in a susceptible host depends partly on its sensitivity, its capacity to react visibly and its vulnerability. Low sensitivity is equivalent to tolerance, which is characterised by mild or nonspecific symptoms (Osterbaan and Fuchs 2019). The sensitivity may also be extremely high such that the originally infected cells or their surroundings are rapidly killed, as evidence by a necrotic local lesion at the site of infection (Barker and Harrison 1984; Valkonen et al. 1996), which can also be described as nonspecific symptoms. The activation of hypersensitivity often induces systemic acquired resistance, which often relies on the salicylic acid (SA)-signalling pathway and the production of pathogenesis-related proteins (Liu et al. 2020; Balint-Kurti 2019). Therefore, nonspecific symptoms may indicate either low sensitivity or hypersensitivity.

As shown in Table S5, of all individuals tested, those with latent infection had thymine, and those infected with symptoms had cytosine at locus 273 of the AT3G44220 gene fragment. Four samples were exceptions to this pattern: Kaisheim 44, Melzower Forst 7, Melzower Forst seedling H, and Schorndorf 36. There are several reasons that may cause these exceptions. First, the genotype we associated with these phenotypes was characterised only by the NDR1/HIN1-like gene. NDR1 (nonrace-specific disease resistance), which is required for proper function of a subclass of R genes (Aarts et al. 1998), is only one key point of highly complex plant disease resistance, and as virus infection is regulated by various factors, other alterations in this complex mechanism may also lead to differences in symptomatology. A second reason could be that despite a thorough investigation of the whole canopy, correct assessment of whether the individual is symptomatic or not is technically challenging in 20–30 m tall specimens and those with extensive canopies, especially in untouched monitoring plots, leading to incorrect phenotyping in some cases.

Considering the homozygous/heterozygous status of all individuals genotyped, seven individuals were found to be heterozygotes, without any or with nonspecific symptoms. If the nonspecificity of the symptoms in these cases reflects hypersensitivity and the asymptomatic status is associated with thymine, as observed for almost all other asymptomatic samples, it is possible that both the homozygous (T/T) and heterozygous (T/C) states are strongly associated with triggering plant defence by virus-localising mechanisms

involving local lesions (Gopalan et al. 1996; Pontier et al. 1999; Takahashi et al. 2004) or by allowing the spread of the virus but inhibiting multiplication or symptom development. Mutation to cytosine (C) in the homozygote state may result in loss of resistance governed by several resistance genes, as described in Century et al. (1997).

The GLM association analysis of one selected marker candidate (AT3G44220) revealed a significant relationship at the conservative level between nucleotide variation at position 183 (locus 273 when a lower number of samples was tested) and the “b” and “c” versions of the numerical phenotype, whereas the correlation with version “a” was not significant. The main difference between versions “a” and “b” or “a” and “c” of the phenotype files was that version “a” did not characterise the infection status of samples, but rather only quantified their symptomatology (see Table 2). In other words, in the first (a) type of clustering, all samples were considered to have the same infection status; more precisely, all samples without symptoms were considered infected. A significant correlation between an SNP and a phenotype clustering in which noninfected asymptomatic and infected asymptomatic samples were in different clusters (b, c type of clustering) may indicate that the T/C nucleotide variation at position 183 can distinguish infected asymptomatic (tolerant) from not infected asymptomatic (either truly uninfected or completely immune) individuals. Consistent with the genotype–phenotype relationship reported in our study, plant NDR1/HIN1-like genes play a role in triggering plant defences (Liu et al. 2020), which are considered signal modulators that are essential for the stimulation of signalling pathways mediated by relevant R proteins (Century et al. 1995; Aarts et al. 1998).

Under field conditions, once infected, trees cannot be cured of virus infection and remain infected throughout their lives. Over the last decade, studies on plant viruses in forest and urban trees have confirmed the assumption that these pathogens contribute to loss of vitality and tree damage, leading to tree decline (Vainio et al. 2024). Although knowledge of the types of viruses is lacking, transmission mechanisms (Rumbou et al. 2021; Vainio et al. 2024; Rehanek et al. 2024) and defence or tolerance mechanisms (Wilson et al. 2023) are increasingly understood. Unfortunately, this knowledge has yet to translate to a way to control virus infections in forest trees in an environmentally friendly way. Given this situation, direct selection of seedlings on the basis of marker data seems rather unrealistic in breeding for virus-tolerant individuals, especially in ash, for which no examples of marker-assisted selection have been reported thus far. All of these findings suggest that selection from a much smaller population of genotypes using marker information would be a more feasible alternative requiring fewer resources. Nonetheless, it remains important to

consider that a desired trait such as virus tolerance or resistance and their inheritance must be carefully evaluated even in marker-assisted selection. The NDR1/HIN1-like gene fragment presented here as a marker candidate is only one single element involved in highly complex plant disease resistance, and virus infection is regulated by various factors. The type or strain of the virus, external conditions, general health status of the tree and genotype, with several other genes participating in the defence cascade, affect the outcome. Our samples collected from adult trees were from seed orchards, a clone archive and IBF plots; the seedlings were only from one IBF plot, Melzower Forst, and not from artificial crossings. Even in the targeted phase of the marker test, we consider the number of samples to be relatively low. However, to ensure at least the same probability of identifying new seedlings with tolerance by our candidate, further tests with selection, even with a smaller number of suitable parents but with carefully selected genotypes, should be performed (Witcombe et al. 2013). In this way, our molecular marker candidate may be the first appropriate tool for the selection of virus-tolerant common ash individuals.

Conclusions

We have shown that a marker candidate on the NDR1/HIN1-like gene fragment associated with virus infection and symptomatology traits in common ash may be suitable for marker-assisted selection in *F. excelsior* breeding.

Improving the efficiency of selection for virus tolerance in common ash breeding by marker assistance could be very important in the future control of virus diseases on ash within shorter breeding cycles. As virus infection alongside many other factors is considered a biotic stress factor possibly interacting with ash dieback, ensuring the growth of virus-tolerant trees could help establish more sustainable management strategies for this fungal disease.

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Author contributions BF and ZAK planned the experiments. SvB, MR and ZAK collected material on sampling tours. MR performed virus and symptom detection; ZAK performed the experiments. ZAK analysed the data and results. ZAK wrote the first draft of the manuscript. BF, SvB, MR and CB revised and proofread the manuscript. BF supervised the research work. All the authors have read and approved the final version of the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare that they have no conflicts of interest, and all the authors have agreed to submit this manuscript to the *European Journal of Forest Research*.

Ethics statement The authors confirm that this work is not under consideration for publication elsewhere and that all the authors and institutions have approved the manuscript for submission.

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References

- Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in Arabidopsis. *Proc Natl Acad Sci USA* 95:10306–10311. <https://doi.org/10.1073/pnas.95.17.10306>
- Aggarwal RK, Allainguillaume J, Bajay MM, Barthwal S, Bertolino P, Chauhan P, Consuegra S, Croxford A, Dalton DL, den Belder E (2011) Permanent genetic resources added to molecular ecology resources database 1 August 2010–30 September 2010. *Mol Ecol Res* 11:219–222. <https://doi.org/10.1111/j.1755-0998.2010.02944.x>
- Ahmar S, Ballesta P, Ali M, Mora-Poblete F (2021) Achievements and challenges of genomics-assisted breeding in forest trees: from marker-assisted selection to genome editing. *Int J Mol Sci* 30:10583. <https://doi.org/10.3390/ijms221910583>
- Almagro L, Gómez Ros LV, Belchi-Navarro S, Bru R, Ros Barceló A, Pedreño MA (2009) Class III peroxidases in plant defence reactions. *J Exp Bot* 60:377–390. <https://doi.org/10.1093/jxb/ern277>
- Ashwath MN, Lavale SA, Santhoshkumar AV, Mohapatra SR, Bhardwaj A, Dash U, Shiran K, Samantara K, Wani SH (2023) Genome-wide association studies: an intuitive solution for SNP identification and gene mapping in trees. *Funct Integr Genomics* 23:297. <https://doi.org/10.1007/s10142-023-01224-8>
- Bai X, Rivera-Vega L, Mamidala P, Bonello P, Herms DA, Mit-tapalli O (2011) Transcriptomic signatures of ash (*Fraxinus* spp.) phloem. *PLoS ONE* 6:e16368. <https://doi.org/10.1371/journal.pone.0016368>

- Balint-Kurti P (2019) The plant hypersensitive response: concepts, control and consequences. *Mol Plant Pathol* 20:1163–1178. <https://doi.org/10.1111/mpp.12821>
- Barker H, Harrison BD (1984) Expression of genes for resistance to potato virus Y in potato plants and protoplasts. *Ann Appl Biol* 105:539–545. <https://doi.org/10.1111/j.1744-7348.1984.tb03080.x>
- Beatty GE, Brown JA, Cassidy EM, Finlay CMV, McKendrick L, Montgomery WI, Reid N, Tosh DG, Provan J (2015) Lack of genetic structure and evidence for long-distance dispersal in Ash (*Fraxinus excelsior*) populations under threat from an emergent fungal pathogen: implications for restorative planting. *TREE GENET GENOMES* 11:1–13. <https://doi.org/10.1007/s11295-015-0879-5>
- Bejerman N, Dietzgen R, Debat H (2023) Novel tri-segmented rhabdoviruses: a data mining expedition unveils the cryptic diversity of cytorhabdoviruses. *Viruses* 15:2402. <https://doi.org/10.3390/v15122402>
- Bengtsson V, Senström A (2017) Ash dieback—a continuing threat to veteran Ash trees? In: Vasaitis R, Enderle R (eds) Dieback of European Ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 262–272. <https://doi.org/10.1079/pavsnr201914025>
- Bos L, Bos L (1970) Symptoms of virus diseases in plants. Wageningen, Netherlands. Centre for agricultural publishing and Documentation, p 206. [https://doi.org/10.1016/0160-9327\(71\)90027-5](https://doi.org/10.1016/0160-9327(71)90027-5)
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633–2635. <https://doi.org/10.1093/bioinformatics/btm308>
- Büttner C, von Barga S, Bandte M, Mühlbach HP (2013) Forest diseases caused by viruses. In: Infectious forest diseases, pp 50–75. <https://doi.org/10.1079/9781780640402.0050>
- Büttner C, Landgraf M, Colino HL, von Barga S, Bandte M (2023) Virus diseases of forest and urban trees. In: Forest microbiol. Academic Press, pp 61–97. <https://doi.org/10.1016/b978-0-443-18694-3.00011-0>
- Caranta C, Palloix A (1996) Both common and specific genetic factors are involved in polygenic resistance of pepper to several potyviruses. *Theor Appl Genet* 92:15–20. <https://doi.org/10.1007/bf00222946>
- Century KS, Holub EB, Staskawicz BJ (1995) NDR1, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. *Proc Natl Acad Sci USA* 92:6597–6601. <https://doi.org/10.1073/pnas.92.14.6597>
- Century KS, Shapiro AD, Repetti PP, Dahlbeck D, Holub E, Staskawicz BJ (1997) NDR1, a pathogen-induced component required for *Arabidopsis* disease resistance. *Science* 278:1963–1965. <https://doi.org/10.1126/science.278.5345.1963>
- Cesari S, Thilliez G, Ribot C, Chalvon V, Michel C, Jauneau A, Rivas S, Alaux L, Kanzaki H, Okuyama Y, Morel JB (2013) The rice resistance protein pair RGA4/RGA5 recognizes the Magnaporthe oryzae effectors AVR-Pia and AVR1-CO39 by direct binding. *Plant Cell* 25:1463–1481. <https://doi.org/10.1105/tpc.112.107201>
- Chira D, Chira F, Tăut I, Popovici O, Blada I, Doniță N, Bândiu C, Gancz V, Biriș IA, Popescu F, Tănăsie Ș (2017) Evolution of ash dieback in Romania. In: Vasaitis R, Enderle R (eds) Dieback of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 185–194. <https://doi.org/10.1079/pavsnr201914025>
- Cooper JI (1975) Arabis mosaic virus, a cause of chlorotic symptoms in leaves of *Fraxinus excelsior* L. *Plant Pathol* 24:114–116. <https://doi.org/10.1111/j.1365-3059.1975.tb01875.x>
- Cortés AJ, Restrepo-Montoya M, Bedoya-Canas LE (2020) Modern strategies to assess and breed forest tree adaptation to changing climate. *Front Plant Sci* 11:583323. <https://doi.org/10.3389/fpls.2020.583323>
- Dietzgen RG (1995) Rhabdoviridae. In: Singh RP, Singh US, Kohmoto K (eds) Pathogenesis and host-parasite specificity in plant diseases: histopathological, biochemical, genetic and molecular basis, vol 3. Pergamon, Oxford, pp 177–197
- Drenkhan R, Agan A, Palm K, Rosenvald R, Jürisoo L, Maaten T, Padari A, Drenkhan T (2017) Overview of ash and ash dieback in Estonia. In: Vasaitis R, Enderle R (eds) Dieback of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 115–124. <https://doi.org/10.1079/pavsnr201914025>
- Enderle R, Fussi B, Lenz HD, Langer G, Nagel R, Metzler B (2017) Ash dieback in Germany: research on disease development and management options. In: Vasaitis R, Enderle R (eds) Dieback of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 89–105. <https://doi.org/10.1079/pavsnr201914025>
- Fraser RSS (1992) The genetics of plant-virus interactions: implications for plant breeding. *Euphytica* 63:175–185. <https://doi.org/10.1007/BF00023922>
- Fraser RSS, Van Loon LC (1986) Genes for resistance to plant viruses. *Crit Rev Plant Sci* 3:257–294. <https://doi.org/10.1080/07352688609382212>
- Gaskin TR, Tischendorf M, Günther I, Rehanek M, Büttner C, von Barga S (2021) Characterization of a novel emaravirus affecting Ash species (*Fraxinus* spp.) in Europe. *Forests* 12:1574. <https://doi.org/10.3390/f12111574>
- George J-P, Sanders TGM, Timmermann V, Potočić N, Lang M (2022) European-wide forest monitoring substantiate the necessity for a joint conservation strategy to rescue European ash species (*Fraxinus* spp). *Sci Rep* 12:4764. <https://doi.org/10.1038/s41598-022-08825-6>
- Gopalan S, Bauer DW, Alfano JR, Loniello AO, He SY, Collmer A (1996) Expression of the *Pseudomonas syringae* avirulence protein AvrB in plant cells alleviates its dependence on the hypersensitive response and pathogenicity (Hrp) secretion system in eliciting genotype-specific hypersensitive cell death. *Plant Cell* 1:1095–1105. <https://doi.org/10.2307/3870354>
- Gray LK, Rweyongeza D, Hamann A, John S, Thomas BR (2016) Developing management strategies for tree improvement programs under climate change: insights gained from long-term field trials with lodgepole pine. *For Ecol Manag* 1:128–138. <https://doi.org/10.1016/j.foreco.2016.06.041>
- Hamacher J, Quadt A (1991) Light- and electron microscopic studies of Cherry leaf roll virus (CLRV) on European ash (*Fraxinus excelsior* L). *J Phytopathol* 131:215–226. <https://doi.org/10.1111/j.1439-0434.1991.tb01191.x>
- Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA (2021) Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *JGEB* 19:128
- Heinze B, Tiefenbacher H, Litschauer R, Kirisits T (2017) Ash dieback in Austria—history, current situation and outlook. In: Vasaitis R, Enderle R (eds) Dieback of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 33–52. <https://doi.org/10.1079/pavsnr201914025>
- ICTV master species List (2023) https://ictv.global/sites/default/files/MSL/ICTV_Master_Species_List_2023_MSL39.v3.xlsx
- Jackson AO, Dietzgen RG, Goodin MM, Bragg JN, Deng M (2005) Biology of plant rhabdoviruses. *Annu Rev Phytopathol* 43. <https://doi.org/10.1146/annurev.phyto.43.011205.141136.623-60>

- Jones OR, Wang J (2010) COLONY: a program for parentage and Sibship inference from multilocus genotype data. *Mol Ecol Res* 10:551–555. <https://doi.org/10.1111/j.1755-0998.2009.02787.x>
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106. <https://doi.org/10.1111/j.1365-294x.2007.03089.x>
- Knepper C, Savory EA, Day B (2011) Arabidopsis NDR1 is an integrin-like protein with a role in fluid loss and plasma membrane-cell wall adhesion. *Plant Physiol* 156:286–300. <https://doi.org/10.1104/pp.110.169656>
- Köbölkuti ZA, Rehanek M, von Bargaen S, Büttner C, Fussi B (2025) Identification and amplification of candidate genes for virus defence in common Ash (*Fraxinus excelsior* L.). *J Plant Dis Prot* 132:30. <https://doi.org/10.1007/s41348-024-01018-5>
- Kvarnheden A, Albert VA, Engström P (1998) Molecular evolution of cdc2 pseudogenes in Spruce (*Picea*). *Plant Mol Biol* 36:767–774. <https://doi.org/10.1023/a:1005901413475>
- Labuschagne C, Nupen L, Kotzé A, Grobler PJ, Dalton DL (2015) Assessment of microsatellite and SNP markers for parentage assignment in ex situ African Penguin (*Spheniscus demersus*) populations. *Ecol Evol* 5:4389–4399. <https://doi.org/10.1002/ec.31600>
- Lee SB, Ham BK, Park JM, Kim YJ, Paek KH (2006) BnNHL18A shows a localization change by stress-inducing chemical treatments. *Biochem Biophys Res Commun* 339:399–406. <https://doi.org/10.1016/j.bbrc.2005.10.210>
- Lefort F, Douglas GC (1999) An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species acer, fraxinus, Prunus and Quercus. *Ann Sci* 56:259–263. <https://doi.org/10.1051/forest:19990308>
- Lefort F, Brachet S, Frascaria-Lacoste N, Edwards KJ, Douglas GC (1999) Identification and characterization of microsatellite loci in Ash (*Fraxinus excelsior* L.) and their conservation in the Olive family (Oleaceae). *Mol Ecol* 8:1088–1089. https://doi.org/10.1046/j.1365-294x.1999.00655_8.x
- Liu C, Liu Y (2024) Accelerating the process of tree breeding: a review and progress of GWAS applications in forest trees. *TGMB* 14. <https://doi.org/10.5376/tgmb.2024.14.0003>
- Liu C, Peng H, Li X, Liu C, Lv X, Wei X, Zou A, Zhang J, Fan G, Ma G, Ma L (2020a) Genome-wide analysis of NDR1/HIN1-like genes in pepper (*Capsicum annuum* L.) and functional characterization of CaNHL4 under biotic and abiotic stresses. *Hort Res* 1:7. <https://doi.org/10.1038/s41438-020-0318-0>
- Liu Y, Sun T, Sun Y, Zhang Y, Radojčić A, Ding Y, Tian H, Huang X, Lan J, Chen S, Orduna AR (2020b) Diverse roles of the Salicylic acid receptors NPR1 and NPR3/NPR4 in plant immunity. *Plant Cell* 32:4002–4016. <https://doi.org/10.1105/tpc.20.00499>
- Mageroy MH, Nagy NE, Steffenrem A, Krokene P, Hietala AM (2023) Conifer defences against pathogens and pests—mechanisms, breeding, and management. *Curr Rep* 2023 9:429–443. <https://doi.org/10.1007/s40725-023-00201-5>
- Maldonado C, Mora-Poblete F, Contreras-Soto RI, Ahmar S, Chen JT, do Amaral Júnior AT, Scapim CA (2020) Genome-wide prediction of complex traits in two outcrossing plant species through Deep Learning and Bayesian Regularized Neural Network. *Front Plant Sci* 11:593897. <https://doi.org/10.3389/fpls.2020.593897>
- Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganai MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* 262:1432–1436. <https://doi.org/10.1126/science.7902614>
- Marwal A, Gaur RK (2020) Host plant strategies to combat against viruses effector proteins. *Curr Genomics* 21:401–410. <https://doi.org/10.2174/1389202921999200712135131>
- Navarro B, Loconsole G, Giampetruzzi A, Aboughanem-Sabanadzovic N, Ragozzino A, Ragozzino E, Di Serio F (2017) Identification and characterization of Privet leaf blotch-associated virus, a novel ideovirus. *Mol Plant Pathol* 18:925–936. <https://doi.org/10.1111/mpp.12450>
- Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol* 3:418–426. <https://doi.org/10.1093/oxfordjournals.molbev.a040410>
- Nienhaus F (1985) Infectious diseases in forest trees caused by viruses, mycoplasma-like organisms and primitive bacteria. *Experientia* 597–603. <https://doi.org/10.1007/bf02007677>
- Noakes AG, Best T, Staton ME, Koch J, Romero-Severson J (2014) Cross amplification of 15 EST-SSR markers in the genus *Fraxinus*. *Conserv Genet Resour* 6:969–970. <https://doi.org/10.1007/s12686-014-0260-2>
- Nürnberg T, Scheel D (2001) Signal transmission in the plant immune response. *Trends Plant Sci* 6:372–379. [https://doi.org/10.1016/s1360-1385\(01\)02019-2](https://doi.org/10.1016/s1360-1385(01)02019-2)
- Osterbaan LJ, Fuchs M (2019) Dynamic interactions between plant viruses and their hosts for symptom development. *Plant Pathol* 101:885–895. <https://doi.org/10.1007/s42161-019-00323-5>
- Pallas V, García JA (2011) How do plant viruses induce disease? Interactions and interference with host components. *J Gen Virol* 92. <https://doi.org/10.1099/vir.0.034603-0.2691-705>
- Pontier D, Gan S, Amasino RM, Roby D, Lam E (1999) Markers for hypersensitive response and senescence show distinct patterns of expression. *Plant Mol Biol* 39:1243–1255. <https://doi.org/10.1023/a:1006133311402>
- Queloz V, Hopf S, Schoebel CN, Rigling D, Gross A (2017) Ash dieback in Switzerland: history and scientific achievements. In: Vasaitis R, Enderle R (eds) Dieback of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 68–78. <https://doi.org/10.1079/pavsnmr201914025>
- Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol* 5:94–100. [https://doi.org/10.1016/S1369-5266\(02\)00240-6](https://doi.org/10.1016/S1369-5266(02)00240-6)
- Rebenstorf K, Candresse T, Dulucq MJ, Büttner C, Obermeier C (2006) Host species-dependent population structure of a pollen-borne plant virus, Cherry leaf roll virus. *J Virol* 80:2453–2462. <https://doi.org/10.1128/JVI.80PMID:16474152;PMCID:PMC1395386>
- Rehanek M, Kubrusli RA, Fernandez H, von Bargaen S, Carmen B (2023) Virusvielfalt in esche. In: Steinhart F, Burzlaff T (eds) Berichte Freiburger Forstliche Forschung—FraxForFuture—Erhalt der Gemeinen Esche (*Fraxinus excelsior* als Wirtschaftsbaumart, vol 106, pp 40–42
- Rehanek M, Al Kubrusli R, Köpke K, von Bargaen S, Büttner C (2024) Detection of viruses in special stands of common ash reveals insights into the Virome of *Fraxinus excelsior*. *Forests* 15:1379. <https://doi.org/10.3390/f15081379>
- Rozas J, Rozas R (1995) DnaSP, DNA sequence polymorphism: an interactive program for estimating population genetics parameters from DNA sequence data. *Bioinform* 1:621–625. <https://doi.org/10.1093/bioinformatics/11.6.621>
- Rumbou A, Vainio EJ, Büttner C (2021) Towards the forest virome: Next-generation-sequencing drastically expands our understanding on virosphere in temperate forest ecosystems. [www.preprints.org. https://doi.org/10.20944/preprints202106.0526.v1](https://doi.org/10.20944/preprints202106.0526.v1)
- Semizer-Cuming D, Finkeldey R, Nielsen LR, Kjær ED (2019) Negative correlation between ash dieback susceptibility and reproductive success: good news for European ashe forests. *Ann Sci* 76:1–9. <https://doi.org/10.1007/s13595-019-0799-x>
- Sioen G, Roskams P, de Cuyper B, Steenackers M (2017) Ash dieback in Flanders (Belgium): research on disease development, resistance & management. In: Vasaitis R, Enderle R (eds) Dieback

- of European ash (*Fraxinus* spp.)—consequences and guidelines for sustainable management. Swedish University of Agricultural Sciences, Uppsala, pp 61–67. <https://doi.org/10.1079/pavsnr201914025>
- Sniezko RA, Liu JJ (2023) Prospects for developing durable resistance in populations of forest trees. *New for* 54. <https://doi.org/10.1007/s11056-021-09898-3>:751–67
- Sollars ESA, Harper AL, Kelly LJ, Sambles CM, Ramirez-Gonzalez RH, Swarbreck D, Kaithakottil G, Cooper ED, Uauy C, Havlickova L, Worswick G (2017) Genome sequence and genetic diversity of European Ash trees. *Nature* 541:212–216. <https://doi.org/10.1038/nature20786>
- Stucky BJ (2012) SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *J Biomol Tech* 23:90. <https://doi.org/10.7171/jbt.12-2303-004>
- Takahashi Y, Berberich T, Yamashita K, Uehara Y, Miyazaki A, Kusano T (2004) Identification of tobacco HIN1 and two closely related genes as spermine-responsive genes and their differential expression during the tobacco mosaic virus-induced hypersensitive response and during leaf-and flower-senescence. *Plant Mol Biol* 54:613–622. <https://doi.org/10.1023/b:plan.0000038276.95539.39>
- Vacek Z, Vacek S, Cukor J (2023) European forests under global climate change: review of tree growth processes, crises and management strategies. *J Environ Manag* 332:117353. <https://doi.org/10.1016/j.jenvman.2023.117353>
- Vainio EJ, Rumbou A, Diez JJ, Büttner C (2024) Forest tree Virome as a source of tree diseases and biological control agents. *Curr Rep* 10. <https://doi.org/10.1007/s40725-024-00214-8>:153–74
- Valkonen JP (2015) Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato. *Breed Sci* 65:69–76. <https://doi.org/10.1270/jsbbs.65.69>
- Valkonen JPT, Jones RAC, Slack SA, Watanabe KN (1996) Resistance specificities to viruses in potato: standardization of nomenclature. *Plant Breed* 115:433–438. <https://doi.org/10.1111/j.1439-0523.1996.tb00952.x>
- Varet A, Parker J, Tornero P, Nass N, Nurnberger T, Dangl JL, Scheel D, Lee J (2002) NHL25 and NHL3, two NDR1/HIN1-Like genes in *Arabidopsis thaliana* with potential role(s) in plant defense. *Mol Plant Microbe Interact* 15:608–616. <https://doi.org/10.1094/MPMI.2002.15.6.608>
- Varet A, Hause B, Hause G, Scheel D, Lee J (2003) The Arabidopsis NHL3 gene encodes a plasma membrane protein and its over-expression correlates with increased resistance to *Pseudomonas syringae* pv. tomato DC3000. *Plant Physiol* 132:2023–2033. <https://doi.org/10.1104/pp.103.020438>
- Varshney RK, Dubey A (2009) Novel genomic tools and modern genetic and breeding approaches for crop improvement. *J Plant Biochem Biotechnol* 18:127–138
- Walker PJ, Freitas-Astúa J, Bejerman N, Blasdel KR, Breyta R, Dietzen RG, Fooks AR, Kondo H, Kurath G, Kuzmin IV, Ramos-González PL (2022) ICTV virus taxonomy profile: rhabdoviridae. *J Gen Virol* 103:001689. <https://doi.org/10.1099/jgv.0.001689>
- Wilson SK, Pretorius T, Naidoo S (2023) Mechanisms of systemic resistance to pathogen infection in plants and their potential application in forestry. *BMC Plant Biol* 23:404. <https://doi.org/10.1186/s12870-023-04391-9>
- Witcombe JR, Gyawali S, Subedi M, Virk DS, Joshi KD (2013) Plant breeding can be made more efficient by having fewer, better crosses. *BMC Plant Biol* 13:1–2. <https://doi.org/10.1186/1471-2229-13-22>

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