الجمهورية الجزائرية الشعبية الديموقراطية
وزارة التعليم العالي والبحث العلمي

Setif 1 University Ferhat Abbas
Faculty of Natural and life sciences

جامعة سطيف 1 فرحات عباس
كلية علوم الطبيعة والحياة

Departement of Agronomy

Laboratory of applied microbiology

N°..... /SNV/2025

THESIS

Submitted by

Nour Elhouda Laidoudi

For the fulfillment of the requirements for the degree of

Third Cycle DOCTORATE

Field: Agriculture sciences

Specialty: Plant protection: bio-pests

TOPIC

Epidemiological study of *Grapevine fanleaf virus* in Algeria

Publicly defended in: 04/08/2026

Members of the jury

President	Mebarkia Abdelkrim	Professor	Setif 1 University Ferhat Abbas
Supervisor	Yahiaoui Bilal	Professor	Setif 1 University Ferhat Abbas
Co-supervisor	Lehad Arezki	MCA	National higher school of agronomy Algiers
Examiners	Laala Samia	MCA	National higher school of agronomy Algiers
	Haddad Benalia	MCA	National higher school of agronomy Algiers
	Belhadj Hani	Professor	Setif 1 University Ferhat Abbas

I am deeply grateful for this achievement, which I owe to my parents for their unwavering love, endless support, and selfless sacrifices that made it possible.

Acknowledgments

Writing the acknowledgements can be a challenging task, as it is difficult to find the right words to fully convey my gratitude. Many individuals have contributed in various ways to the completion of this work, and I want to express my sincere thanks to each of them for their invaluable support and encouragement.

First of all, I would like to thank Prof. **Mebarkia Abdelkrim** to do me the honor of chairing of the jury, and Prof. **Laala Samia**, Prof. **Haddad Benalia** and Prof. **Belhadj Hani**, members of the jury, for agreeing to judge and evaluate this work.

I would like to express my gratitude to Professor **Bilal Yahiaoui**, my thesis supervisor, for generously sharing his knowledge and experience with me. His insightful suggestions and constructive criticism were invaluable throughout the process. I greatly appreciated his work ethic, efficiency, and enthusiasm. In short, he truly deserves the title of thesis director.

I would also like to thank Dr. **Arezki Lehad**, my thesis co-director, with whom I had the pleasure of working at the National High School of Agronomy in Algiers. He provided me with the trust, time, and resources essential for my journey as a doctoral student. His optimism and excellent sense of humor made our collaborative moments enjoyable, supportive, and inspiring.

I would like to extend my sincere gratitude to our collaborators at the Institute for Sustainable Plant Protection, National Research Council of Italy, especially Dr. **Minafra Angelantonio**, Dr. **Deluca Francesca**, Dr**. Fanelli Elena**, and Dr. **Troccoli Alberto**, for their warm welcome and invaluable support during my internship research stay. Their expertise, kindness, and dedication significantly contributed to the success of this work.

Special thanks go to my dear friends and laboratory colleagues **Anfel Djenaoui**, **Roufaida Segmane, Meriem Daichi**, **Imene Mahdid**, and **Rahil Neggaz** for their friendship, cooperation, and all the wonderful moments we shared together.

Finally, I would like to extend my heartfelt gratitude to all the farmers and agricultural workers who have supported our efforts in their vineyards. I also sincerely thank the laboratory engineers of the Microbiology Laboratories at UFAS and ENSA El Harrach for their technical assistance. Additionally I would also like to thank Mr. **Derradji** from the forest district of Ain Azel for granting us permission to use the nursery and greenhouses, which greatly facilitated our work.

Abstract

Prevalence and genetic diversity of *Grapevine fanleaf virus* (*Nepovirus foliumflabelli*, GFLV) were determined in vineyards and grape varieties in Algeria. Samples (435) from different cultivars and viticulture areas were screened using DAS-ELISA and partially confirmed by RT-PCR, revealing 20% infection incidence. In Ahmer Bou Amer the greatest incidence of infection was recorded (61%). Some vines, confirmed to be GFLV-infected, had characteristic symptoms of leaf yellowing, chloroses, and mosaic patterns, reducing vine vigour and fruit quality. High throughput sequencing and bioinformatics analyses of a single GFLV-infected accession obtained a nearly complete *Grapevine fanleaf virus* RNA1 consensus sequence of 5,979 nt, and an RNA2 with complete consensus sequence of 3,711 nt. Phylogenetic analyses of an amplified fragment of the GFLV coat protein gene from some of the accessions indicated close genetic relationships between Algerian and Russian/ United States of America GFLV isolates, suggesting potential shared origins or transmission pathways. The nematological analysis indicates that *Xiphinema index*, the vector of GFLV, was absent at the sampled site. However, the soil samples revealed the presence of *Meloidogyne incognita*, which represents an additional threat to grapevines. These results emphasize the need for implementing strict phytosanitary measures (e.g. use of virus-free planting material) to mitigate GFLV spread and its detrimental effects on grapevine production in Algeria.

Keywords. Grapevine, GFLV, DAS-ELISA, High Throughput Sequencing, RT-PCR, Vector

Résumé

La prévalence et la diversité génétique du *Grapevine fanleaf* virus (*Nepovirus foliumflabelli*, GFLV) ont été étudiées au cours des vignobles et des variétés de la vigne en Algérie. Quatre cent trente-cinq échantillons prélevés sur divers cultivars et régions viticoles ont été analysés par la méthode DAS-ELISA et ont fait l'objet d'une confirmation partielle par RT-PCR, ce qui a mis en évidence une incidence d'infection de 20 %. L'incidence d'infection la plus élevée (61 %) a été observée à Ahmer Bou Amer. Certaines vignes, dont l'infection par le GFLV a été confirmée, présentaient des symptômes caractéristiques tels que, jaunissement des feuilles, chloroses et de mosaïques, entrainant une diminution de la vigueur des vignes et la qualité des fruits. High throughput sequencing et les analyses bioinformatiques d'une seule accession infectée par le GFLV ont permis d'obtenir une séquence consensus presque complète de l'ARN1 du *Grapevine fanleaf virus* de 5 979 nt, et un ARN2 avec une séquence consensus complète de 3 711 nt. Les analyses phylogénétiques d'un fragment amplifié du gène de la protéine capsidiale du GFLV obtenus à partir de certaines accessions, ont révélé des relations génétiques étroites entre les isolats algériens et russes/américains du GFLV, suggérant la possibilité d'origines ou des voies de transmission potentiellement partagées. L'analyse nématologique a révélé l'absence de *Xiphinema index*, le vecteur du GFLV, sur les sites échantillonnés. Cependant, les échantillons de sol ont montré la présence de *Meloidogyne incognita*, un nématode phytopathogène qui représente une menace supplémentaire pour les vignes. Ces résultats soulignent la nécessité de mettre en œuvre des mesures phytosanitaires strictes (par exemple, l'utilisation de matériel de plantation exempt de virus) pour atténuer la propagation du GFLV et ses effets néfastes sur la production de vigne en Algérie. Ces résultats soulignent la nécessité de mettre en œuvre des mesures phytosanitaires rigoureuses (par exemple, l'utilisation de matériel de plantation exempt de virus) afin de limiter la propagation du GFLV et ses effets néfastes sur la production de vigne en Algérie.

Mots-clés: Vigne, GFLV, DAS-ELISA, High throughput sequencing, RT-PCR, vecteur

الملخص

تم تحديد الانتشار والتنوع جيني لفيروس الأوراق المروحية (*Nepovirus* *Grapevine fanleaf virus* (*foliumflabelli*, GFLV) في مزارع الكروم وأصناف العنب في الجزائر. لقد تم فحص عدد من العينات يقدر ب 435 من عدة أصناف ومناطق مختلفة الخاصة بزراعة الكروم باستخدام DAS-ELISA وتأكيدها جزئيًا بواسطة RT-PCR ، كشفت النتائج عن حدوث إصابة بنسبة 20٪. وقد سُجلت أعلى نسبة إصابة في أحمر بوعمر قدرت ب (61٪). أظهرت بعض أصناف العنب التي تأكدت إصابتها بفيروس GFLV ، أعراضًا مميزة تتمثل في اصفرار الأوراق، والكلوروز، وأنماط الفسيفساء، مما يؤدي إلى انخفاض القدرة الانتاجية للكروم وجودة الثمار. أدى التسلسل عالي الإنتاجية والتحليلات الحيوية المعلوماتية High throughput sequencing لعينة واحدة مصابة بفيروس GFLV إلى الحصول على تسلسل إجماعي شبه كامل لفيروس الأوراق المروحية للكرمة RNA1 يبلغ 5979 نت، وتسلسل إجماعي كامل لـ RNA2 يبلغ 3711 نت. أشارت التحليلات الفيلوجينية لجزء مكثف من جين بروتين غلاف GFLV لبعض العينات إلى وجود علاقات وراثية وثيقة بين عزلات GFLV الجزائرية والروسية/الأمريكية، مما يشير إلى احتمال وجود أصول مشتركة أو مسارات انتقال مشتركة. يشير التحليل النيماتولوجي إلى أن *Xiphinema index* ، الناقل لفيروس GFLV ، كان غائبًا في الموقع الذي تم أخذ العينات منه. ومع ذلك، كشفت عينات التربة عن وجود *Meloidogyne incognita* ، الذي يمثل تهديدًا إضافيًا للكروم. تؤكد هذه النتائج على الحاجة إلى تنفيذ تدابير صارمة للصحة النباتية (مثل استخدام مواد زراعية خالية من الفيروسات) للتخفيف من انتشار فيروس GFLV وآثاره الضارة على إنتاج الكروم في الجزائر.

الكلمات المفتاحية: العنب، GFLV، DAS-ELISA، High throughput put sequencing، RT-PCR، الناقل

Table of contents

Materials and methods

Results and discussion

List of abbreviations

AGVd	Australian grapevine viroid
BBR	Botrytis Bunch Rot
CEVd	Citrus Exocortis viroid
CP	Coat Protein
CTAB	Cetyltrimethylammonium bromide
DAS-ELISA	Double Antibody Sandwich- Enzyme Linked Immune Sorbent Assay
DTT	Dithiothreitol
EPPO	European and Mediterranean Plant Protection Organization
GAMaV	Grapevine Astound Virus
GFKV	Grapevine Fleck virus
GFLV	Grapevine Fanleaf virus
GHVd	Grapevine Hammerhead Viroid-Like RNA
GLRVs	Leafroll-Associated Viruses
GLVd	Grapevine Latent Viroid
GRBV	Grapevine Red Blotch Virus
GRSPaV	Rupestris Stem Pitting Virus
GRVFV	Grapevine Red Leaf Virus
GSyV-1	Grapevine Synergistic Virus 1
GVA	Grapevine virus A
GVB	Grapevine virus B
GYSVd	Grapevine Yellow Speckle Viroid
HSVd	Hop Stunt Viroid
HTS	High Throughput Sequencing
IAPSC	Inter- African Phytosanitary Council
ITAFV	Institute techniques d'arboriculture fruitiers et de la vigne
JGVd	Japanese Grapevine Viroid
LOC	Lab On Chip
MMLV	Moloney Murine Leukemia Virus
NAPPO	North American Plant Protection Organization
NGS	Next Generation Sequencing

OIV	International Organization of Vine and wine
ORF	Open reading frame
P1	RNA1 Polyprotein
P2	RNA 2 Polyprotein
PD	Pierce's Disease
RT-LAMP	Reverse Transcription Loop Mediated Isothermal Amplification Assay
VPg	Genome-Linked Viral Protein
1A	Protease cofactor
1BHe	Helicase
1CVPg	Genome-linked protein
1DPro	Protease
1EPol	RNA-dependent RNA polymerase
2AHP	Homing protein
2BMP	The movement protein

List of Figures

List of boxes

List of tables

Introduction

Introduction

The grapevine (*Vitis vinifera* L.) is one of the oldest domesticated fruit crops, with a history of cultivation dating back more than 6,000 years in the Near East and it has since spread globally, influencing agriculture, culture and the global economy (Meng *et al.*, 2017; Khan *et al.*, 2020). Today, it is grown on all continents except Antarctica and represents one of the most important fruit crops worldwide (Butiuc-Keul and Coste, 2023). Grapes are consumed fresh as table fruit, dried as raisins, or processed into juice and wine, the latter being of major economic and cultural significance (Gharate *et al.*, 2025; Paul *et al.*, 2025). According to the International Organization of Vine and Wine (OIV) in 2024, the global grapevine cultivation area reached 7.5 million hectares, marking a decline of about 0.6% compared to the previous year.

Historically, Algeria was one of the major vine-growing countries. During the colonial period, vineyard area reached some 350,000 hectares in the early 1960s, and grape production exceeded 1,900,000 tones (FAO, 2025). Currently the viticulture in Algeria covers nearly 69,000 hectares, with annual production estimated at over 600,000 tones, highlighting its contribution to national agriculture and rural economies (OIV, 2024).

Grapevines are subjected to various pests and pathogens, particularly infectious intracellular agents (viruses, viroids, and phloem and xylem-limited prokaryotes), causing serious crop losses. The most significant viral diseases are grapevine degeneration and decline due to nepoviruses, leafroll disease, the rugose wood complex, and fleck disease (Fuchs, 2024).

The *Grapevine fanleaf virus* (*Nepovirus foliumflabelli*, GFLV) is one of the 15 viruses associated with fanleaf degeneration, a serious and prevalent disease affecting grapevines (Schmitt-Keichinger *et al.*, 2017). This virus can decrease vineyard yields, damage the quality of fruit, and shorten vine longevity (Krebelj *et al.*, 2015). GFLV belongs to the Nepovirus genus (Secoviridae) and is transmitted by the dagger nematode *Xiphinema index* (M'rabet Samaali *et al.*, 2024) in a nonpersistent, non-circulative way (Demangeat, 2007; Fuchs *et al.*, 2017). The soil-borne nature of the vector means that once a vineyard becomes infested with *X. index*, the virus can persist for decades, even if infected vines are removed. This makes GFLV particularly difficult to manage compared to viruses with airborne vectors.

Globally, the epidemiology of GFLV has been studied in several major viticulture regions. In Europe, studies have been conducted in France, Italy, Spain, Bosnia and Herzegovina and Serbia) (Andret-Link *et al.*, 2004; Crnogorac *et al.*, 2021; Panno *et al.*, 2021; Bagi *et al.*, 2022). research has focused on Iran and Kazakhstan Asia (Hajizadeh *et al.*, 2015; Frolov *et al.*, 2025).

In North America, surveys have been carried out in the United States and Canada (Mekuria *et al.*, 2009; Poojari *et al.*, 2020). These studies have identified key risk factors associated with disease incidence, such as vineyard age, the use of non-resistant rootstocks, planting of uncertified propagation material, and the presence of previous vine crops on the same site.

Accurate and early diagnosis of grapevine virus infections is critical for successful disease management, ensuring quality and productivity in viticulture. Traditional methods, such as DAS-ELISA, remain widely used because they allow for relatively inexpensive and rapid screening of large numbers of samples. In contrast, the use of reverse transcription polymerase chain reaction (RT-PCR) and related molecular methods offers significantly higher sensitivity and specificity. These methods enable the detection of smaller amounts of viral RNA, identification of mixed infections, and the capacity for genetic characterization and phylogenetic analyses (Constable *et al.*, 2012; Zhou *et al.*, 2015; Bruisson *et al.*, 2017).

Next generation sequencing (NGS) is a technology for analyzing genomic data (Mokili *et al*., 2012; Massart *et al*., 2014; Brister *et al.*,2015), and identifying virus pathogens *de novo* (Adams *et al*., 2009; Kreuze *et al*., 2009; Barba *et al*., 2014; Al Rwahnih *et al*., 2015). A study on Iranian grapevine cultivars by using high throughput sequencing (HTS) identified thirteen viruses and viroids, with grapevine red blotch, satellite, grapevine leafroll associated virus 1, and *Grapevine fanleaf virus* dominating (Gholampour *et al*., 2024).

The GFLV situation has received less molecular documentation compared to other classical viticulture regions in Algeria. While serological and symptomatic surveys have been conducted, indicating the presence of GFLV in Algerian vineyards (Tahirine *et al.*, 2020), comprehensive molecular characterizations and complete-genome sequences from Algerian isolates have been limited or absent in public databases until recently.

The overall aim of this study is to provide a thorough epidemiological characterization of *Grapevine fanleaf virus* in Algeria by combining serological, molecular, nematological and genomic approaches. The specific objectives are:

1. To evaluate the prevalence and spatial distribution of GFLV infection across major grapevine-growing regions and cultivars in Algeria, using DAS-ELISA and RT-PCR.
2. To characterize the position of Algerian GFLV isolates and to establish phylogenetic relationships with isolates from other geographic regions by sequencing genomic regions.
3. To use High Throughput Sequencing (HTS) to obtain complete viral genome of GFLV.

4. To investigate the nematological context of surveyed vineyards, identifying root-parasitic nematodes (with particular attention to *Xiphinema* spp.) and assessing their potential role in virus transmission within the Algerian viticultural landscape.

Literature review

Literature review

1. Overview on Grapevine and viticulture

Grapevine (*Vitis vinefera*), belongs to the Vitaceae family and are considered one of the major and oldest fruit in the world. Grapevines are climbers, which need support to grow. The vine is composed of several morphological structures, including the trunk, cordon, shoots, and bunches. A grape cluster consists of multiple components, such as peduncles, rachis, pedicels, and berries. Botanically fruit of a grape is known as a berry (Botineau, 2010; Somkuwar *et al*., 2024).

1.1 Importance of viticulture in the world

According to the International Organization of Vine and Wine (OIV), in 2024, the world's wine-growing area, corresponding to the total surface area planted with vines, including areas not yet in production, for all destinations (wine and juice, table grapes and raisins), is estimated at 7.5 million hectares.

Grapevine production is mainly located in Europe and Asia (Figure 1), China ranks first in grape production (Figure 2), producing 13,500,000 tonnes followed by Italy, France, the United States, Spain, and India. In Africa, South Africa is the biggest producer, with a production of 1,973,818.52 tonnes (FAOSTAT, 2025.)

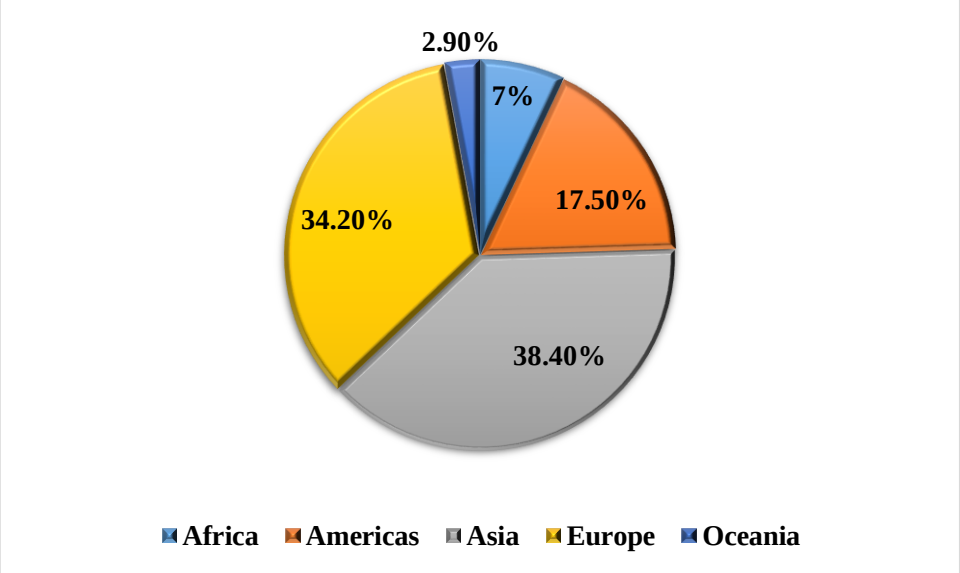


Figure 1: Grapevine world production region in 2023 (FAOSTAT, 2025)

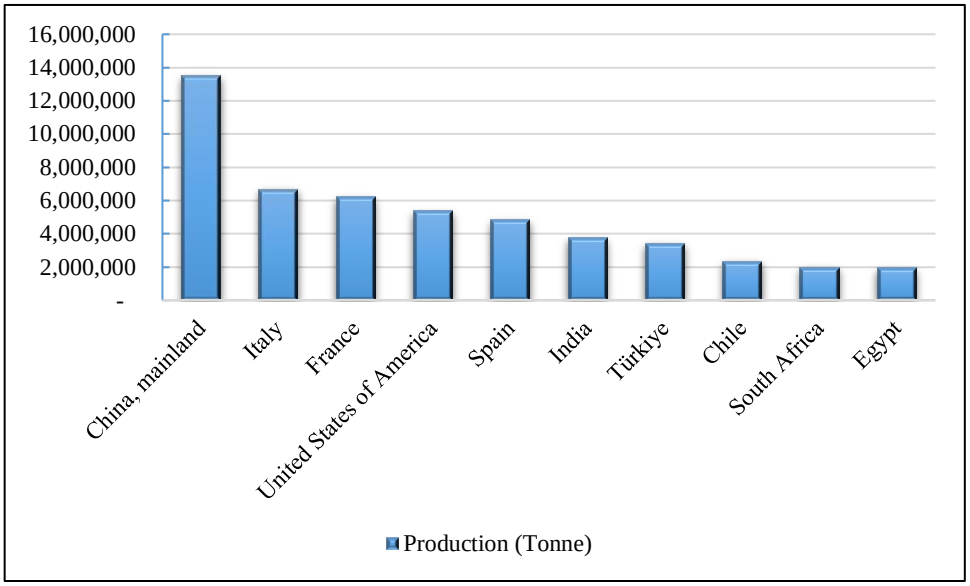


Figure 2: Global grapevine production by coutry (FAOSTAT, 2025).

1.2 Viticulture in Algeria

1.2.1 Economic importance of viticulture

During the French colonization of Algeria, viticulture underwent major development and was oriented almost exclusively toward European wine production (Isnard, 1969; Levadoux et *al.*, 1971). Consequently, by 1960, Algeria had become the fourth-largest wine producer globally and the world's largest wine exporter, accounting for approximately one-quarter of the international wine trade volume. However, this situation drastically changed following Algeria's independence in 1962, as the country subsequently lost its wine export markets (Rahali *et al.*, 2019) and the cultivation of this crop experienced a significant decline in terms of production and surface area until 1990. Between the early 1990s and mid-2000s, the area harvested and grape production stabilized at relatively low levels, (around 50000 ha and 146000 tones). From around 2006 until 2023, grape production shows signs of gradual recovery and incremental growth, surpassing harvested area, this suggests improved productivity or more intensive agricultural practices (Figure 3) (FAOSTAT, 2025).

1.2.2 Algerian grapevine Cultivars

Grapevine cultivars in Algeria comprise a diverse array of autochthones and allochthones varieties, utilized primarily for wine production, fresh table consumption and drying purposes. Based on their maturation periods, these cultivars can be categorized into three distinct groups: early-ripening (2nd week of July, mid-season) seasonal (last July and mi-September), and late-ripening varieties (maturity after mi September).

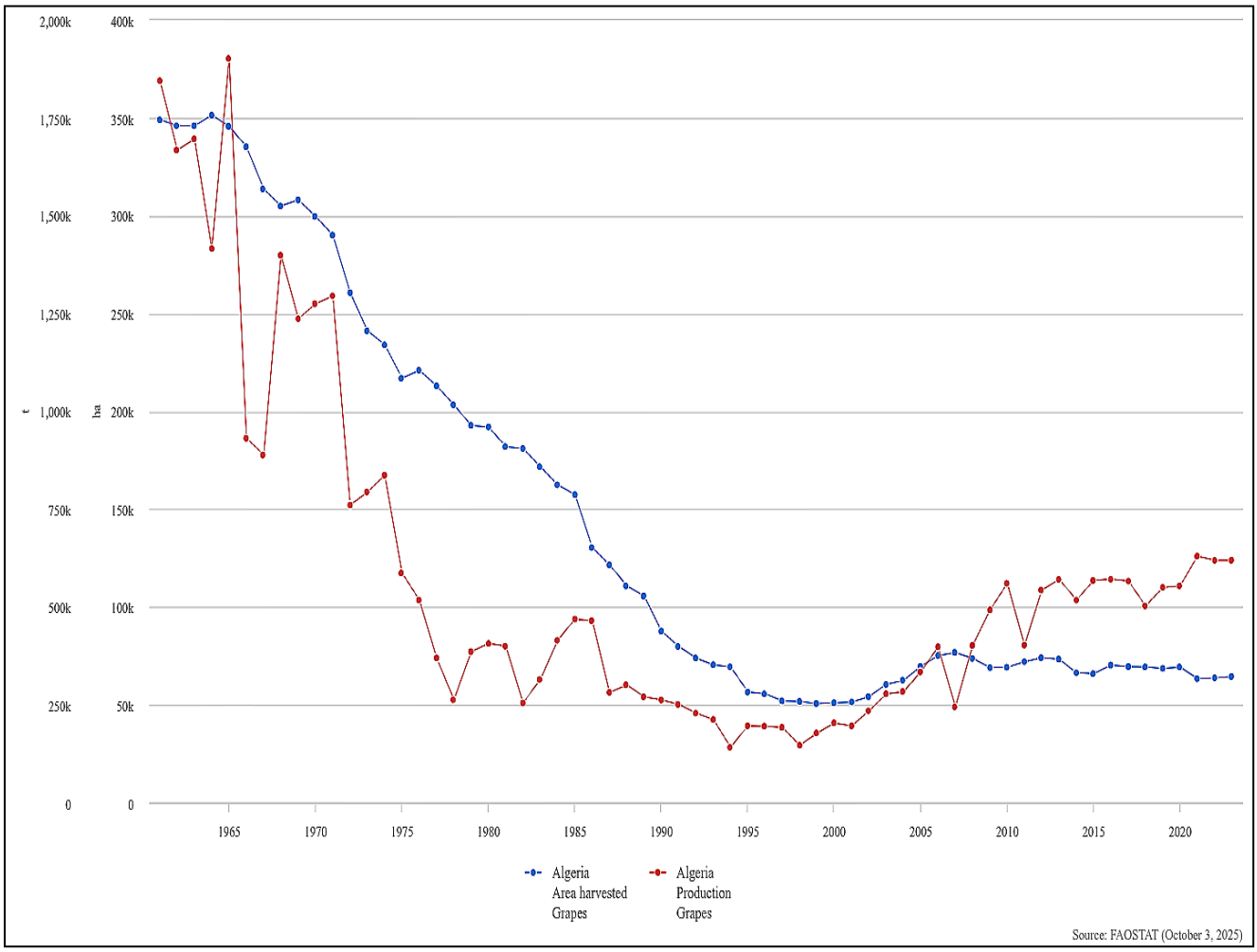


Figure 3 : Evolution of grapevine area harvested (ha) and production (t) in Algeria between 1961 and 2023, based on FAOSTAT (2025).

For the autochthones cultivars, ITAFV (Institut Technique de l'Arboriculture Fruitière et de la Vigne) of Algeria has an important collection as reference for the genetic diversity of grapevine in Algeria.

According to the Official Journal of the Algerian Republic (No. 52, dated September 21, 2011), grapevine cultivars in Algeria include 39 varieties designated for fresh consumption (table grapes), five cultivars specifically intended for drying, and 31 wine grape varieties. The wine grape cultivars are further classified into two distinct categories based on berry coloration: black or pink grapes, and white grapes (Appendix 1).

2. Major grapevine disease and pests

2.1 Main pests

Grapevines are susceptible to a variety of arthropod pests that can significantly affect yield and fruit quality. The main arthropods pests of grapevine are Phylloxera (*Daktulosphaira vitifoliae)*, mealybugs and leafhoppers.

- **Phylloxera:**

  Grape **phylloxera (*Daktulosphaira vitifoliae*)** is considered the most economically important pest impacting grapevines (Powell *et al.*, 2013; Savoi *et al.*, 2020). This aphid-like insect (Figure 4), native to North America, has been accidentally disseminated to vineyards worldwide. It damages grapevines chiefly by feeding on them, causing the development of galls roots and leaves. However, the most significant damage results from root infections, which can progressively weaken the plant and, in extreme instances, lead to vine death (Agarwal *et al.*, 2020). Historically, the introduction of phylloxera from North America to Europe in the 19th century led to the collapse of nearly 70% of French vineyards, causing massive financial losses and necessitating the widespread adoption of resistant rootstocks (Powell *et al.*, 2013). Moreover, the costs of managing phylloxera, including vineyard replanting with resistant rootstocks and ongoing monitoring, add to the financial burden for growers. In California, the replacement of susceptible rootstocks has been estimated to cost millions of dollars annually (Lund *et al.*, 2017).

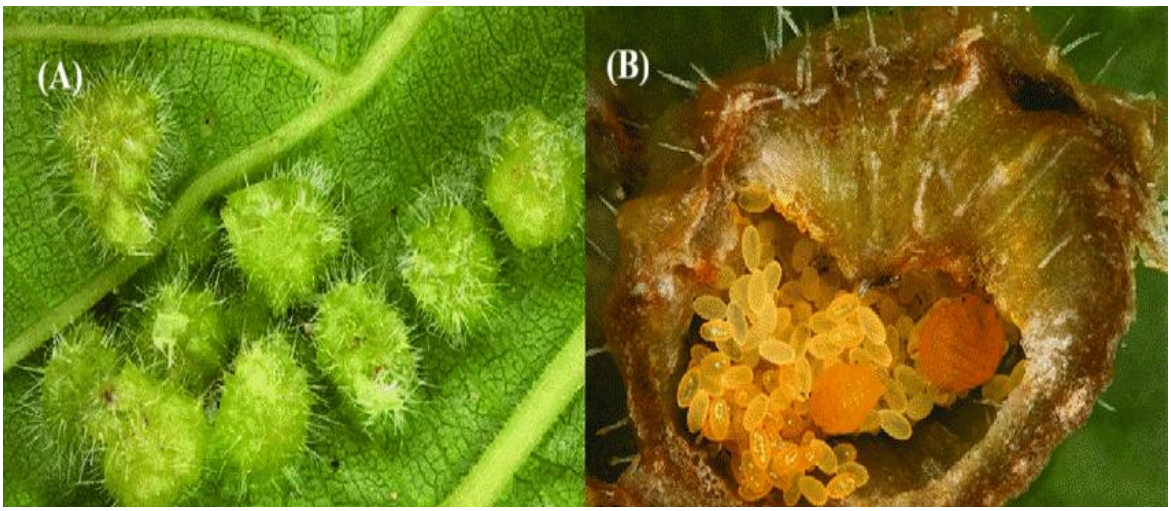


Figure 4 : Grape phylloxera (*Daktulosphaira vitifoliae*) (A) Leaf-galls proliferate on the underside of the leaf. (B) Section of a gall containing eggs and gallecoles *(*Pantaleoni *et al.*, 2012)

- **Mealybugs:**

Mealybugs are significant pests of grapevines worldwide, causing both direct and indirect damage. They are known for their role in transmitting plant viruses and affecting the growth and yield of grapevines. Mealybugs belong to the Family Pseudococcidae (Hemiptera). The most significant species is the vine mealybug (*Planococcus ficus*) a phloem-feeding insect from the Mediterranean area, that has become a serious pest in many grape-growing regions worldwide (Schulze-Sylvester *et al.*, 2021). High infestation levels of *P. ficus* can significantly reduce leaf and stem biomass in grapevines, indicating a direct impact on the plant's vegetative growth (Castillo and Bello-Bedoy, 2022). Additionally, *Pseudococcus maritimus* is also considered a grapevine pest, particularly due to its role in transmitting grapevine leafroll-associated viruses (GLRaVs), which can lead to significant economic losses resulting from reduced grape quality and yield (O'Hearn and Walsh, 2021).

In Algeria the study of grapevine pest was limited. Bissaad *et al.*, 2017 noted the high prevalence of *Planococus ficus* in the Metidja vineyard during summer and early autumn, which are their favorite climatic conditions (Ji *et al.*, 2020).

- **Leafhoppers:**

Leafhoppers (Hemiptera: Cicadellidae) are small insects that often occur in high-density populations across various crops. They feed on the leaves and can simultaneously transmit pathogens, which damages the plants and decreases overall yield. Within viticulture leafhoppers are also prevalent within grapevine ecosystems and can inflict considerable damage (Jarrell *et al.*, 2020).

Leafhopper species of the subfamily Typhlocybinae are included among the most common insect pests of vineyards that substantially affect grape growth and productivity (Prazaru *et al.*, 2023). Several species are reported to infest vineyards of the Mediterranean basin and worldwide where vines are cultivated. *Arboridia adanae* (Dlabola, 1957), *Asymmetrasca decedens* (Paoli, 1932), *Erasmoneura vulnerata* (Fitch, 1851), *Jacobiasca lybica* (Bergevin and Zanon, 1922) and *Zygina rhamni* (Ferrari, 1882) are included in the species that very often are reported damaging vineyards (Evangelou *et al.*, 2023). Moreover, some species are vectors of viruses, viroids or phytoplasmas like the leafhopper *Scaphoideus titanus* (Ball) is the most economically important pest affecting Northern Italian vineyards and is the main vector of the phytoplasma associated with Flavescence dorée which are devastating pathogens of vines, and they greatly reduce grape production (Prazaru *et al*., 2023; Tamborindeguy *et al*., 2023). Another species of leafhopper that plays a major role in the transmission of *Xylella fastidiosa,*

the causal agent of Pierce's disease of grapevine, is *Homalodisca coagulata* (Almeida and Purcell., 2003), *Draeculacephala Minerva* (Almeida and Purcell., 2008).

2.2 Grapevine parasitic nematodes

Plant-parasitic nematodes are among the most significant soil-borne pests affecting grapevines (*Vitis spp*.) worldwide, causing substantial economic losses through direct feeding damage and, in some cases, virus transmission. Key genera include *Meloidogyne* spp. (root-knot nematodes), which are sedentary endoparasites that induce the formation of characteristic galls on roots (Figure 5) , leading to stunted growth, chlorosis, and reduced yields (Yang *et al.*, 2021; Malik *et al.*, 2022; Esmenjaud *et al.*, 2023). *Pratylenchus* spp. (root-lesion nematodes), which are migratory endoparasites that cause necrotic lesions and restrict root system development (Askary *et al.*, 2018). The damage inflicted by these nematodes can lead to vine decline, reduced fruit quality, and yield losses estimated at 12.5% globally. Management strategies focus on an integrated approach combining cultural practices (e.g., fallow periods), the use of certified nematode-resistant rootstocks, and, where necessary, chemical control (Khan, 2023).

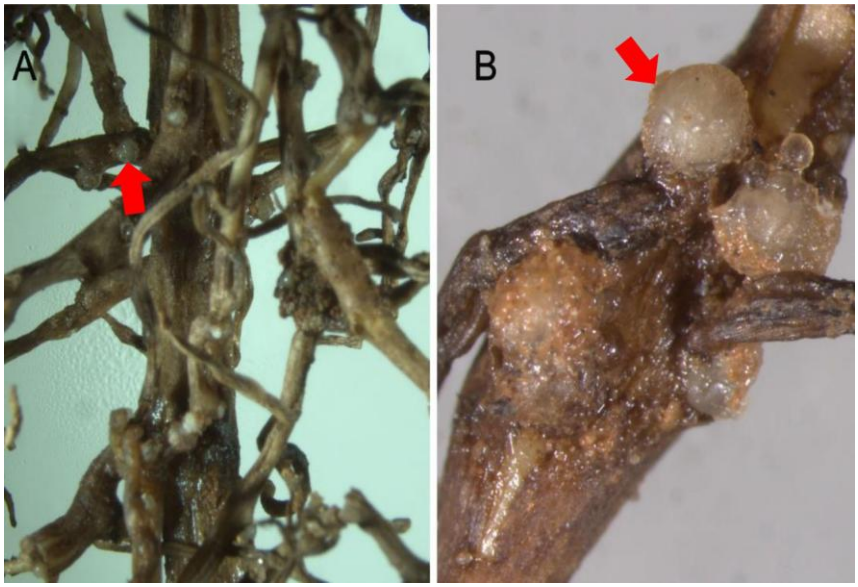


Figure 5 : Root symptom of diseased *Vitis vinifera*. (A) and (B) all show the root symptom of diseased *Vitis vinifera*, the arrow of fig show eggs of root-knot nematodes (Yang et *al*., 2021)

2.3 Grapevine pathogens

2.3.1 Fungal diseases

Fungal pathogens pose a continual challenge to viticulture, affecting vines from the green shoots and leaves to the ripening fruit and even the perennial wood. Downy mildew, powdery

mildew, and Botrytis gray mold stand out as globally significant threats that demand routine monitoring and control to prevent devastating crop losses (Thind *et al.*, 2004; Lopez Pinar *et al.*, 2016). Table 1 presents the fungal pathogens affecting grapevine worldwide.

Several studies carried out in Algeria show the occurrence of cryptogamic disease, Ammad *et al.*, 2014 study the diversity of fungal trunk pathogens associated with grapevine Dieback of grapevine in Algeria, showing significant pathogenic variability impacting vineyard health (Figure 5). Furthermore (Arkam *et al.*, 2021) study the diversity of Botryosphaeriaceae causing grapevine trunk diseases and their spatial distribution under different climatic conditions in Algeria. Recently Belkacem *et al.*, 2025 assessed grapevine trunk diseases, particularly Botryosphaeria dieback, in various grape varieties and rootstocks. Results show greater resistance in Cherchali and *Ferrana* blanche varieties, indicating the importance of selecting resistant cultivars for sustainable farming practices.

Table 1: Fungal pathogen that affect grapevine in the words.

Disease	Agent causal	Symptoms and damage	References
Downy mildew	*Plasmopara viticola*	• *P. viticola* causes leaf discoloration, necrosis and defoliation subsequently reduce nutrient composition, sugar accumulation in berries, capacity for buds overwintering and decreasing overall crop yields. • Infect young, tender green leaf, shoot, seeds, and fruit tissues, leading to significant losses in a short time, with optimal conditions causing 40-90% destruction of plants in fields.	(Fröbel and Zyprian, 2019) (Koledenkova *et al.*, 2022)
Powdery Mildew	*Erysiphe necator*	• Symptoms appears as a white or grayish powdery coating on leaves, shoots, and grapes. Affected leaves often become distorted, curl inward, and may prematurely drop from the vine. Grapes infected show cracks, shrivel, or fail to ripen properly, resulting in significant reductions in yield and fruit quality and can negatively affect the quality of the wine.	(Gadoury *et al.*, 2012) (Kunova *et al.*, 2021) (Rienth *et al.*, 2021)
Botrytis bunch rot	*Botrytis cinerea* pers Fr (B. cinerea)	• The BBR infects ripe berries, rendering them soft and watery; under high humidity, they become coated with the grey sporulation of the fungus, transform into mummies, and subsequently detach. • The economic impact, particularly in humid conditions, includes reduced yields and higher costs for fungicides.	(Reglinski *et al.*, 2010) (Hed and Centinari, 2024)
Black rot	*Guignardia bidwellii*	• Black rot can affect all aerial green parts of grapevine (leaves, shoots, and fruit). Foliar infections are characterized by reddish-brown lesions with distinctive black centers. Berries darken and shrivel, resembling small mummies. • In heavily infected vineyards, yield loss can sometimes reach up to 100% in the absence of adequate plant protection and during extreme weather conditions, such as a predominantly humid, warm year.	(Kellner *et al.*, 2022; Szabó *et al.*, 2023)
Grapevine Trunk Diseases : Eutypa Dieback and Esca	Eutypa dieback: *Eutypa lata* and *Eutypella vitis*	• Characterized by stunted shoots, leaf deformation (small, cupped, chlorotic leaves), shortened internodes, wedge-shaped necrosis in the wood, and eventual dieback of cordons and spurs. Generally impacts older, mature vines.	(Calzarano *et al.*, 2021; Del Frari *et al.*, 2021; Kenfaoui *et al.*, 2022; Muntean *et al.*, 2022)
	Esca: *Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Fomitiporia mediterranea*	• The leaves symptoms present interveinal chlorosis progressing to necrotic spots, resulting in a "tiger-stripe" look. Internally, the wood exhibits degradation, characterized by brown to black streaks or necrosis. The disease affects fruit productivity and quality, often causing berry shriveling or discoloration. Over time, infected vines weaken, develop shoot dieback, and eventually can die.	
Anthracnose	*Elsinoe ampelina*	• Commonly called "birds eye," it produces dark, sunken lesions with grayish centers on foliage, stems, and berries. Impacted berries have circular, depressed lesions, frequently resulting in desiccation and fissuring. In the presence of severe infection, early defoliation, fruit drop, and delayed fruit development and ripening may occur.	(Li *et al.*, 2021)

2.3.2 Bacterial diseases

Among grapevine diseases, bacterial pathogens play a crucial role due to their substantial economic and agricultural impact in grape-growing regions worldwide. The primary bacterial diseases affecting grapevines include bacterial blight caused by *Xylophilus ampelinus*, Pierce's disease caused by *Xylella fastidiosa*, and crown gall caused by *Agrobacterium vitis* (Szegedi and Civerolo, 2011). These diseases represent significant challenges for viticulture, necessitating ongoing research and effective management strategies to mitigate their impact (Figure 6).

- **Bacterial blight**: The bacterial blight of grapevine, caused by the bacterium *Xylophilus ampelinus*, significantly impacts grapevine production, causing yield reduction and reduced vitality. Infected grapevines can lose up to 80% of total yield. The disease is transmitted through vascular tissues and inoculum from infected cuttings (Prokić *et al.*, 2018). Recently, the bacterium is regulated as a quarantine pest in the regions of NAPPO (North American Plant Protection Organization) and IAPSC (Inter- African PhytoSanitary Council) and is included in the A2 list of pests recommended for regulation as quarantine pests by EPPO (European and Mediterranean Plant Protection Organization) (Carminati *et al.*, 2025).
- **Pierce's disease**: Pierce's disease is a severe bacterial infection of grapevines caused by the bacterium *Xylella fastidiosa*. Symptoms include leaf scorching, dried fruit, cordon dieback, and can lead to vine mortality (Armijo *et al.*, 2016). Despite stringent quarantine regulations implemented to protect the European viticulture industry (Directive 2000/29/EC), recent identification of PD in grapevines on the island of Majorca, Spain, marks its initial occurrence within Europe. This discovery, along with reports of PD detection in Taiwan, has raised significant concerns about the potential spread of the disease to continental Europe and other prominent global wine-producing regions, highlighting the need for increased vigilance and preventive measures (Kyrkou *et al.*, 2018; Giménez-Romero *et al.*, 2022).
- **Crown gall**: primarily caused by the bacterium *Agrobacterium vitis*, represents a significant phytopathological issue affecting grapevine production globally. The disease is characterized by tumor formation on aerial parts of the grapevine, accompanied by root necrosis, which severely impacts plant health (Gelvin, 2018). Crown gall infections can substantially diminish plant vigor and reduce grape yield

by up to 40%, contingent upon the severity of infection. In cases of severe infestation, the disease may lead to cane dieback or even the death of entire plants, resulting in substantial economic losses in viticulture (Gelvin, 2018; Faist, 2023).

2.3.3 Virus and viroids

Grapevines (genus *Vitis*) are highly susceptible to a diverse array of viral pathogens, with a total of 102 distinct virus species reported globally. These viruses represent exemplary isolates from species across 44 genera within 21 distinct virus families. The extensive diversity and prevalence of these viral infections underscore the exceptional vulnerability of *Vitis* species, solidifying their status as the cultivated crop hosting the greatest number of viral species (Fuchs, 2024). However, not all are economically important. The major viral diseases impacting viticulture include the complexes associated with **rugose wood complex, leafroll**, **fanleaf degeneration**, as well as **fleck disease**, and emerging diseases like **red blotch**, each caused by one or more virus species (Basso *et al.*, 2017; Martelli, 2017; Sabella *et al.*, 2018).

- **Grapevine rugose wood complex:**

  Grapevine Rugose Wood is a complex viral disease that severely affects grapevines, causing a degradation of the woody trunk and graft union. Considered as one of the most economically damaging viral disease complexes in viticulture, it causes significant losses in all major grape-growing regions worldwide (Basso *et al.*, 2017). The disease is linked to three viruses belonging to the family Betaflexiviridae, which induce four specific syndromes: **Rupestris stem pitting** (RSP), attributed to the *Grapevine rupestris stem pitting-associated virus* (GRSPaV); **Kober stem grooving**, caused by *Grapevine virus A* (GVA); **LN33 stem grooving**; and **Corky bark**, resulting from *Grapevine virus B* (GVB) (Martelli, 2014; Bachir *et al.*, 2019; Orfanidou *et al.*, 2021). The main symptom of rugose wood is that the affected vines may show reduced vigor, delayed bud opening in spring, and decline within a few years from plantation. Grafted vines may show swelling and a difference in scion and rootstock diameter. Corky rugose wood, a thick, spongy bark, may occur on scion or rootstock, with severity varying based on scion/stock combinations (Martelli, 2014; 2017).

  In Algeria, Bachir et *al* 2019, studied the prevalence of the three viruses of rugose wood (GVA, GVD and GRSPaV). The results showed that 68% of the viruses were present, with GRSPaV predominating.

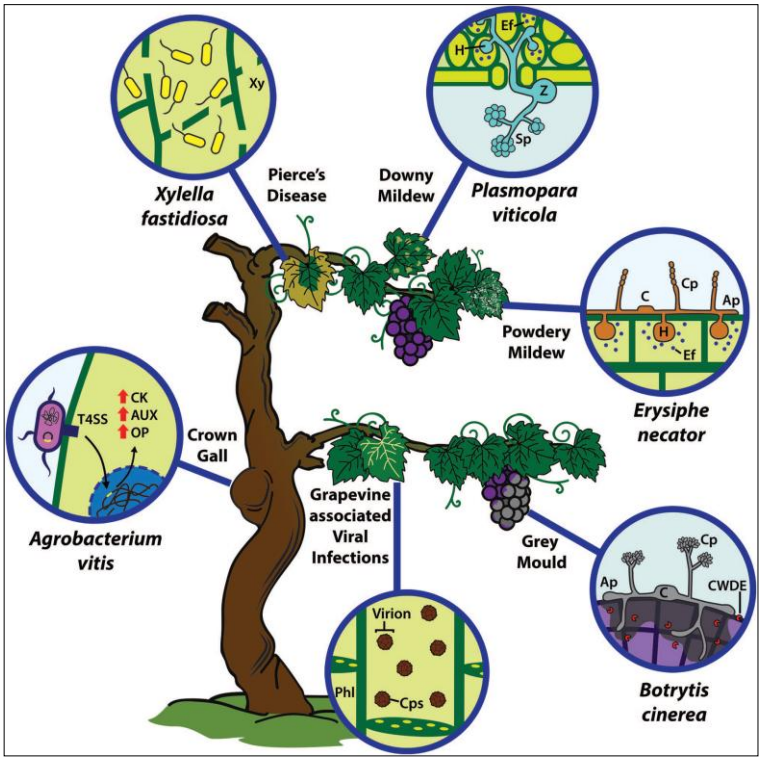


Figure 6 : Infection strategy of main grapevine pathogenic microorganisms and disease-associated symptoms (Armijo *et al*., 2016)**.**

- **Grapevine leaf roll disease:**

  Grapevine Leafroll Disease (GLD) is one of the most widespread and economically significant viral diseases affecting grapevines globally. It is associated with a six distinct viruses known as Grapevine leafroll-associated viruses (GLRaVs) (Naidu *et al.*, 2015; Song *et al.*, 2021), with GLRaV-3 being the most prevalent and impactful (Committee on Assistance to the California Department of Food and Agriculture Pierce's Disease/Glassy-Winged Sharpshooter Board on Grapevine Viruses and Grapevine Disease Research *et al.*, 2025). GLD viruses belonging to the family Closteroviridae, with GLRV1, 3, 4 and 13 assigned to the genus *Ampelovirus*, GLRV2 to the genus *Closterovirus* and GLRV7 to the genus *Velarivirus* (Ito and Nakaune, 2016; Habili *et al.*, 2023). These viruses are phloem-limited and are transmitted through vegetative propagation and by insect vectors such as mealybugs (Hemiptera: Pseudococcidae) and soft scale insects (Hemiptera: Coccidae) in semi-persistent manner (Donda *et al.*, 2023).

  The GLRV infection could reduce yield by 30% to 50%. Additionally, it can affect the chemical quality of the berries, juice, and wine. Moreover, chronic infections

contribute to the premature decline in vine vigor and longevity, thereby reducing the productive lifespan of vineyards (Martelli, 2017; Song *et al.*, 2021).

- **Grapevine Fleck disease**

  Grapevine fleck virus (GFkV) is a prevalent viral pathogen affecting grapevines on a global scale. This virus is classified within the genus *Maculavirus* of the family Tymoviridae and demonstrates phylogenetic affinities with other notable grapevine viruses, including *Grapevine Red Blotch Virus* (GRBV), *Grapevine Red Leaf Virus* (GRVFV), *Grapevine Astound Virus* (GAMaV), and *Grapevine Synergistic Virus 1* (GSyV-1) (Naidu and Mekuria, 2010; De Souza *et al.*, 2024; Komínková *et al.*, 2024). GFkV typically induces latent infections in *Vitis* spp; however, it may cause foliar symptoms in indicator hosts such as *V. rupestris* (Naidu and Mekuria, 2010; Martelli, 2017). The significance of GFkV is underscored by its association with the rugose wood complex, particularly manifesting as corky bark and stem grooving. Transmission of GFkV occurs primarily through the propagation of us material, as no natural vector has been identified to date (Martelli, 2017; De Souza *et al.*, 2024). This mode of transmission underscores the importance of implementing certified virus-free propagation programs and strict sanitary practices to limit the spread of the virus in viticultural systems (Martelli, 2017; De Souza *et al.*, 2024).

- **Viroids**

  Viroids are the smallest recognized infectious agents, consisting exclusively of one short strand of circular, single-stranded, non-coding RNA molecules. Unlike viruses, viroids lack a protein coat and do not encode proteins, but they can autonomously replicate in host cells by hijacking host enzymatic machinery (Di Serio *et al.*, 2017). In grapevines (*Vitis vinifera* and related species), several viroids have been identified that can affect grape quality, yield, and overall plant health. The main viroids infecting grapevines include *Hop stunt viroid* (HSVd), *Grapevine yellow speckle viroid* 1 and 2 (GYSVd-1, GYSVd-2), *Australian grapevine viroid* (AGVd), *Japanese grapevine viroid* (JGVd), *Grapevine latent viroid* (GLVd), *Citrus exocortis viroid* (CEVd), and *Grapevine hammerhead viroid-like RNA* (GHVd) (Di Serio *et al.*, 2017; Navrotskaya *et al.*, 2021; Morgan *et al.*, 2022).

3. Fanleaf degeneration: *Grapevine fanleaf virus*

Grapevine fanleaf virus (GFLV) is one of the viral pathogens associated with fanleaf degeneration, a major viral disease that significantly influence grapevine health and productivity worldwide (Andret-Link *et al.*, 2004).

GFLV is taxonomically classified within the genus *Nepovirus* of the family Secoviridae. A defining characteristic of GFLV is its transmission by the ectoparasitic dagger nematode *Xiphinema index*, a discovery that established the first documented case of nematode transmission for a plant virus and marked a pivotal advance in the field of plant virology (Andret-Link *et al.*, 2004; Schmitt-Keichinger *et al.*, 2017; Al Rwahnih *et al.*, 2021). Moreover, GFLV was the first nepovirus for which full-length, infectious cDNA clones were developed. This achievement has enabled detailed molecular dissection of virus–vector–host interactions and has significantly propelled the creation of novel diagnostic tools and control measures (Schmitt-Keichinger *et al.*, 2017).

3.1 Symptomatology

Grapevine fanleaf virus (GFLV) is a globally significant pathogen of grapevines (*Vitis* spp.) and the primary causal agent of fanleaf degeneration, a severe disease also known as "court-noué" (Digiaro *et al.*, 2017; Kubina *et al.*, 2022). Infection induces a broad spectrum of morphological, physiological, and anatomical abnormalities in the host plant. The phenotypic expression of the disease—encompassing both symptom type and severity—is highly variable and is modulated by an interplay of factors. These include the specific grapevine cultivar, GFLV strain virulence, the presence of other pathogens (co-infections), prevailing environmental conditions, and the developmental stage of the vine at the time of infection.

3.1.1 Macroscopic symptoms

- **Leaves**

Symptoms typically emerge in early spring and are commonly classified into two major syndromes, one of which involves **foliar discoloration**. This syndrome is characterized by chlorotic mottling and yellow mosaic patterns, often localized along the veins or interveinal regions (Box 1). Additionally, vein banding and vein clearing are frequently observed, particularly in susceptible grapevine cultivars (Cigsar *et al.*, 2003; Hajizadeh *et al.*, 2015). The second major syndrome is **leaf malformation**, which is characterized by distinct bilateral asymmetry of the leaf blades. Affected leaves often exhibit closely spaced primary veins,

jagged or deeply serrated margins, and an enlarged petiolar sinus. These structural deformities collectively give the leaf a fan-like appearance, from which the virus derives its name (Digiaro *et al.*, 2017; Kubina *et al.*, 2022).

- **Shoots**

Grapevine fanleaf virus (GFLV) infection induces a suite of shoot-related symptoms that severely compromise vine development and architecture (Box 1). A primary and diagnostic symptom is the pronounced shortening of internodes, resulting in overall shoot stunting and a bushy growth habit. This stunting is often coupled with aberrant branching, a phenomenon termed "double nodding," characterized by the emergence of secondary shoots at or near the nodes of the primary shoot. Furthermore, certain cultivars exhibit a distinctive zig-zag growth pattern of the main shoot axis, a direct consequence of asymmetrical or irregular internodal elongation (Schmitt-Keichinger *et al.*, 2017; Courty *et al.*, 2024; Konup *et al.*, 2025)..

- **Fruits**

Infected grapevines exhibit poor fruit set and significant berry deformation, often resulting in small, loosely formed clusters composed of irregularly shaped berries. Fruit development is typically asynchronous, with uneven ripening patterns accompanied by altered biochemical composition, including reduced sugar accumulation and imbalanced acidity levels (Oliver and Fuchs, 2011b; Krebelj *et al.*, 2015; Kubina *et al.*, 2022). These physiological disruptions collectively lead to a decline in overall fruit quality, adversely affecting both yield and the enological potential of the harvested grapes.

3.1.2 Microscopic symptoms

Grapevine fanleaf virus (GFLV) infections, similar to those caused by other nepoviruses affecting grapevines, induce distinct cytopathological alterations that can be categorized into three main types. The first involves the formation of vesiculate-vacuolate cytoplasmic inclusions, frequently located in close association with the nucleus (Figure 7). The second type consists of tubules filled with aligned rows of virus particles, indicative of intracellular viral transport or aggregation. The third alteration is characterized by abnormal cell wall outgrowths, which may reflect disrupted cell wall integrity or host defense responses. These structural modifications are commonly observed in infected tissues and serve as cytological markers of GFLV infection (Martelli and Boudon-Padieu E., 2006; Meng *et al.*, 2017).

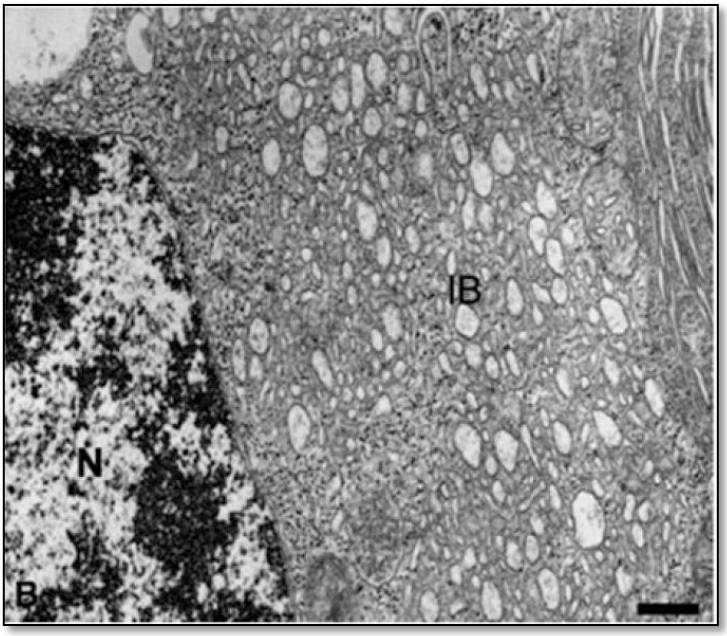
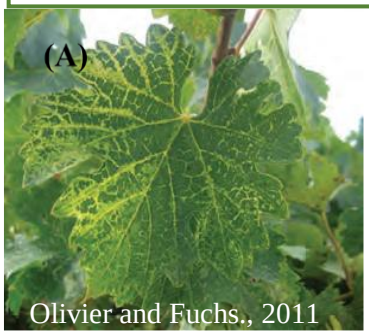
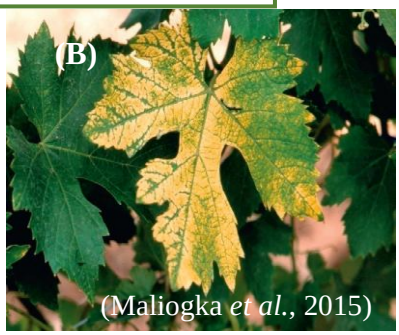
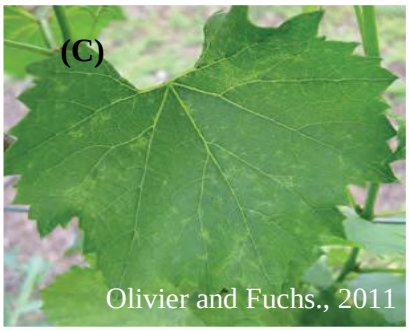


Figure 7: Vacuolate-vesiculate inclusion body (IB) next to a nucleus (N) in a GFLV-infected cell (Bar = 250nm) (Digiaro *et al*., 2017).

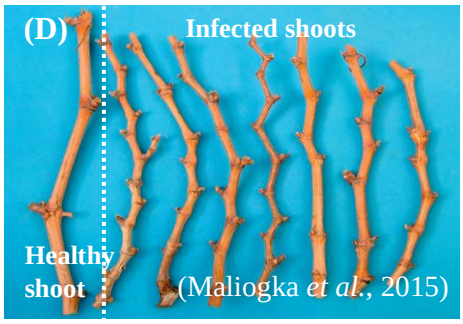
Box 1: Symptoms of grapevine Fanleaf virus

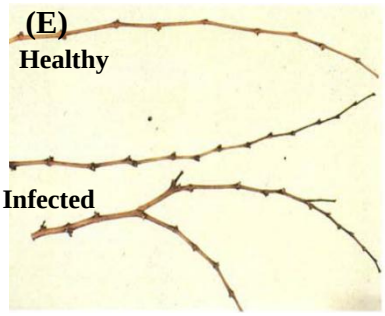




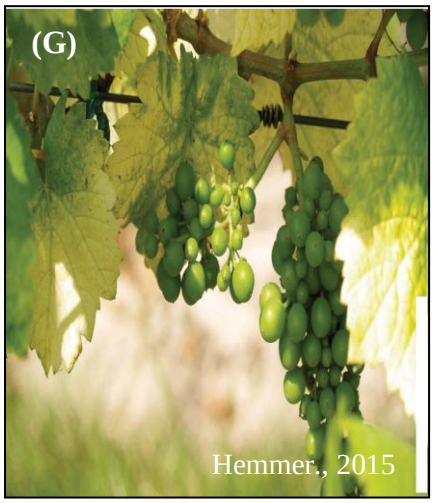


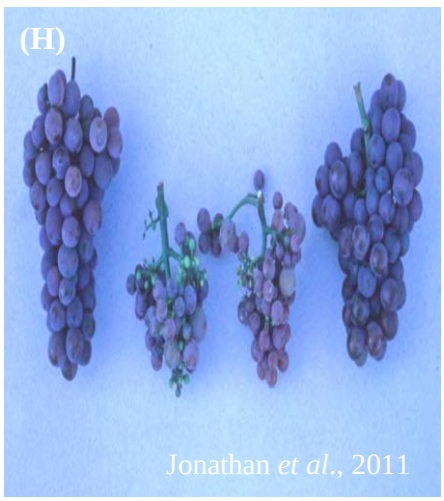
Appearance of leaf syndromes, First Foliar discoloration; (A) vein banding, (B) chlorosis, (C) vein clearing, the second syndrome Leaf malformation (D) Fan-like morphology of an infected leaf (right) compared to a healthy leaf (left).





Stems from infected vines showing short internodes, double nodes and zig-zag growth between nodes compared to cane from healthy vines(D). Short internodes and abnormal bifurcations Branching (E and F).





(G) Small clusters with shot berries in the middle GFLV-infected *Vitis vinifera* compared to healthy clusters on the left and right, (H) The image illustrates grapevine clusters exhibiting **poor fruits** (middle), characterized by the presence of both normally developed and undersized, seedless berries.

3.2 Genome structure

Grapevine fanleaf virus (GFLV) is a plant-infecting virus with a positive-sense single-stranded RNA genome. It belongs to the family Secoviridae and genus *Nepovirus*. The particles of GFLV are polyhedral with diameter of 30nm (Figure 8) (Oliver and Fuchs, 2011; Digiaro *et al.*, 2017). The genome is bipartite, comprising two RNA segments—RNA1 and RNA2—each enclosed in its own icosahedral capsid (Martelli and Boudon-Padieu E., 2006; Meng *et al.*, 2017). The reference strain GFLV-F13, originally isolated in France, contains RNA1 with an estimated length of 7,342 nucleotides, while RNA2 measures around 3,774 nucleotides (Andret-Link *et al.*, 2004). Both genomic RNAs are monocistronic, meaning each codes for one major open reading frame (ORF) (Mekuria *et al.*, 2009). Beyond the primary RNA segments, certain GFLV variants harbor a satellite RNA that spans roughly 1114 nucleotides. This RNA is linear in structure and encodes a single ORF responsible for a non-structural protein, whose function remains unidentified. Notably, this satellite does not appear to influence the virus's pathogenic behavior (Gottula *et al.*, 2013; Čepin *et al.*, 2016; Kubina *et al.*, 2022).
RNA 1 contains a single open reading frame that encodes polyprotein 253 kDa (P1), which is cleaved into five individual proteins: a putative protease cofactor (1A), a helicase ($1B^{Hel}$), a genome-related protein ($1C^{VPg}$), a proteinase ($1D^{Pro}$), and an RNA-dependent RNA polymerase ($1E^{Pol}$). **RNA 2** also encodes a single polyprotein that is cleaved into three key proteins: the homing protein (2A), the movement protein (2B), and the coat protein (2C). Both RNAs are polyadenylated at their 3' ends and have a small protein (VPg) covalently linked to their end (Figure 9) (Meng *et al.*, 2017; Kubina *et al.*, 2022).

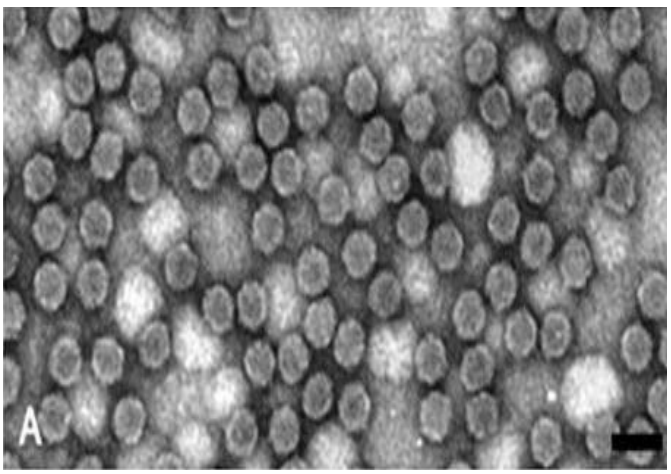


Figure 8 : GFLV particles from a purified unfractionated virus preparation (Bar=50 nm) (Digiaro *et al*., 2017)

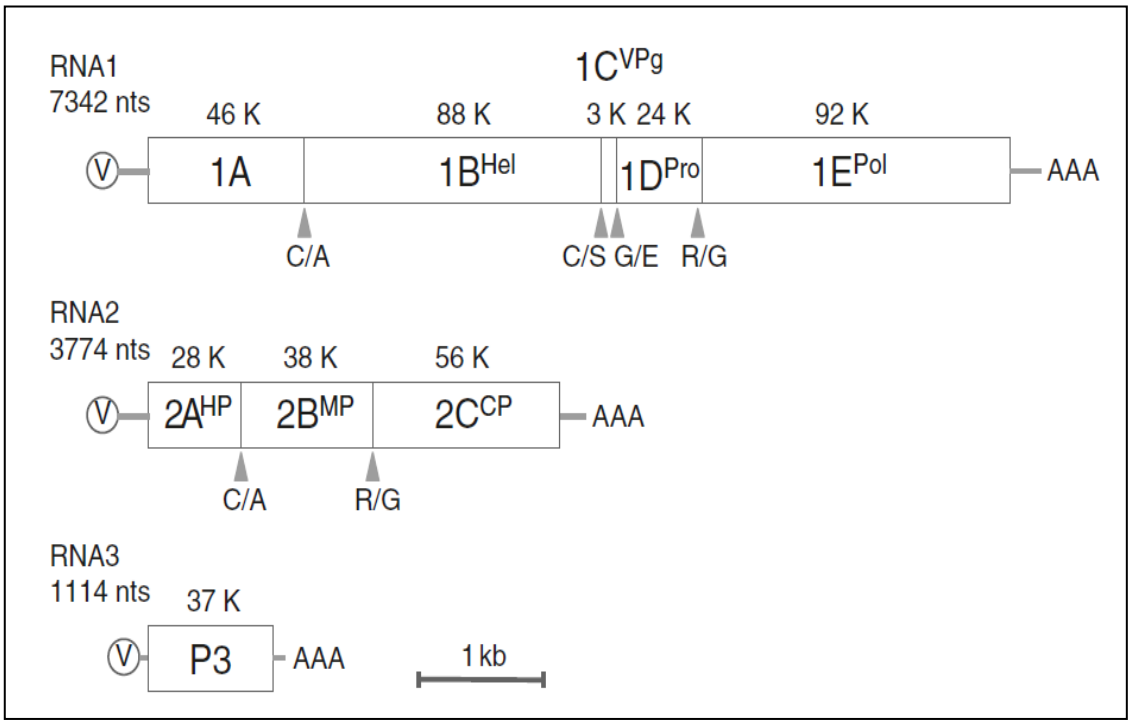


Figure 9 : Genomic organization of *Grapevine fanleaf virus* (Schmitt-Keichinger *et al*., 2017)

3.3 Mechanism of infection

Grapevine fanleaf virus (GFLV) enters grapevine root cells through the feeding activity of the ectoparasitic nematode *Xiphinema index*, which introduces the viral particles directly into the cytoplasm. This marks the initiation of the viral replication cycle (Andret-Link *et al.*, 2004).

3.3.1 Replication

After being introduced directly into the cytoplasm of grapevine root cells, the *Grapevine fanleaf virus* (GFLV) initiates its replication cycle (Martelli *et al*., 2006). The viral capsid disassembles, releasing the two genomic RNAs, RNA1 and RNA2, each bearing a 5′ genome-linked viral protein (VPg) and a 3′ polyadenylated tail (Fuchs *et al.*, 2017). RNA1 is translated into a large polyprotein that undergoes proteolytic cleavage by the virus-encoded 3C-like protease (1D) to produce functional replication proteins, including the helicase (1B), protease, and RNA-dependent RNA polymerase (1E) (Andret-Link *et al.*, 2004). These proteins localize to the endoplasmic reticulum, where they form membrane-associated replication complexes (Box 2) (Ritzenthaler *et al.*, 2002)

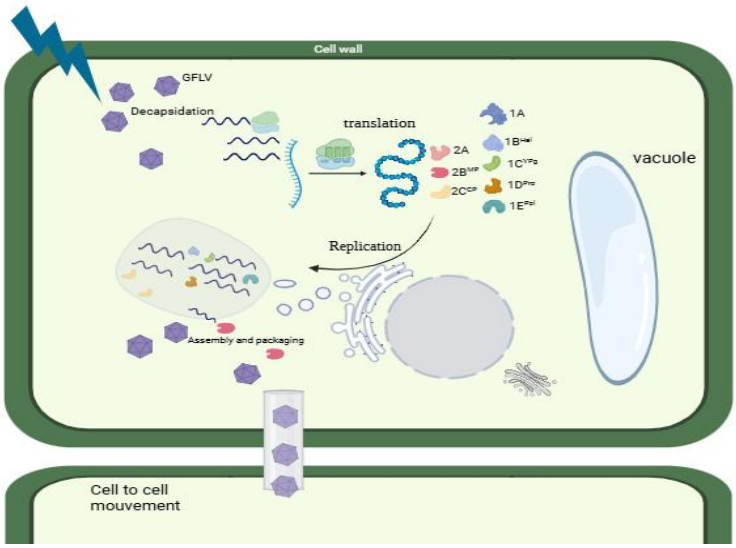
The RNA polymerase synthesizes negative-strand RNA intermediates, which serve as templates for generating new positive-sense genomic RNAs. Concurrently, RNA2 is translated

to produce the 2A homing protein, which directs RNA2 to the replication sites, facilitating its recognition and replication by the RNA1-encoded replication machinery (Gaire *et al.*, 1999).

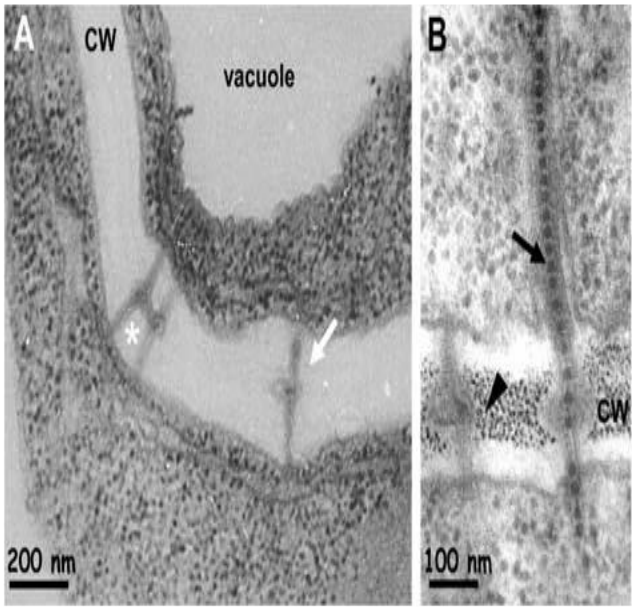
3.3.2 Intercellular movement and encapsidation phase

Following replication, GFLV relies on specific proteins encoded by RNA2 for intracellular movement and systemic infection. The movement protein (2B) facilitates the virus's passage through plasmodesmata by assembling tubular structures that enable direct cell-to-cell transport of virions or viral ribonucleoprotein complexes (Ritzenthaler and Hofmann., 2007; Schmitt-Keichinger *et al.*, 2017)

Box 2: *Grapevine fanleaf virus* cycle in plant cell



Intracellular Replication and Movement Cycle of *Grapevine fanleaf virus* (GFLV) in a Plant Cell



Microscopic image of plasmodesmata in Chenopodium quinoa leaf tissue. (A) cross-section through leaf showing single (white arrow) and branched plasmodesmata. (*) Straddling the cell wall. (B) Plasmodesma in GFLV-infected leaf tissue modified by a tubule filled with individually detectable icosahedral GLFV virions (black arrow). As indicated by the black arrowhead, infected tissues also contain unmodified plasmodesmata.

(Ritzenthaler and Hofmann, 2007)

Simultaneously, the coat protein (2C) encapsidates newly synthesized RNA1 and RNA2 into separate icosahedral particles, approximately 30 nm in diameter, stabilizing the genomes and preparing them for intercellular movement and long-distance trafficking through the plant's vascular system (Andret-Link *et al.*, 2004; Ritzenthaler, 2011)

Both the movement and coat proteins are essential for systemic spread and for ensuring that GFLV can be acquired by nematode vectors, thus completing its biological cycle and enabling subsequent transmission to new host plants (Ritzenthaler, 2011).

3.4 Diagnosis and detection

Accurate diagnosis of viral infections is fundamental to the effective management of grapevine health (Basso *et al.*, 2017). Visual assessment based on symptomatology is inadequate for reliable diagnosis, as many grapevine viruses establish latent infections or induce nonspecific symptoms that overlap with abiotic stress or other biotic agents. Consequently, sensitive and specific laboratory-based techniques are essential for the definitive detection of viral pathogens in both symptomatic and asymptomatic vines.

Diagnostic approaches for grapevine viruses are broadly categorized into three groups: biological indexing, serological assays, and molecular techniques. While time-consuming, biological indexing remains a critical tool, particularly within certification schemes and for investigating novel or uncharacterized viral diseases. In contrast, serological and molecular methods represent the most practical approach for large-scale diagnostics and surveillance. Their superior speed, ease of implementation, and scalability make them the most feasible option for routine testing (Maliogka *et al.*, 2015).

3.4.1 Biological indexing

Biological indexing, a classical diagnostic method for grapevine viruses, is based on the propagation of viral inoculum onto susceptible woody or herbaceous indicator plants and subsequent observation of induced symptoms. This technique detects only biologically active agents through specific phenotypes—such as leaf deformation, chlorosis, or vein clearing—making it indispensable for virus characterization and certification protocols characterization (Al Rwahnih *et al.*, 2015; Digiaro *et al.*, 2017). Its major limitations, however, include low throughput, high resource requirements, and a reliance on symptomatic expression, which inherently restricts its sensitivity. Consequently, biological indexing often fails to detect latent or low-titer infections that are readily identified by molecular methods (Digiaro *et al.*, 2017; Zherdev *et al.*, 2018).

3.4.2 Serological assays

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) is among the most widely used serological technique for the detection of grapevine viruses, based on antigen-antibody specificity, including *Grapevine fanleaf virus* (GFLV) (Digiaro *et al.*, 2017; Zherdev *et al.*, 2018). Its broad application in certification programs and large-scale epidemiological surveys, is due to its procedural simplicity, cost-effectiveness, and high-throughput nature, facilitating the parallel analysis of hundreds of samples (Digiaro *et al.*, 2017; Vigne *et al.*, 2018). However, despite these advantages, the sensitivity of DAS-ELISA is limited, particularly when virus titers are low or unevenly distributed within plant tissues, which can lead to false-negative results (Buja *et al.*, 2022; Kanapiya *et al.*, 2024).

3.4.3 Molecular methods based on nucleic acids

Molecular techniques are widely employed for the detection of grapevine viruses due to their superior sensitivity compared to serological assays such as ELISA (Maliogka *et al.*, 2015). These methods target the viral nucleic acids, enabling the identification of infections even at low viral titers. Among them, reverse transcription polymerase chain reaction (RT-PCR) is one of the most commonly used tools for detecting RNA viruses infecting grapevines, including *Grapevine fanleaf virus* (GFLV) (Maliogka *et al.*, 2015; Zhou *et al.*, 2015). RT-PCR is often used to confirm serological results and provides a reliable approach for routine diagnostics (Maliogka *et al.*, 2015; Zherdev *et al.*, 2018).

3.4.4 High throughput sequencing (HTS)

High-Throughput Sequencing (HTS)**,** also referred to as next-generation sequencing (NGS), is an advanced molecular technique that enables the simultaneous sequencing of millions of DNA or RNA fragments in a single run (Navrotskaya *et al.*, 2021; Soltani *et al.*, 2021). HTS facilitates the detection of a wide range of viruses and viroids, the identification of novel species, and the characterization of complex mixed infections (Diaz-Lara *et al.*, 2023). Due to its superior sensitivity, expansive diagnostic breadth, and scalability, HTS is becoming an indispensable tool for comprehensive grapevine health surveys, virus discovery, and advanced certification protocols (Vigne *et al.*, 2018; Stevens and Al Rwahnih, 2024).

In addition to conventional serological and molecular methods, Lab-on-a-Chip (LOC) platforms have emerged as advanced diagnostic tools for grapevine viruses, including GFLV. These microfluidic devices integrate and miniaturize laboratory processes onto a single chip.

One prominent application is the use of immunosensors; for instance, Buja *et al.* (2022) developed an electrochemical microfluidic chip capable of detecting GFLV through impedance changes following specific antibody binding. A complementary molecular approach on LOC platforms is isothermal amplification, such as Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). As demonstrated by Almasi. (2015) for GFLV detection, RT-LAMP offers significant advantages, including rapid results, operational simplicity, the elimination of RNA extraction steps, and the avoidance of expensive thermocycling equipment. These characteristics make LOC technologies promising for high-throughput, portable, and resource-efficient diagnostics.

3.5 Transmission

Grapevine, a high-value perennial crop propagated vegetatively, is affected by a wide range of viral pathogens, among which *Grapevine fanleaf virus* (GFLV) is one of the most economically damaging. GFLV distributed globally where *Vitis vinifera* and its hybrid rootstocks are cultivated, and has likely associated with viticulture since ancient times (Maliogka *et al.*, 2015). Due to the phloem-limited nature of the virus and the vegetative propagation practices used in grapevine cultivation, its transmission predominantly occurs through two main pathways: (1) dissemination by soil-borne nematode vectors and (2) the use of infected plant material during propagation, such as grafting or cuttings (Andret-Link *et al.*, 2004; Maliogka *et al.*, 2015).

The existence of this transmission pathways contributes to the persistence of GFLV in vineyards and presents significant challenges for its eradication, posing major challenges for vineyard health management and long-term grapevine productivity.

3.5.1 Vector mediated transmission

The transmission of *Grapevine fanleaf virus* (GFLV) is a highly specific process, primarily mediated by the ectoparasitic dagger nematode *Xiphinema index* (Figure 10). This vector transmits the virus from grapevine to grapevine in a non-circulative, semi-persistent manner. As a member of the family Longidoridae (order Dorylaimida), *X. index* feeds on the actively growing root tips of grapevines, facilitating direct virus acquisition and inoculation (Maliogka *et al.*, 2015; Digiaro *et al.*, 2017). This specificity is determined by precise molecular interactions, wherein specific amino acid residues in the GFLV coat protein bind to putative receptors within the nematode's feeding apparatus a critical determinant for successful transmission (Andret-Link *et al.*, 2004; Schellenberger *et al.*, 2010).

- **Mechanism of transmission**

Virus acquisition occurs when *X. index* feeds on an infected root system, during which viral particles bind to specific, putative receptor sites within the nematode's feeding apparatus, particularly the stylet and esophageal lumen. Transmission to a healthy plant is accomplished during a subsequent feeding event, typically on a young, growing root tip, where the retained virions are released into susceptible host cells (Singh *et al.*, 2020). The vector's ability to persist in vineyard soils for years, while retaining transmissible virus particles even in the absence of a host, presents a major obstacle to effective long-term disease management (Demangeat *et al.*, 2005).

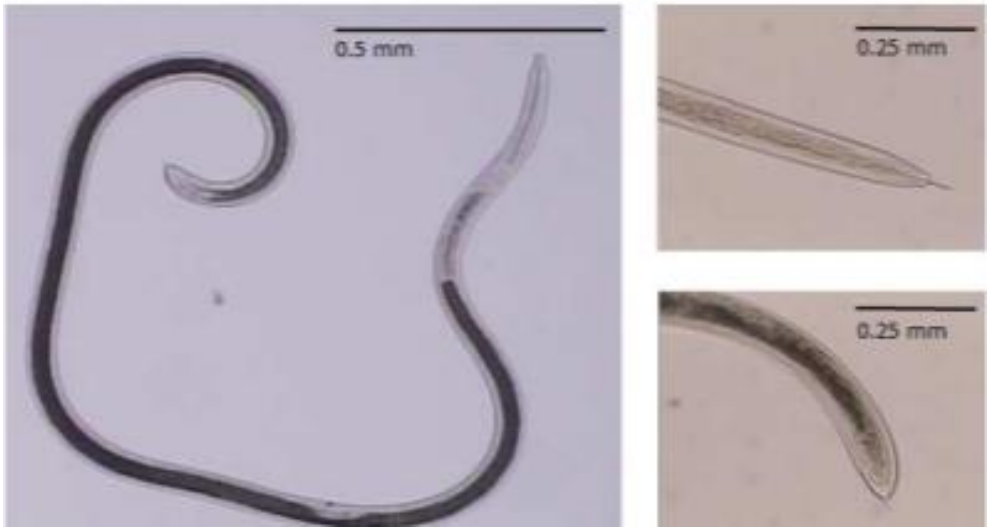


Figure 10 : Typical morphology of *Xiphinema index*, left: the full body length, right up: the stylet, right down: the tail (Canchignia *et al.*, 2017).

- **Biology and ecology of *Xiphinema index***

Xiphinema index is a migratory ectoparasitic nematode that inhabits the rhizosphere of perennial plants and feeds on the root tips of its hosts (Esmenjaud *et al.*, 2014). While its primary host is the grapevine (*Vitis vinifera*), which is also the natural reservoir for GFLV, it can also feed on alternative hosts such as fig trees—a plant species that does not support viral replication (Esmenjaud *et al.*, 2013). This nematode thrives in deep, humid soils under warm climatic conditions, which favor the establishment of persistent populations. Its life cycle involves females depositing eggs in the soil near feeding sites; juveniles undergo four successive molts, feeding on roots at each developmental stage before reaching adult (Van Zyl *et al.*, 2016; Nguyen *et al.*, 2019). Reproduction occurs predominantly through meiotic

parthenogenesis, although rare instances of sexual reproduction have been documented when males are produced (Jones *et al.*, 2013; Van Zyl *et al.*, 2016).

- **Symptoms**

The ectoparasitic feeding activity of *Xiphinema index* induces characteristic pathological changes in the root systems of host plants. Using its protractible stylet, the nematode penetrates the apical meristem of young root tips and injects enzymatic saliva that degrades cell walls, facilitating the ingestion of cytoplasmic contents. This direct feeding causes localized necrosis at the puncture sites and disrupts normal root morphogenesis, leading to macroscopic symptoms such as root tip swelling and impaired elongation (Singh et al., 2020). At a cellular level, *X. index* infection stimulates the formation of plurinucleate cells within hypertrophic tissues, often resulting in the development of gall-like structures (Figure 11). Chronic feeding pressure from established nematode populations exacerbates these pathological effects, culminating in significant architectural damage, including reduced root biomass and compromised overall root function (Hao et al., 2012; Singh et al., 2020).

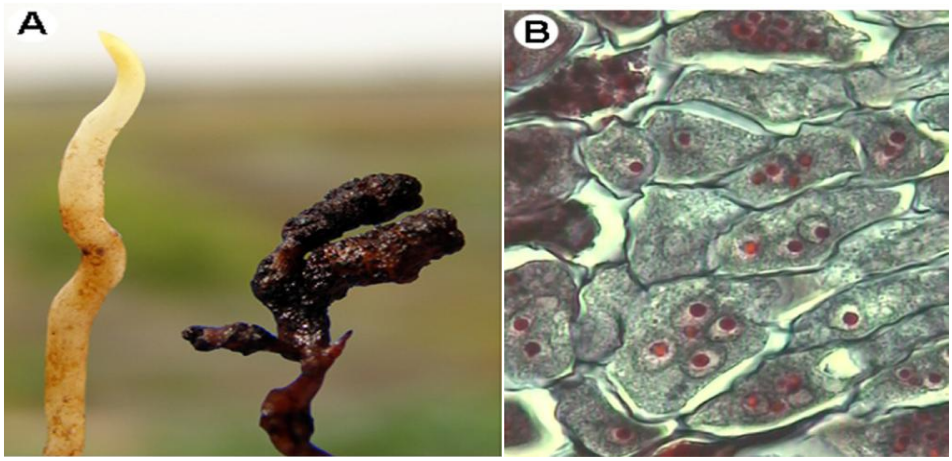


Figure 11 : Parasitism of *Xiphinema index* on roots of *Vitis spp.*: (A) Characteristic swellings on infected root tips compared with a healthy root. (B) Multinucleate cells induced by *X. index* in *Vitis* rootstock (Jones *et al.*, 2013).

3.6 Management and control strategies

Effective management of *Grapevine fanleaf virus* (GFLV) is profoundly challenging, primarily due to the absence of direct antiviral treatments in perennial hosts and the biological complexity of its transmission cycle, which involves the soil-borne ectoparasitic

nematode *Xiphinema index*. Consequently, management must rely exclusively on preventive, integrated, and sustainable long-term strategies.

3.6.1 Use of certified plant material

Prevention is fundamental backbone in the managing of grapevine viral diseases, including those caused by *Grapevine fanleaf virus* (GFLV). GFLV is classified in the EPPO database under the species Nepovirus foliumflabelli (family Secoviridae), with EPPO code GFLV00, and it is generally considered a regulated non-quarantine pest (RNQP) in the Europe. Given the primary role of infected propagation material in virus spread, the establishment of clean, virus-tested plant stocks is fundamental to ensuring vineyard phytosanitary status and long-term productivity (Martelli and Boudon-Padieu E., 2006; Digiaro *et al.*, 2017). This principle is implemented through certification schemes that propagate and distribute certified virus-free planting material (scions and rootstock). These programs rely on the systematic indexing of mother vines and propagation stocks using validated serological (e.g., DAS-ELISA) and molecular (e.g., RT-PCR) diagnostics to confirm the absence of GFLV and other economically significant viruses. The rigorous adoption of certified material is the most effective strategy to prevent the initial introduction of viral inoculum into vineyards and to avert the establishment of GFLV in previously uninfected areas (Maliogka *et al.*, 2015; Fuchs, 2020).

- **Thermotherapy**

Thermotherapy is a well-established technique for the sanitation of grapevine propagation material and the production of plants free from *Grapevine fanleaf virus* (GFLV) (Maliogka *et al.*, 2015). The method is based on exposing entire plants or plant parts to elevated temperatures, typically within a range of 35–38°C, for an extended period of several weeks. This thermal regime capitalizes on the differential heat sensitivity between the host plant's meristematic tissues and the virus, selectively inactivating the pathogen while preserving plant viability (Křižan *et al.*, 2009; Panattoni *et al.*, 2013). The technique of thermotherapy was often used in combination with meristem culture, as heat treatment reduces viral concentration, enabling subsequent isolation of virus-free meristem tips. Thermotherapy is particularly valuable in certification programs, as it can be applied *in vivo* to potted plants or integrated *in vitro* with meristem culture to eliminate viruses from explants, thereby generating certified virus-free planting stock (El Sayed *et al.*, 2023).

- **Meristem culture**

Meristem culture is a highly effective technique for eliminating *Grapevine fanleaf virus* (GFLV) from infected grapevines. The method involves the sterile excision and *in vitro* cultivation of apical meristems (typically 0.2–0.7 mm) or larger shoot tips (5–10 mm) on specialized growth media (Panattoni *et al.*, 2013). Due to the absence of developed vascular tissue in these explants, coupled with intense metabolic activity, they frequently escape viral infection. Cultivation under controlled conditions allows for the regeneration of complete, pathogen-free plants. The regeneration period varies from several months for smaller meristem explants to 1–2 months for larger shoot tips (Maliogka *et al.*, 2015). This technique can achieve up to 100% virus elimination, a success rate that is further enhanced when combined with prior thermotherapy treatment (Fayek *et al.*, 2009; Shatnawi *et al.*, 2011; Miljanić *et al.*, 2022; El Sayed *et al.*, 2023).

- **Somatic embryogenesis**

Somatic embryogenesis, a technique developed for plant regeneration, is also employed for virus elimination. The process involves culturing explants (e.g., anthers, ovaries, leaf tissue) on a callus-induction medium, followed by embryo differentiation to generate somatic embryos, which can then regenerate into whole plants suitable for *in vitro* propagation (Panattoni *et al.*, 2013; Maliogka *et al.*, 2015). The effectiveness of somatic embryogenesis in eradicating GFLV has been demonstrated in several studies, with some grapevine cultivars showing near 100% virus elimination when the protocol is rigorously optimized and regenerated plants are subjected to long-term monitoring (Gambino *et al.*, 2008; Nuzzo *et al.*, 2022). The integration of thermotherapy prior to or during somatic embryogenesis significantly enhances its efficacy, resulting in virus-free plantlets, whereas plants regenerated without this treatment often retain the infection (Olah *et al.*, 2022).

- **Chemotherapy**

Chemotherapy is an *in vitro* sanitation strategy that involves supplementing culture media with antiviral chemical compounds to eliminate viruses from infected plant tissue. Many compounds used for this purpose, such as ribavirin, were originally developed against human viruses and later repurposed for plant pathogen control (Weiland *et al.*, 2003; Maliogka *et al.*, 2015). In grapevine, studies have evaluated agents including ribavirin, oseltamivir, and salicylic acid for their efficacy against nepoviruses like *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV), as well as other grapevine viruses. These compounds are typically

applied in combination with biotechnological techniques such as meristem culture or somatic embryogenesis to enhance overall virus eradication rates (Guţă *et al.*, 2017; Aiter *et al.*, 2020; Khassein *et al.*, 2024; Turcsan *et al.*, 2025).

3.6.2 Vector management

Effective management of *Xiphinema index*, the nematode vector of GFLV, is a critical but challenging component of controlling grapevine fanleaf disease.

- **Nematicides**

Effective management of *Xiphinema index*, the nematode vector of GFLV, is a critical but challenging component of controlling grapevine fanleaf disease. Historically, chemical control in infested soils relied on broad-spectrum fumigant nematicides. However, these compounds provide only temporary suppression, exhibit high environmental toxicity, and many have been banned due to carcinogenic properties and risks to human health (Maliogka *et al.*, 2015; Van Zyl *et al.*, 2016). Consequently, research focus has shifted toward developing sustainable, non-chemical alternatives. Among the most promising strategies are the use of plant-derived nematicidal compounds and the cultivation of antagonistic cover crops, which aim to suppress nematode populations without adverse ecological impacts (see Table 2).

Table 2 : Nematicidal effects of garlic extract and Fabaceae plant derivatives on nematode populations.

Nematicides	Methods	Results	References
Garlic extract	In vitro and pot-based experimental study.	Garlic extract at 2 mL/L caused nearly 100% nematode mortality in 2 hours and reduced nematode populations in soil.	(D'Addabbo *et al.*, 2023)
Plants Fabaceae	In vitro experiment and metabolomic analysis	Fabaceae plant parts and sainfoin pellets showed nematicidal effects on *X. index* in vitro	(Negrel *et al.*, 2022)

- **Cultural and agronomic practices**

Cultural and agronomic practices offer sustainable strategies for suppressing *Xiphinema index* populations. Extended fallow periods and the strategic use of antagonistic cover crops between vineyard rotations can effectively reduce nematode densities. The persistence of the virus-vector complex, however, presents a significant challenge; GFLV has been detected in viruliferous *X. index* surviving in host-free soil for up to four years (Demangeat *et al.*, 2005). Therefore, selecting effective cover crops is critical. Studies have identified specific plants including white lupin, marigold, and nyjer that significantly reduce *X. index* populations in controlled conditions. Field trials further support the efficacy of marigold and hairy vetch in lowering nematode counts within established vineyards (Villate *et al.*, 2012). These practices disrupt the nematode life cycle and reduce soil inoculum, thereby contributing to long-term disease management.

- **Biological control**

Biological control offers an environmentally sustainable strategy for managing *Xiphinema index*. Research has explored the use of antagonistic organisms, including nematode-trapping fungi, parasitic bacteria, and arbuscular mycorrhizal fungi, to suppress nematode populations (Table 3).

Table 3 : Influence of fungi and microbial species on nematode populations.

Fungi	Influence	references
Arthrobotrys flagrans	Show significantly reducing *X. index* juveniles in pot cultures, and can be formulated into soil-applied pellets.	(Wernet and Fischer, 2023)
***Trichoderma* species, (*T. virens*, *T. atroviride*, and *T. rossicum*)**	have demonstrated innate ability to decrease *X. index* populations in vitro, sometimes outperforming the commonly used *T. harzianum*	(Daragó *et al.*, 2013)
***Bacillus* and *Pseudomonas* species**	The use of Rhizobacteria such as Bacillus and Pseudomonas species, when applied as consortia or isolated strains, have reduced root damage and suppressed nematode populations in grapevine roots	(Canchignia *et al.*, 2017; Aballay *et al.*, 2020)
Arbuscular mycorrhizal	*Glomus intraradices* (*Rhizophagus intraradices*) reduce nematode numbers and gall formation.	(Hao *et al.*, 2012)

3.6.3 Resistance rootstock and cultivars to the virus and their vector

- **Resistance to the virus**

Natural sources of resistance to GFLV within *Vitis vinifera* are exceptionally rare. A major breakthrough, however, was the identification of a single recessive resistance factor, rgflv1, in the Riesling variety. Located on chromosome 1, this gene confers resistance to the virus but does not prevent nematode feeding; the plant remains susceptible to the vector, *Xiphinema index*. As the first and only known natural source of GFLV resistance in grapevine, rgflv1 offers a promising genetic basis for breeding resistant rootstocks (Djennane et al., 2021).

Biotechnological approaches have also shown promise. For example, the use of nanobodies (single-domain antibodies) specific to GFLV has conferred strong resistance to a broad range of GFLV isolates in both model plants and grapevine rootstocks. This resistance is effective even when the virus is transmitted by nematodes (Hemmer *et al.*, 2018; Orlov *et al.*, 2020). Despite these advances, practical deployment of virus resistance in vineyards remains limited, and further work is needed to translate laboratory findings into field-ready solutions.

- **Resistance to *Xiphinema index***

Resistance to *Xiphinema index* has been identified primarily in non-*vinifera Vitis* species and the related genus *Muscadinia* (Oliver and Fuchs, 2011). This trait has been successfully introgressed to develop commercially available, nematode-resistant rootstocks, such as the UCD GRN series, which exhibit broad tolerance to multiple nematode pests (Ferris *et al.*, 2012). While these rootstocks do not confer complete immunity, they significantly reduce nematode feeding efficiency and viral transmission rates. Consequently, they substantially delay GFLV infection in the scion, extending the productive lifespan of vineyards planted on infested soils (Oliver and Fuchs, 2011).

Materials and Methods

Materials and Methods

1. Virus source

Field inspections and systematic sampling were conducted across Algeria's principal viticultural regions (Southern, Central, Eastern, and Western zones) (Figure 12). A total of 435 grapevine samples were collected during December 2021 and January 2022. *Grapevine fanleaf virus* (GFLV) isolates were subsequently obtained from this material. The sampling strategy encompassed commercial, autochthonous vineyards, and Saharan vineyards.

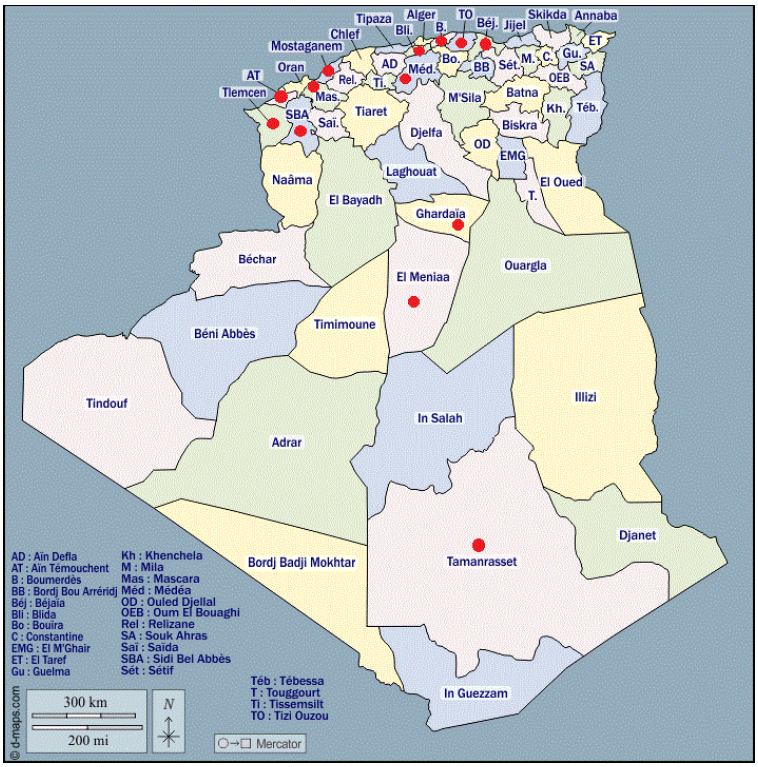


Figure 12: Algerian map highlighting the principal sampling regions; the red circle indicates the area where the samples were carried out.

Mature canes were randomly selected from each sampled vine. From each cane, a composite sample consisting of four dormant cuttings was collected. Each composite sample was subsequently divided into two homogenous subsamples, which were individually labeled and stored in paper bags at 4°C. To preserve tissue integrity and prevent desiccation, the paper bags were periodically moistened. These subsamples were preserved for subsequent serological and molecular analyses.

2. Serological detection

2.1 Double antibody ELISA

To determine the incidence of *Grapevine fanleaf virus* (GFLV) in Algerian vineyards, all collected samples were analyzed using Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). The assay was performed at the Laboratory of phytopathology, Department of Bontanic, National high school of agronomy,Algiers**,** using a commercial GFLV-specific antiserum kit (Complete 480, Bioreba AG, Switzerland). The procedure followed the manufacturer's recommended protocol (Appendix 3), which is adapted from the standard DAS-ELISA method originally described by Adams and Clark, 1977 (Figure13).

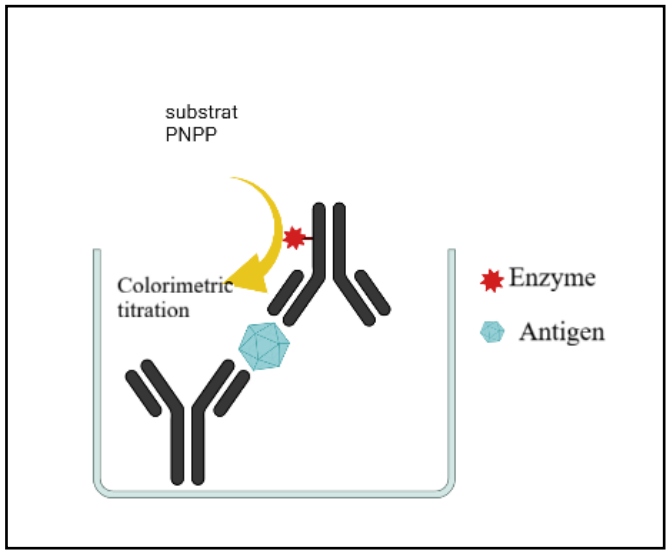


Figure 13: Schematic representation of DAS-ELISA created by BioRender.com

2.1.1 Interpretation of the results

The results of the DAS-ELISA test were interpreted by comparing the optical densities obtained for each extract against a positivity threshold.

According to Astier et *al*. (2001), the sensitivity and specificity of virus detection by ELISA depend on the choice of the positivity threshold. They report that if the samples to be identified as infected contain a high viral concentration, choosing the positivity threshold poses no difficulty (for example, 3 times the average optical density of healthy reference samples: the test's specificity is excellent, while its sensitivity is low).

In interpretation of our results; Sample optical density (OD) values were classified relative to a calculated positivity threshold derived from negative controls. Two threshold values were established:

Threshold 1= mean OD of negative controls×3

Threshold 2= mean OD of negative controls×2

Samples with an OD below Threshold 2 were classified as negative (GFLV-undetected), while those with an OD above Threshold 3 were classified as positive (GFLV-infected). Samples yielding OD values between the two thresholds were considered indeterminate. All samples serologically identified as positive for GFLV were retained at 4°C for subsequent molecular characterization.

2.1.2 Statistical analysis

All statistical analyses were performed using IBM SPSS statistics softwar. A chi-square goodness-of-fit test was first applied to assess the global infection percentage. Then, chi-square tests of independence were used to evaluate the association between infection status and region, as well as between infection status and cultivar.

3. Vegetative propagation

To preserve plant material for subsequent analyses, a subset of samples previously identified as GFLV-positive, along with selected negative controls, were maintained through propagation by cuttings. The propagation process was conducted in the greenhouse of the nursery of Ain Azel (Setif, Algeria) according to the standardized steps described by (Waite *et al.*, 2015), ensuring the conservation of viable material for future experimental investigations:

- **Selection of plant material**: Dormant and mature stems were collected during the resting season. Selection criteria included straight, well-lignified, and possessed healthy buds with an optimal diameter ranging between 0.5 and 1 cm.
- **Preparation of cuttings:** Selected canes were surface-sanitized and sectioned into nodal cuttings using sterilized pruning shears. Each cutting unit was standardized to a length of 20–25 cm, containing a minimum of three intact buds, with the basal cut made approximately 1 cm below the lowest node.
- **Rooting hormone treatment**: the cutting were oriented in the right direction. The basal end (lower 2–3 cm) of each cutting was treated with a commercial rooting powder containing 3-indolebutyric acid (IBA). Prior to hormone application, cuttings were briefly hydrated in water to prevent desiccation and ensure uniform uptake of the rooting compound.
- **Planting in rooting media**: treated cuttings were planted vertically in suitable rooting medium (perlite) in nursery beds. Planting depth was standardized so that two buds

remained above the substrate surface, with the lower nodes and the treated basal section buried. The substrate moisture was maintained at approximately 70% field capacity, and beds were kept under ambient greenhouse conditions (20–25°C) to optimize callusing and root initiation.

- **Rooting and Acclimatization:** Substrate moisture was maintained at approximately 70% field capacity through regulated irrigation. Root initiation, confirmed by the emergence of root primordia from the basal callus, was typically observed 4–8 weeks post-planting. The duration of this phase exhibited variability dependent upon cultivar and ambient greenhouse conditions.
- **Transplanting**: Following confirmation of a developed root system (3 to 6 weeks), successfully rooted cuttings were carefully extracted and transplanted into individual 3-liter pots containing potting soil. Regular irrigation was maintained to support initial growth post-transplant.
- **Post-Transplant Management**: Young vines were provided with vertical support and maintained in a protected nursery area. A integrated management program was implemented, including manual weed control, preventative fungicide applications, and periodic training of primary shoots to promote optimal architecture and establishment.

Box 3 : Vegetative propagation

4. Molecular detection

The technique used to detect GFLV is RT-PCR (Reverse transcription-Polymerase Chain Reaction), which is widely used to detect fruit tree viruses, including grapevine GFLV (Oliver *et al.*, 2010).

4.1 Total nucleic acid extraction

Total nucleic acids (TNA) were extracted from a subset of 87 grapevine samples that tested positive for GFLV via DAS-ELISA. The extraction was performed using a cetyltrimethylammonium bromide (CTAB)-based protocol adapted from (Setiono *et al.*, 2018). Plant tissue (100 mg of phloem) was cryogenically ground to a fine powder in liquid nitrogen using a sterile mortar and pestle. The powdered tissue was homogenized with 1 mL of pre-heated (65°C) extraction buffer (2% CTAB [w/v], 2.5% PVP-40 [w/v], 2 M NaCl, 100 mM

Tris-HCl [pH 8.0], 24 mM EDTA [pH 8.0]). The homogenate was incubated at 60°C for 1 hour, followed by centrifugation at 13,000 ×g for 15 minutes.

The supernatant was subjected to a purification step involving an equal volume of chloroform-isoamyl alcohol (24:1, v/v). The mixture was emulsified by gentle agitation for 45 minutes and then centrifuged at 13,000 ×g for 15 minutes at 4°C. The aqueous phase was carefully transferred to a new tube, and nucleic acids were precipitated by the addition of 0.7 volumes of ice-cold isopropanol. After gentle inversion until a white pellet formed, the sample was incubated at -20°C for 20 minutes and centrifuged at 13,000 ×g for 10 minutes.

The supernatant was carefully removed, and the pellet was washed with 500 µL of 70% cold ethanol. The sample was incubated at room temperature for 20 min, followed by centrifugation at 13,000 × g for 5 min. The ethanol supernatant was discarded, and the pellet was washed again with 500 µL of cold ethanol. A final centrifugation (5 min at 13,000 ×g) was performed, and the supernatant was removed. The tubes were air-dried for 20 min, and the pellet was resuspended in 100 µL of sterile distilled water.

The integrity and approximate concentration of the extracted TNAs were assessed by agarose gel electrophoresis. A 7 µL aliquot of each sample was mixed with 3 µL of loading dye and resolved on a 1% agarose gel prepared in 1× TAE buffer, stained with Gel red. Electrophoresis was performed at a constant voltage of 100 V for 25 minutes. Nucleic acid bands were visualized under UV transillumination.

4.2 Reverse transcription polemerase chain reaction

4.2.1 Synthesis of DNA complementary

Complementary DNA (cDNA) was synthesized from the extracted total nucleic acids via reverse transcription. For each reaction, 10 µL of TNA template was combined with 1 µL of random hexamer primers (50 µM; Eurofins Genomics) and 5.5 µL of nuclease-free water (Mix I). Two no-template controls (NTCs), containing nuclease-free water in place of TNA, were included. The mixture was incubated at 65°C for 5 minutes to denature secondary RNA structures and then immediately placed on ice.

For reverse transcription, Mix II was prepared containing, per reaction: 2 µL of 5x First-Strand Buffer containing dithiothreitol (DTT), 0.5 µL of a dNTP mix (20 mM), and 1 µL (200 units) of Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase. Mix II (7.5 µL) was added to each denatured primer-template mixture from the previous step, yielding a final reaction volume of 20 µL. The complete reaction was incubated at 25°C for 10 minutes,

followed by 50 minutes at 39°C. The reaction was terminated by heating at 70°C for 10 minutes. The synthesized cDNA was stored at –20°C until use for subsequent PCR amplification.

4.2.2 Polymerase chain reaction

Amplification of the GFLV coat protein (CP) gene fragment was performed using a conventional PCR assay. The reaction utilized *HOT FIREPol* DNA polymerase (Solis BioDyne) and the specific primer pair described by MacKenzie *et al.* (1997), which amplifies a 312 bp fragment of the CP gene (Table 4).

The PCR master mix was prepared on ice with the following components per 20 µL reaction: 2 µL of 5X Buffer B2, 2 µL of 25 mM $MgCl_2$, 1 µL of dNTP mix (10 mM), 0.5 µL each of forward and reverse primers (10 µM), 0.25 µL of *HOT FIREPol* DNA polymerase (5 U/µL), 11.75 µL of nuclease-free water, and 2 µL of cDNA template.

Amplification was carried out in a thermal cycler with the following program: an initial denaturation and polymerase activation step at 92°C for 10 min; followed by 35 cycles of denaturation at 90°C for 30 s, primer annealing at 52°C for 45 s, and extension at 72°C for 1 min; concluding with a final extension at 72°C for 7 min. All PCR runs included appropriate negative controls.

Table 4 : Primers used for GFLV detection

Region	Sequences	Size	Reference
Coat protein	C3310 5′-GATGGTAACGCTCCCGCTGCTCTT-3′	312 bp	(MacKenzie *et al.*, 1997)
	H2999 5′-TCGGGTGAGACTGCGCAACTTCCTA-3′		

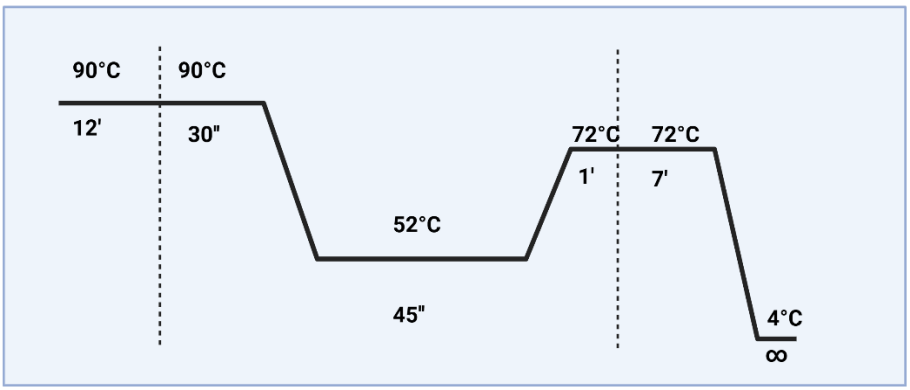


Figure 14 : Polymerization conditions of GFLV primers for amplification of the coat protein (CP) gene segment.

4.2.3 Revealing and examining bands

PCR amplicons were resolved by electrophoresis on a 1.2% agarose gel prepared in 1X TAE buffer and stained with Gel red. A 100 bp DNA ladder was included in each run as a molecular size standard. Electrophoresis was performed at a constant voltage of 100 V for 30 minutes. DNA bands were visualized and documented using a UV transilluminator imaging system.

4.3 Sequences analysis

The amplified products were purified by agarose gel extraction using the NucleoSpin® Gel & PCR Clean-up Mini Kit (MACHEREY-NAGEL), following the manufacturer's instructions (Appendix 4)

4.3.1 Cloning and sequencing

The PCR product was cloned into pGEM-T Easy vector System II (Promega, Madison, USA) following the manufacturer's instruction (Appendix 5)

4.3.2 Plasmid isolation (Miniprep)

Plasmid DNA was isolated from bacterial cultures using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol (Appendix 6).

5. High throughput sequencing

5.1 Total RNA extraction and Illumina-NGS

One cane sample from autochthonous cultivar "Cherchalli" was processed by scarping the bark, and cutting it into small pieces of ~ 1cm. The pieces were placed in microcentrifuge tubes, immediately submerged in a sterile RNAlater solution to preserve RNA integrity, and stored at 4°C until extraction.

RNA was extracted from the phloem-enriched tissue using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol for plant tissues. An Illumina-compatible sequencing library was then prepared from the total RNA using the TruSeq Stranded Total RNA Library Prep Kit (Illumina).

The quality and integrity of the extracted RNA were assessed using an Agilent 2100 Bioanalyzer with the RNA 6000 Nano Kit (Agilent Technologies). Only high-quality RNA (RNA Integrity Number, RIN > 7.0) was used for library preparation. Sequencing was

performed on an Illumina NovaSeq 6000 platform, generating paired-end reads (2 × 101 bp) (Kearse *et al.*, 2012b), raw reads were trimmed (Kearse *et al.*, 2012a).

5.2 Map to reference

The cleaned reads were mapped to reference sequences using the "Map to Reference" tool in Geneious Prime® 2024.0.5 (Kearse *et al.*, 2012), with the Geneious RNA Mapper plugin set to medium-low sensitivity. Consensus sequences were generated from mappings against suspected viral genomes. In parallel, a comprehensive screening was performed by mapping the entire RNA-seq dataset against a custom plant virus reference database. This database was constructed by concatenating 5,040 representative plant virus sequences retrieved from GenBank, yielding a total reference length of 76,145,671 nucleotides. The mapping reports for all analyses were evaluated based on key metrics, including: the number of reads assembled to the reference, the percentage of total reads used, the depth of coverage, and the pairwise sequence identity between the consensus and the reference sequence.

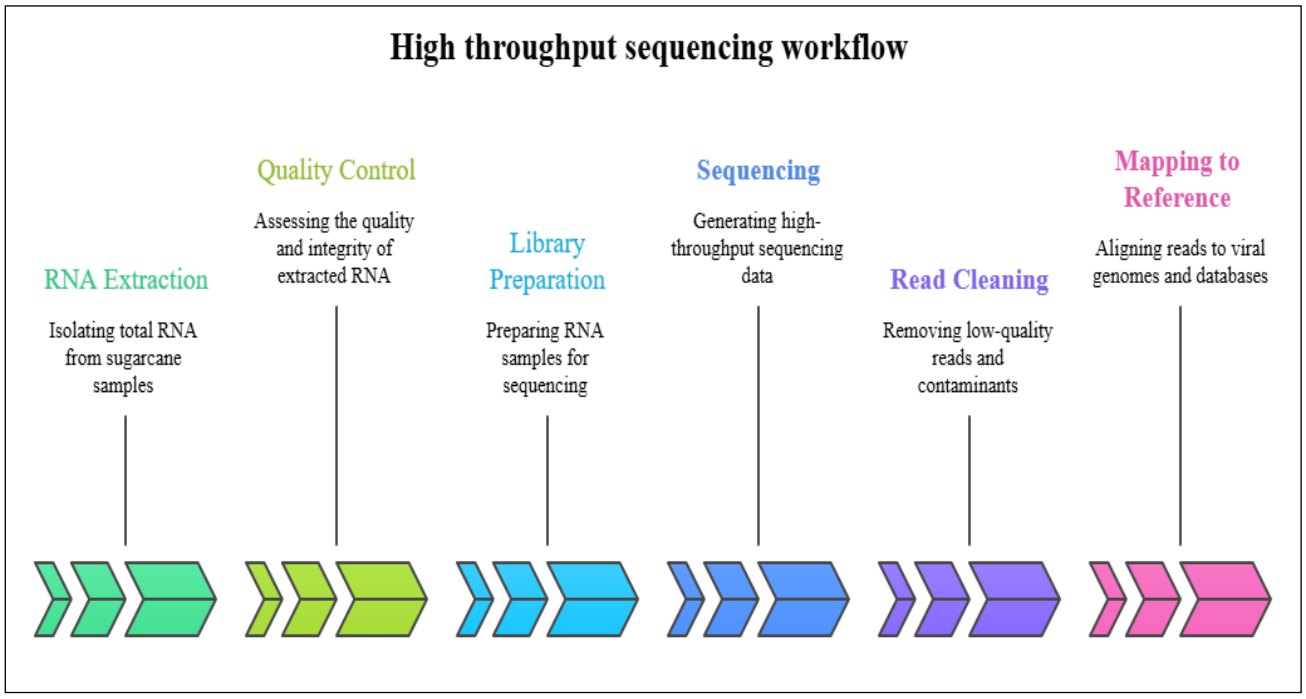


Figure 15: Schematic overview of the RNA-seq workflow from sample processing to bioinformatic analysis.

6. Phylogenetic analysis

This study aimed to compare the nucleotide sequences obtained from Algerian isolates with global variants available in public databases. For each viral sequence generated in this study, a Basic Local Alignment Search Tool (BLASTN) analysis was performed against the NCBI GenBank nucleotide database (www.ncbi.nlm.nih.gov) to identify homologous sequences and assess genetic similarity.

The four Sanger-derived sequences and one high-throughput sequencing (HTS)-derived consensus from this work have been deposited in GenBank under accession numbers (Table8). For phylogenetic analysis, the Coat Protein (CP) gene sequences from the Algerian *Grapevine fanleaf virus* (GFLV) isolates were aligned with representative GFLV sequences from different global origins. Multiple sequence alignment was performed using the ClustalW algorithm implemented in MEGA11 software (Tamura *et al.*, 2021). Phylogenetic trees were inferred using the Neighbor-Joining (NJ) method with 1,000 bootstrap replicates to assess branch support. The tree was rooted using a *Grapevine fleck virus* (GFkV) isolate (MT48, NC_003347.1) as an outgroup.

7. Nematological soil analysis

To determine the potential vector of *Grapevine fanleaf virus* (GFLV), a nematological analysis was conducted with the aim of identifying the presence of *Xiphinema index*. The selection of sampling sites was based on regional conditions and logistical feasibility in collaboration with local farmers. Samples were collected from a vineyard located in the eastern region of Algeria, specifically in Souk El Tenin, Béjaïa.

7.1 Soil sampling

During the spring season, sampling was focused on vineyard sites that had previously tested positive for *Grapevine fanleaf virus* (GFLV) via DAS-ELISA. To ensure a more representative assessment of the nematode vector population, additional rhizosphere soil samples were collected from the immediate vicinity of these positive sites. A total of 18 soil samples were analyzed from the cultivar 'Seibel', of which 2 were infected with GFLV. For the cultivar 'Muscat of Italy', 9 samples were examined, with 1 testing positive for GFLV. This strategy allowed the detection of *X. index* not only directly within virus-infected foci but also in the surrounding buffer zones, which can act as reservoirs for the vector population and potential pathways for the nematode-mediated spread of the virus.

7.2 Soil nematodes extraction method

The selection of a nematode extraction method depends on several factors, including the available equipment, the type of sample matrix (e.g., soil, roots), and the target nematode species. For this work, nematodes were extracted using a modified Cobb's sieving and decanting method (also referred to as a flotation-sedimentation technique) based on the protocol by Flegg (1967), this method relies on the physical separation of nematodes from soil particles using a series of sieves with specific mesh sizes, often followed by additional purification steps (Figure 15).

- **Samples preparation**

Soil samples were collected using an auger. At each sampling point, soil cores were taken from the rhizosphere of grapevine plants at a depth of 30 cm. The samples were subsequently prepared by breaking up the large clumps and removing all stones, roots and other debris.

- **Soil suspension**

Approximately **1kg** of soil was placed in a 10-liter bucket**.** The soil was then suspended in 5 to 7 liters of water and vigorously agitated with a sturdy rod to ensure complete homogenization and to dislodge nematodes from soil particles.

- **Decanting**

The soil suspension was allowed to settle for 20–30 seconds to permit sand and coarse silt particles to sediment. The supernatant, containing finer particles and nematodes, was then carefully poured through a stack of sieves, leaving the coarse sediment behind.

- **Sieving**

The soil suspension was successively passed through a stacked series of sieves. The suspension was first poured through a coarse sieve (710 µm aperture) to remove large debris, and then through a fine sieve (40 µm) designed to retain nematodes. The nematodes and fine particulate matter collected on the fine sieve were gently rinsed with a gentle stream of water into a small sieves covered with tissue paper or a paper towel to concentrate the organisms. These small sieves were placed in Petri dishes fulling of water and maintained at room temperature for 48 hours to allow nematodes to migrate into the water for collection and observation under stereomicroscope.

- **Observation**

Nematodes were examined under a stereomicroscope (OPTIKA) for initial observation and handling. For detailed morphological identification, specimens were mounted on glass slides and observed under a compound light microscope with differential interference contrast (DIC)

or phase-contrast optics. Key diagnostic characters for *Xiphinema* identification were measured and assessed, including: body length and ratios (a, b, c, c'), lip region morphology, amphidial aperture shape, guide ring position, and odontostyle and odontophore lengths (following the descriptions in Archidona-Yuste *et al.*, 2016).

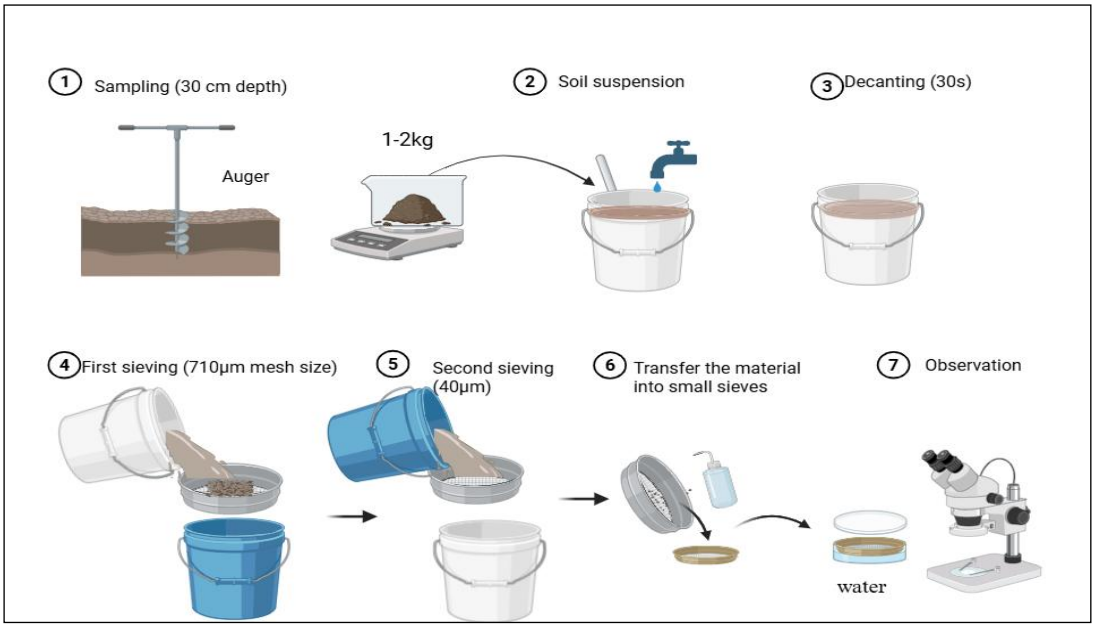


Figure 16 : Soil nematodes extraction using Cobb's method created by Biorender.com

7.3 Molecular identification of nematodes

To confirm species identity, molecular analyses were performed on nematodes extracted from soil samples. Following morphological identification, a subset of 29 individual nematodes was selected for molecular characterization. The selected specimens were divided into four groups for downstream analysis (Table 5).

Table 5: Soil samples divided into four groups according to grapevine cultivar and number of samples collected

Group	cultivar	Number of individual
S1	Seibel	13
S2	Seibel	5
M	Muscat	7
R	Red glob	4

7.3.1 DNA extraction

Genomic DNA was extracted from individual nematodes using a modified proteinase K lysis protocol (De Luca *et al.*, 2004). Individual specimens were hand-picked under a stereomicroscope, transferred to a clean glass slide, and suspended in 25–30 µL of lysis buffer**.** The lysis buffer consisted of: 2M KCl, 1M $MgCl_2$, 1M Tris, 10%Trion X-100 and Proteinase K. Each nematode was macerated thoroughly with a sterile syringe needle. The lysate was then transferred to a sterile 0.2 mL PCR tube. Lysis was performed by incubating the tube at 60°C for 60 minutes, followed by enzyme inactivation at 95°C for 5 minutes. The crude lysate was centrifuged briefly and used directly as template for PCR amplification, or stored at -20°C**.**

7.3.2 PCR Amplification

PCR amplifications were performed using primer sets targeting the D2–D3 expansion segments of the 28S ribosomal RNA (rRNA) gene and species-specific SCAR markers for *Meloidogyne incognita* (Inc) and *Meloidogyne javanica* (Jav). Each reaction had a total volume of 100 µL, containing 10 µL of DNA template, 20 µL of 1X PCR buffer, 6 µL of 25 mM $MgCl_2$, 2 µL of 10 mM dNTP mix, 2 µL of each primer (10 µM), 0.3 µL of *Taq* DNA polymerase G2 Flexi (5 U/µL), and nuclease-free water to volume. The thermal cycling protocol consisted of an initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 40 s, annealing at 60 °C for 40 s (D2–D3) or 56 °C for 30 s (SCAR markers), and extension at 72 °C for 40 s; followed by a final extension at 72 °C for 5 min. Amplification products were held at 4 °C prior to analysis.

Table 6: Primers used for Nematodes identification

	primer		**Size (bp)**	**Ref**
SCAR INC	inc-K14-F	5’-CCCGCTACACCCTCAAC-3’	399	(Fanelli *et al.*, 2017)
	inc-K14-R	5’-GGGATGTGTAAATGCTCCTG-3’		
SCAR JAV	Fjav	GGTGCGCGATTGAACTGAGC	670	(Fanelli *et al.*, 2017)
	Rjav	CAGGCCCTTCAGTGGAACTATAC		
D2-D3	D2A	ACAAGT ACCGTGGGGAAAGTTG		(Troccoli *et al.*, 2024)
	D3B	TCG GAAGGAACCAGCTACTA		

Amplification products were separated by electrophoresis on 1.5% (w/v) agarose gels prepared in 1× TBE buffer, with GelRed® incorporated into the gel matrix prior to casting. A 100 bp DNA ladder served as the molecular weight standard. Electrophoresis was performed at 100 V

for 45–60 min in 1× TBE running buffer. Bands were visualized under ultraviolet (UV) light using a gel documentation system.

7.3.3 Sequencing and phylogenetic analysis

PCR amplicons were excised and purified from agarose gels using the NucleoSpin Gel and PCR Clean-up kit (MACHEREY-NAGEL), following the manufacturer's protocol (see section 5). Purified products were submitted to Eurofins Genomics for commercial Sanger sequencing. For the D2–D3 region, six representative amplicons—one from S1, two from S2, two from R, and one from group M—were selected for bidirectional sequencing. Resulting chromatograms were assembled, edited, and the consensus sequences were subjected to BLAST analysis against the NCBI nucleotide database.

Results and discussion

Results and discussion

1. Survey results and observed symptomatology

Most of the surveyed vineyards were located in the central region of Algeria in the regions of Boumerdes and Medea, composed primarily of Ahmer Bou amer, Cardinal and Seibel cultivar. Symptoms were observed during Mai 2023. These cultivars exhibited a range of viral disease symptoms; the leaves appear asymmetric and distorted with sharply toothed margins, closer primary veins and an open petiolar sinus. Other foliar symptoms include vein banding with light-green to chrome-yellow chlorotic bands along the veins, chlorotic mottling and yellow mosaic with partially or completely chrome- yellow leaves. In the Seibel cultivar, the leaves exhibit a Fanleaf like deformation, characterized by open petiolar sinuses and acute denticulation. The Ahmer Bou Amer cultivar exhibited yellow chlorosis and mosaic patterns on the leaves, with irregularly distributed discoloration across the leaf surface.

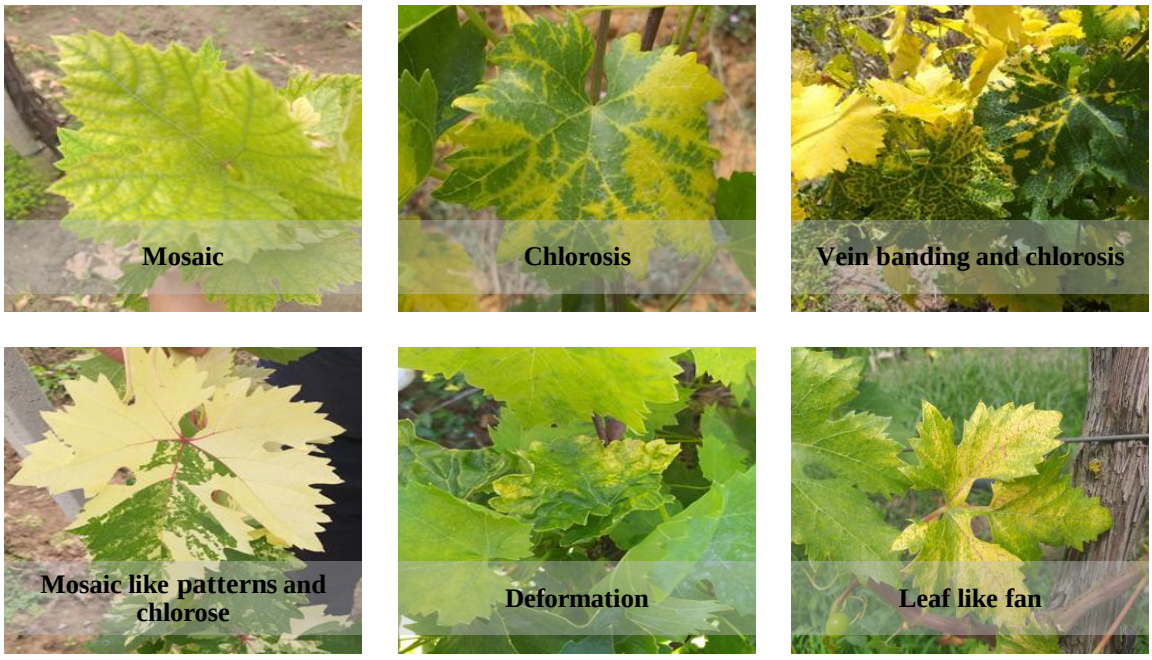


Figure 17: Representative foliar symptoms observed on grapevine cultivars leaves in central Algeria showing mosaic, chlorosis, vein banding with chlorosis, mosaic-like patterns, leaf deformation, and fan-shaped leaves (observed in May 2023).

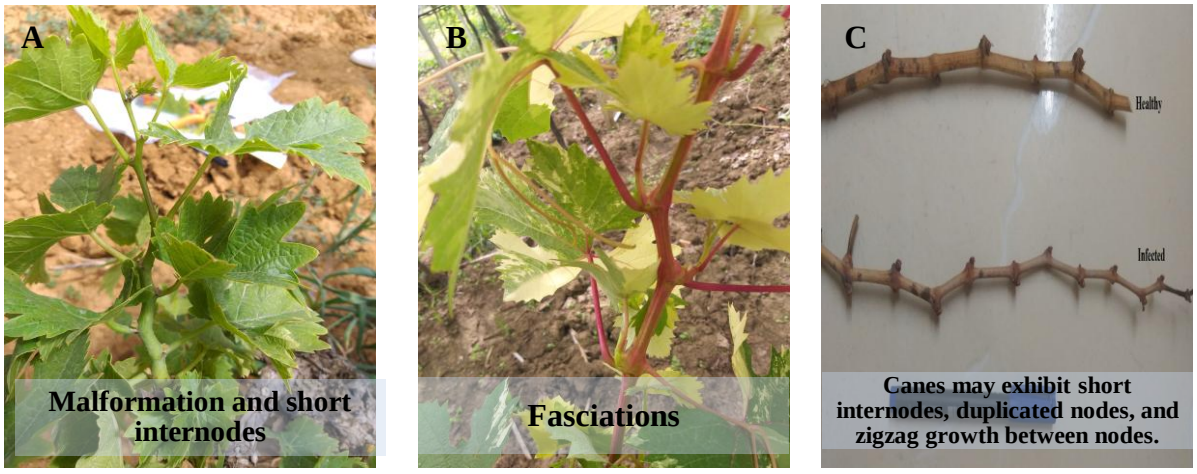


Figure 18: *Grapevine Fanleaf virus* symptoms on canes of the samples. (A, B) observed in May 2023 and (C) in December 2023.

1.1 Discussion of symptomatology analysis

The survey conducted across vineyards in Algeria revealed the presence of symptoms indicative of viral infections in several grapevine cultivars. Such symptoms, including leaf deformation, vein banding, mottling, and yellow mosaic, are characteristic of viral diseases and are commonly linked to the *Grapevine fanleaf virus* (GFLV) and other virus like *Grapevine leaf roll* (GLRV), which are well-documented threats to grapevine health and productivity worldwide (Andret-Link *et al.*, 2004; Martelli, 2014). The asymmetrical and deformed leaves identified in the examined vineyards, practically in the Seibel cultivar, distinguished by serrated margins, large petiolar sinuses and fanleaf-like deformation, are indicative of GFLV infections (Martin *et al.*, 2021; Kubina *et al.*, 2022). The other symptoms observed in the Ahmer Bou Amer cultivar, such as irregular yellow chlorosis and mosaic patterns, could be indicative of GLRV, GFLV, or mixed infections, which disrupt chlorophyll production and decrease vine vigor (Allaya and Abaab, 2006; Naidu *et al.*, 2015). The differential responses of the cultivars suggest variability in susceptibility to viral pathogens, indicating the influence of cultivar-specific resistance and environmental factors on disease expression. These viral infections significantly impact grapevine health and productivity, leading to reduced yield and compromised fruit quality (Maliogka *et al.*, 2015; Meng *et al.*, 2017). North Algeria is characterized by moderate climatic conditions and intensive viticulture, both of may facilitate the spread of GFLV through its vector, the nematode *Xiphinema index* (Martelli, 2014; El Sayed *et al.*, 2023). Furthermore, the lack of rigorous virus-free certification programs for planting

material likely contribute to the dissemination of infected propagation stocks (Panno *et al.*, 2021).

2. Serological and molecular results

2.1 Serological test

2.1.1 Prevalence of GFLV in Algerian vineyards

The results of the enzyme-linked immunosorbent assay (DAS-ELISA), conducted on 435 samples collected from the surveyed regions, revealed that 20% of these samples (corresponding to 86 samples) tested positive for GFLV. This result establishes the overall GFLV infection rate in the surveyed Algerian vineyards (Figure 20). A Chi-square goodness-of-fit test indicated that the observed infection frequency differed significantly from the expected distribution ($Z = 10.43$, $p < 0.001$), confirming a highly significant overall prevalence of GFLV.

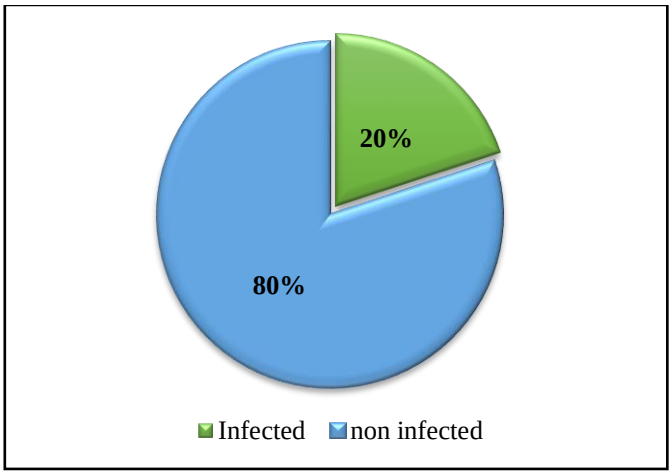


Figure 19 : Prevalence of GFLV in Algerian vineyards

2.1.2 Infection frequency of vineyard samples by region

The overall infection rate of GFLV in Algerian vineyards was established at 20%. The highest frequency was observed in the central region at 27% (50 out of 185 sample), followed by the western region with 16% (33 out of 210 sample) of infected samples and the east region by 14% (3 out of 21 samples). All samples from the southern region tested negative for GFLV (Figure 21). A Chi-square test of independence revealed a significant association between geographic region and GFLV occurrence ($\chi^2 = 13.40$, $df = 3$, $p = 0.004$), indicating that infection distribution differed significantly among regions (Table 7).

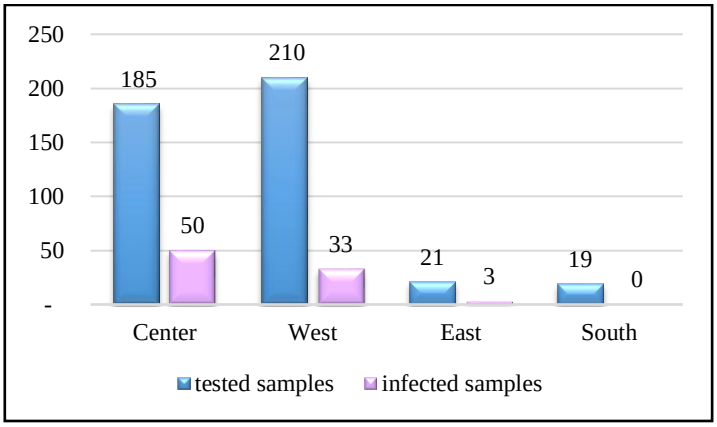


Figure 20: Regional Prevalence of GFLV Infection in Algerian Vineyards

Table 7 : Regional infection rates with chi-square independence test result.

Region	Infection rate%
Center	27
West	15.7
South	00
East	14.3%
significance	p= 0.004

2.1.3 Infection Frequency of Vineyard Samples by Cultivars

A notable variability in infection frequency was observed among cultivars; Ahmer Bou Amer presents the highest incidence at 62%, followed by Carignon at 45%. Other cultivars also showed varying degrees of infection: Alicante (40%), Cardinal (30%) and Cinsault (18%), Muscat Italy (26%), Seibel (23%), Michel Palliri and Adari (20%), Alphose lavalee (Gros Noir) (10%), Dattier (7%) and Muscat (5%). Conversely, the varieties Mersguerra, Valensi, Metllili, Sebseb, and Issers were found to be completely free of GFLV infection (Table 8). A Pearson chi-square test of independence revealed a highly significant association between cultivar and infection status ($\chi^2(19) = 81.067$, $p < 0.001$). Conversely, the varieties Mersguerra, Valensi, Metllili, Sebseb, and Issers were found to be completely free of GFLV infection.

Table 8: Infection rates of *Grapevine fanleaf virus* (GFLV) in selected grapevine cultivars in Algeria.

Cultivars	tested samples	Infected samples	Infection rate
Ahmeur bouameur	34	21	62%
Carignon	20	9	45%

➔ Continued			
Alicante	10	4	40%
Cardinal	37	11	30%
Muscat Italie	35	9	26%
Seibel	35	8	23%
Adari	20	4	20%
Michel palliri	10	2	20%
Cinsault	60	11	18%
Gros noir	29	3	10%
Dattier	45	3	7%
Muscat	20	1	5%
Anonyme	8	0	0%
Vigne commun	5	0	0%
Issers	4	0	0%
Mersguerra	10	0	0%
Metlili	4	0	0%
Red Globe	27	0	0%
Sebseb	2	0	0%
Valensi	20	0	0%
Significance	p=0.00*		

2.2 Discussion of serological analysis

The DAS ELISA test findings indicated an infection rate of 20% from 435 samples and showed a significant prevalence of GFLV in the examined regions. Compared to the prevalence in other regions of the Mediterranean basin, this infection rate places Algeria in the middle. For instance, in northern Tunisia, a survey detected GFLV in about 36% of vines by serology (Samaali *et al.*, 2019). In contrast, a study in Sicily (Italy), covering 617 grapevines across 11 cultivars, found only 48 positive samples (approximatively 7.8%) via combined serological and molecular screening (Panno *et al.*, 2021). In the Balearic Islands in Spain, among conserved local cultivars, GFLV occurred in about 25.4% of vines in a germplasm bank setting (El Aou-ouad *et al.*, 2022). This variation in infection rates indicates the differing prevalence of GFLV across the Mediterranean region, which may be influenced by local agricultural practices, climate conditions, and the genetic diversity of grapevine cultivars. The 20 % rate implies that

roughly one in five vines may harbor the virus, a prevalence that cannot be dismissed as marginal.

The results of DAS-ELISA testing across four regions of Algeria; central, western, eastern, and southern; reveal significant regional variation in *Grapevine fanleaf virus* (GFLV) prevalence, with infection rates ranging from 14% to 27%. The central region exhibited the highest overall rate (27%), followed by the western (15.6%) and eastern (14%) regions. Within the central region, notable local increases were observed: the wilaya of Medea (Ben Chicaou locality) recorded the highest infection rate at 54%, followed by Tizi Ouzou (Tadmit locality) with 23%.

The elevated frequency of infection could be attributed to several factors. The central region is a crucial area for viticulture in Algeria, which likely aids in the spread of the virus through dense vineyard networks, increased movement of plant material, and higher populations of vectors. Additionally, *Xiphinema index*, the primary nematode vector of GFLV, thrives in regions with moderate to high temperatures and moisture levels, conditions that may be prevalent in the central area, thereby facilitating viral transmission (Everaert *et al.*, 2024). Furthermore, Tahirine *et al.*, 2020 identified a GFLV infection rate of 47% in central Algeria. The current study may indicate a decrease in infection incidence, potentially due to sampling from diverse vineyards or the limited distribution of propagation materials.

The western region exhibited a moderate infection rate of 16%, particularly in the wilayas of Mostaganem (Abdelmalek Ramdane locality) and Aïn Témouchent (El Amria locality). This suggests a more localized presence of GFLV compared to the central region. Several factors may account for this moderate prevalence.

The western region may have a less dense concentration of vineyards, which would limit the movement of infected plant material and reduce the chances of viral transmission. Furthermore, local environmental conditions, such as temperature and humidity, may not be as favorable for the nematode vector as in the central region, which could result in lower infection rates. The relatively lower infection rate in the western region may also be indicative of better vineyard management practices, including the use of virus-free planting material. Nevertheless, the presence of GFLV in this region, even at a lower prevalence, underscores the need for ongoing monitoring and targeted control measures to prevent further spread of the virus.

The eastern region, based on samples collected from Bejaia, demonstrated the lowest infection rate at 14%. While this rate is noteworthy, it reflects a comparatively lower viral pressure than that seen in the central and western regions. Bejaia's environment is similar to that of other central areas, such as Boumerdes, which share comparable climate and agricultural conditions.

This similarity indicates that other factors, including vineyard management practices or the distribution of disease vectors, may play a more significant role in the lower prevalence observed in this region.

It is important to note that the sample size from Bejaia was insufficient to accurately represent the larger eastern region, Which likely limits the generalizability of the results. A small sample size may lead to the underrepresentation of infection hotspots or distort the results, especially in regions where GFLV infection is irregular or concentrated. Although the 14% prevalence provides an initial indication of GFLV's presence in Bejaia (Souk El Tenin locality), further studies with larger sample sizes are needed to determine whether this prevalence is consistent across the entire eastern region of Algeria.

The absence of GFLV infection in the southern region is particularly interesting. Several factors may contribute to this observation. The southern region of Algeria is characterized by a more arid climate, featuring higher temperatures and lower humidity—conditions that are generally less conducive to the survival of Xiphinema nematodes. Additionally, the limited number of samples collected may be attributed to the difficulties associated with sampling in the southern area. In the southern region, no reported cases of GFLV infection, revealing that high temperatures may significantly contribute to the suppression or inactivation of the virus. This finding is consistent with the principles of thermotherapy, a widely used method for virus eradication in grapevines, which relies on exposing infected plant material to elevated temperatures to eliminate viral infections (Panattoni and Triolo, 2010; Miljanić *et al.*, 2022; El Sayed *et al.*, 2023).

Infection rates of *Grapevine fanleaf virus* varied significantly among the surveyed grapevine cultivars in Algeria, as confirmed by a significant Chi-square test of independence ($\chi^2 = 81.07$, df = 19, $p < 0.001$), indicating marked heterogeneity in infection distribution among cultivars. The cultivar Ahmeur bou Ameur exhibited the highest incidence (62%), whereas no GFLV infection was detected in Issers, Mersguerra, Metlili, Sebseb, and Valensi. This absence of infection may suggest reduced susceptibility or possible tolerance in these cultivars; however, caution is required, as the absence of field detection does not constitute direct evidence of genetic resistance and may also reflect localized agronomic practices, including the use of healthy propagation material, lower vector pressure, or environmental effects. The broad distribution of GFLV across Algerian vineyards is likely facilitated by the exchange of infected propagation material and the widespread occurrence of its nematode vector, *Xiphinema index*, as reported in Algerian surveys (Smaha *et al.*, 2023).). Conversely, the high to moderate infection incidence observed in Ahmeur bou Ameur, Alicante Bouschet, and Carignan suggests

cultivar-dependent differences in susceptibility. Such variability is of particular interest for integrated management of Grapevine fanleaf disease, as apparently less susceptible cultivars may represent valuable genetic resources for future resistance breeding programs. Similar cultivar-related responses have been reported in autochthonous cultivars from Bosnia and Herzegovina (Crnogorac *et al.*, 2021) and selected cultivars in Italy (Panno *et al.*, 2021).

3. Molecular analysis results

3.1 PCR Detection and Cloning of GFLV Isolates

Molecular detection was performed via reverse transcription-polymerase chain reaction (RT-PCR) targeting the coat protein (CP) gene of *Grapevine fanleaf virus* (GFLV). Agarose gel electrophoresis of the RT-PCR products revealed successful amplification of the target fragment in twelve samples, yielding a single, distinct amplicon of the expected size (312 base pairs).

To validate the specificity of the amplicons and to facilitate subsequent sequencing, four representative products—each originating from a distinct cultivar (Ahmer Bou Amer, Muscat Italy, Seibel, and Dattier)—were selected for cloning. Colony PCR performed on selected transformants confirmed the presence of recombinant plasmids containing the desired insert (Figure 22).

Recombinant plasmid DNA was subsequently purified from positive bacterial clones using a standard alkaline lysis miniprep protocol. This purification step served to isolate high-quality template DNA, thereby confirming the successful insertion of the target GFLV CP gene fragment prior to Sanger sequencing.

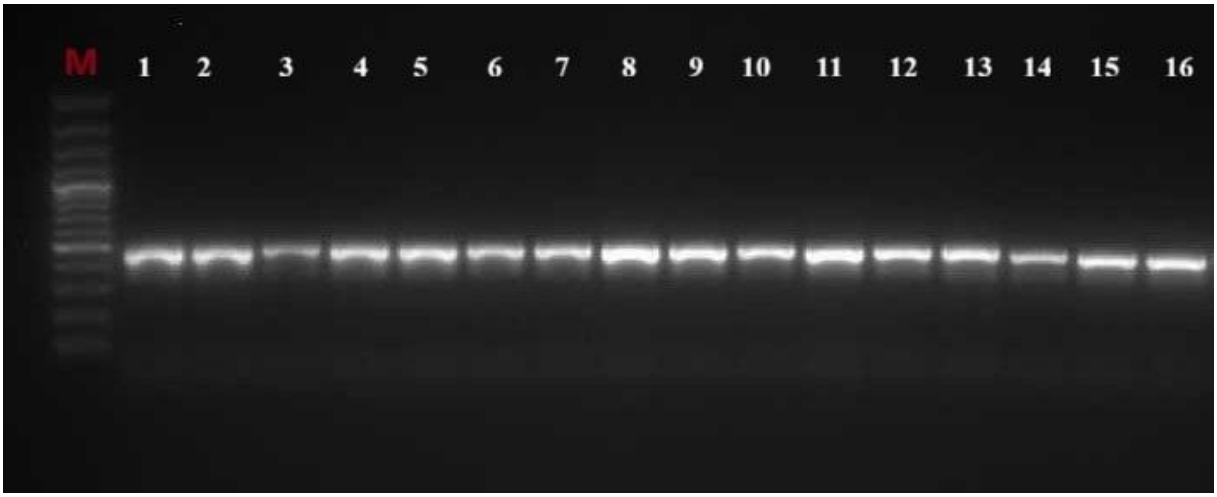


Figure 21: Agarose gel electrophoresis of PCR products confirming recombinant clones. Line **M** represent the DNA size Marker (100bp), Lane 1-4: Ahmer Bou Amer, Lane 5-8: Muscat Italy, 9-12: Seibel, lane 13-16: Dattier.

3.2 Sequencing

The clones used for sequencing were obtained from the isolates Seibel "1," Ahmer Bou Amer "230," Dattier "202," and Muscat italy "300." The obtained sequences are registered in the NCBI gene bank, and the accession number has been assigned for each one (Table 9). Prior to phylogenetic analysis, all sequences were screened for potential contamination from the cloning vector using the VecScreen tool (http://www.ncbi.nlm.nih.gov/tools/vecscreen/) available through the NCBI platform.

Table 9: The accessions numbers of the Algerian cultivars

Isolates	Origin	Cultivar	Accession Number
1	Boumerdes	Seibel	PP983213
230	Medea	Ahmer bou Amer	PP983215
202	Sidi Belabas	Dattier	PP983214
300	Medea	Muscat Italy	PP983212

3.3 HTS analysis

The Illumina sequencing platform generated 47,034,642 short reads of a total of 101 bases. Using Geneious software, all RNA reads were paired, and subsequently mapped to the reference genomes of the suspected viruses and viroids.

A total of 228,166 reads (0.48%) were assembled into a virtually full consensus sequence contig of 5,979 bp against *Grapevine fanleaf virus* RNA1. Two parts were missing, which were

not covered by the reads. The initial part measured 15 bp, extending between 5,980 and 5,994 bp, while the second part was 73 bp, extending from 6,801 to 6,873 bp. The coverage was 98.8%, while the GC content was 46.8% (Figure 23).

The sequence covered 5,721 bp of the total 6,855 bp of the polyprotein region, was submitted to GenBank with accession number PP976049, and was assigned as ALG100. The partial genomic fragment reconstructed through read assembly contained a single open reading frame (ORF) encoding an RNA helicase and an RNA-dependent RNA polymerase. This ORF spans 5,721 nucleotides and translates into a protein comprising 1,907 amino acid residues.

The assembled reads were 301,093 against the sequence of *Grapevine fanleaf virus* RNA2 (0.64%), and produced a complete consensus sequence of 3,711 bp. The coverage was 97.5% and the GC content was 49.9%. The sequence was 3,333 bp long, that encodes 1,111 residues representing the polyprotein P2 region, and was deposited in GenBank under accession number PP976050, and named ALG101. The P2 sequence codes for a typical Nepovirus subgroup A polyprotein and coat protein. In addition to the complete GFLV genome, two other viroids were identified as *Grapevine yellow speckle viroid* (PP977156) and *Hop stunt viroid isolate* (PP977157). Furthermore, *Grapevine Rupestris stem pitting-associated virus* (GRSPaV) and *Grapevine Pinot gris virus (GPGV)* were also detected. These viruses will be further characterized in a separate study.

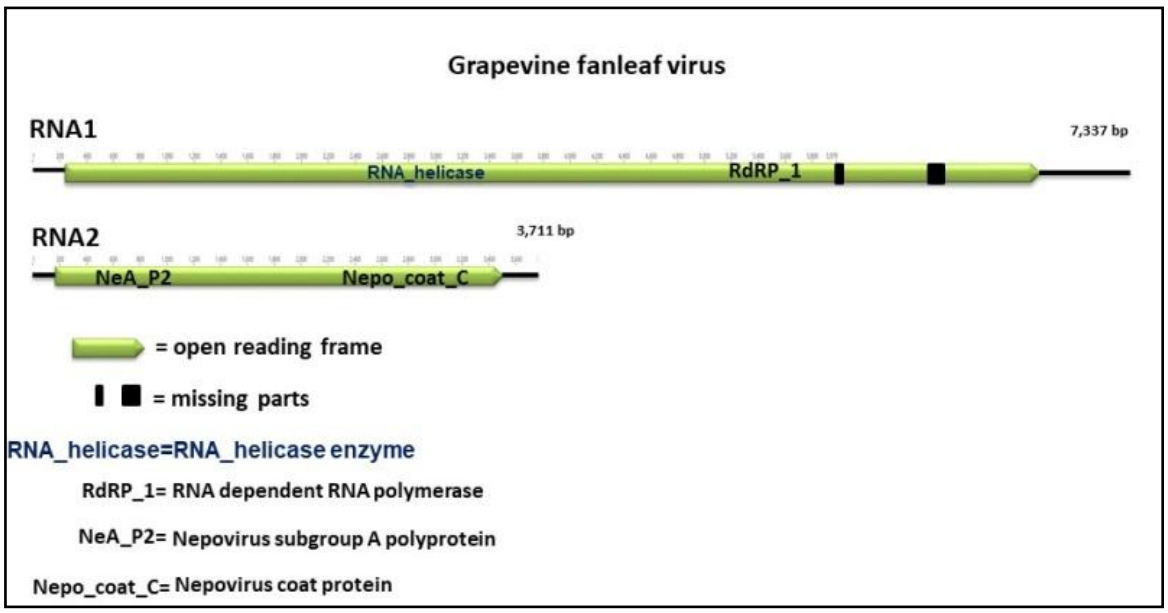


Figure 22: Genome organization of the two segments of *Grapevine fanleaf virus* genome shows major protein domains and also two missing parts over RNA1.

3.4 Phylogenetic analyses

To elucidate the phylogenetic relationships of the Algerian isolates, five Algerian coat protein (CP) gene sequences were aligned with homologous sequences originating from other regions. The comparative dataset included 13 sequences from France, two from Russia, eight from the United States of America, one from Iran, and one from Türkiye, all obtained from the GenBank database. The resulting phylogenetic tree revealed that most Algerian isolates, particularly Seibel and Ahmer Bou Amer, shared a close genetic affinity with European isolates, especially those originating from Russia. In contrast, the Muscat and Dattier isolates clustered with isolates from the United States of America, whereas the GFLV ALG 101 isolate grouped with a French isolate (Figure 24).

Pairwise sequence similarity analysis was conducted among the five grapevine isolates The similarity between isolates ranged from 81% to 98.99%. The highest similarity was observed between Seibel and Ahmer Bou amer (99%), while the lowest similarity was detected between ALG101RNA2 (Cherchalli) and Ahmer bou amer (82%) (Table 10).

Table 10*:* Percentage similarity between nucleotide sequences of grapevine isolates

	PP983212Muscat_Italy	**PP983213_Seibel**	**PP983214_Dattier**	**PP983215_Ahmer_Bouamer**	**PP976050.1ALG101RNA2**
PP983212Muscat_Italy	100,00%				
PP983213_Seibel	90%	100,00%			
PP983214_Dattier	92%	90%	100,00%		
PP983215_Ahmer_Bouamer	91%	90%	91%	100,00%	
PP976050.1ALG101RNA2	87%	87%	85%	82%	100,00%

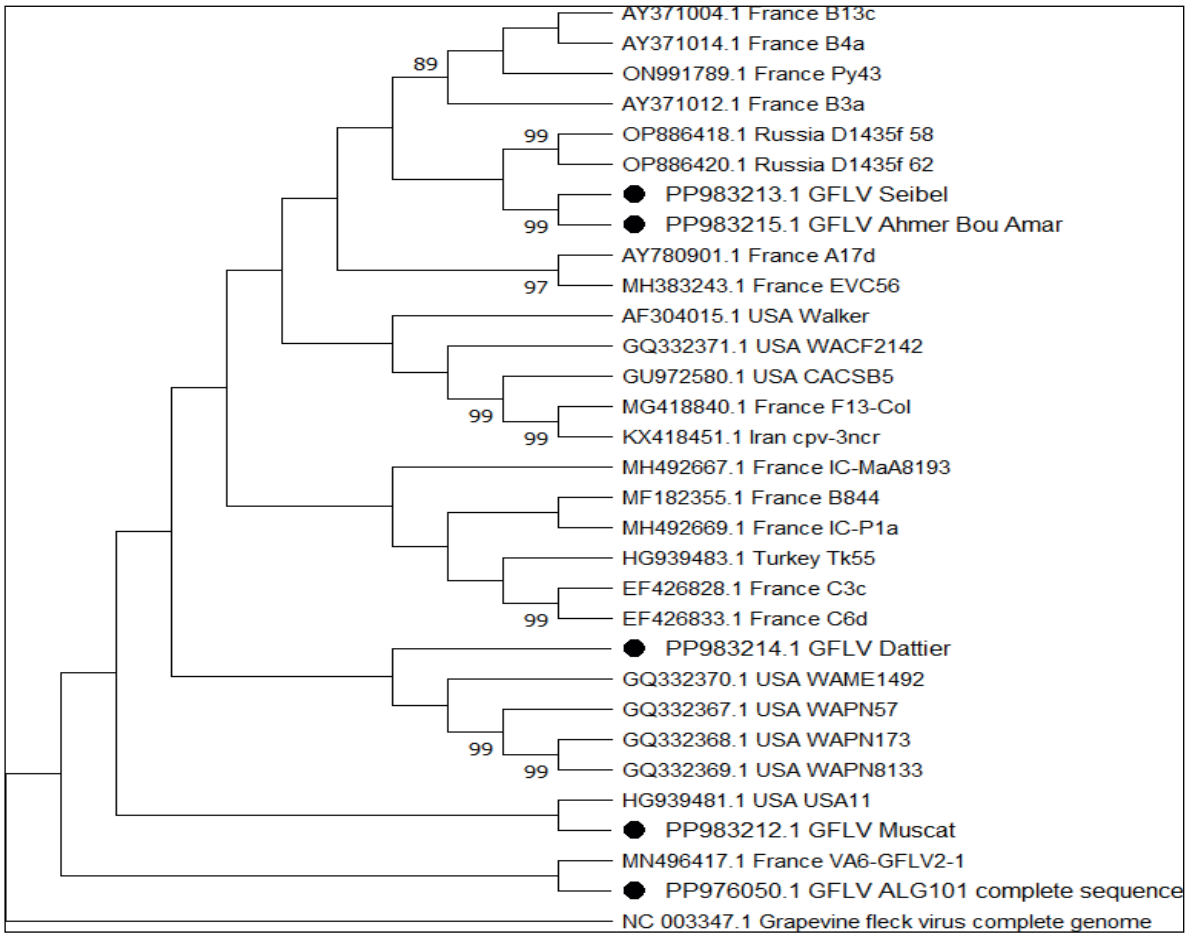


Figure 23: Phylogenetic tree of *Grapevine fanleaf virus*, constructed with sequences of a 312nt fragment of the viral capsid protein (CP), constructed using the neighbor-joining method with 1,000 bootstrap replicates. ● indicates Algerian isolates.

3.5 Discussion of molecular and phylogenetic analyses

ELISA and RT-PCR are essential methods for viral detection in grapevines. RT-PCR provides enhanced sensitivity and is especially appropriate for confirmation and multiplex assays.

Conversely, ELISA is essential for regular, large-scale screenings. The combination of both techniques may substantially enhance the precision and reliability of grapevine health management (Erilmez and Kaya, 2016; Vigne *et al.*, 2018).

In this study, the prevalence of *Grapevine fanleaf virus* (GFLV) across various regions was assessed by using the Enzyme-Linked Immunosorbent Assay (ELISA). This technique enabled us to detect and quantify GFLV in the sampled plant tissues, providing a solid preliminary analysis of the virus's distribution. After the initial screening with ELISA, Reverse Transcription Polymerase Chain Reaction (RT-PCR) was conducted on a subset of the samples. This molecular confirmation was crucial for validating the presence of GFLV, as RT-PCR

offers greater specificity and sensitivity compared to serological methods (Constable *et al.*, 2012). The subsequent sequencing of the viral RNA confirmed the virus's identity and allowed for a detailed analysis of its genetic diversity within the sampled regions.

Five GFLV isolates were selected to represent a diverse grapevine cultivars and geographic regions. Phylogenetic analyses indicated no correlation between the clustering of these isolates and their geographic origins. The Algerian isolates clustered in different clades, revealing sequence variability among each other and indicating potential differences at origins and spread of a panmictic population. The 'Seibel' and 'Ahmer Bou Amer' isolates clustered with Russian isolates, while the 'Dattier,' 'Muscat,' and GFLV ALG 101 isolates grouped with several isolates from the United States. These phylogenetic relationships indicate the high genetic diversity of Algerian GFLV isolates.

A primary aim of this study was to generate the first complete genome sequences of GFLV from Algeria using High throughput sequencing (HTS). This method is important for detecting grapevine viruses due to its elevated sensitivity and throughput, along with its ability to concurrently identify both known and novel viruses (Al Rwahnih *et al.*, 2015). Another study of Hily *et al.*, in 2018 used total RNA-seq to reconstruct complete genomes of several grapevine viruses and viroids from field samples and identified a novel Tymovirales-like virus that was consistently present in sequencing data but only sporadically detected in grapevines, suggesting infection of a grapevine-associated organism rather than the host itself. This study demonstrates the ability of HTS-based viromics to reveal both known grapevine viruses and components of the grapevine phytobiome. Vigne *et al.*, 2018 compared RNA-seq and small RNA-seq high-throughput sequencing (HTS) approaches with traditional diagnostic methods; including DAS-ELISA, RT-qPCR, IC-RT-PCR, and vsiRNA northern blot; for the detection of Grapevine fanleaf virus (GFLV) in vineyard samples. The study demonstrated that small RNA-seq is optimal for broad virome detection, whereas RNA-seq is more suitable for full-length genome assembly. Furthermore, the findings confirmed that conventional tools, particularly DAS-ELISA, remain robust; however, HTS provides added value in detecting mixed infections, discriminating viral variants, and enabling comprehensive virome characterization.

The genomic data generated in this study provide an important reference framework for future molecular and epidemiological investigations. The Algerian GFLV isolates exhibited notable genetic similarities to isolates from Russia, the United States, and France. These phylogenetic affinities may reflect historical introductions of GFLV-infected propagation material originating from diverse viticultural regions, thereby contributing to the dissemination of the virus across geographically distant grapevine populations. This proves the importance of

monitoring and controlling the movement of plant materials to prevent the further dissemination of such pathogens. Additionally, understanding these genetic connections can aid in developing targeted strategies for managing viral infections in vineyards worldwide.

4. Nematological analysis

4.1 Morphological analaysis

Morphological examination did not reveal the presence of *Xiphinema index*, the known vector of GFLV. However specimens belonging to the gene *Meloidogyne* were observed and identified based on their characteristic morphological features. Juveniles (J2) were slender with a distinct stylet (Figure 25), while adult females exhibited a pear-shaped body and a prominent perineal pattern (Figure 25-A-). These diagnostic traits are consistent with descriptions of *Meloidogyne* spp. reported in the literature, confirming the presence of this root-knot nematode in the surveyed vineyards. The morphological identification was performed in collaboration with nematology specialist Dr. Alberto Troccoli (IPSP C.N.R, Italy), whose expertise further confirmed the accuracy of the identification.

Figure 24:(A) female adult of Meloidogyne spp. (B) second stage juvenile (J2) recovered from the soil and visualized under microscope at 40X magnification.

4.2 Molecular analysis

PCR amplification of targeted ribosomal regions yielded amplicons of the expected size, approximately 800 bp for the D2–D3 expansion segments. Species identity of the isolates was confirmed by SCAR-PCR using species-specific primers: a diagnostic band of ~720 bp was produced with the SCAR-JAV primer set, while no amplification was observed with the SCAR-INCO primers (Figure 26).

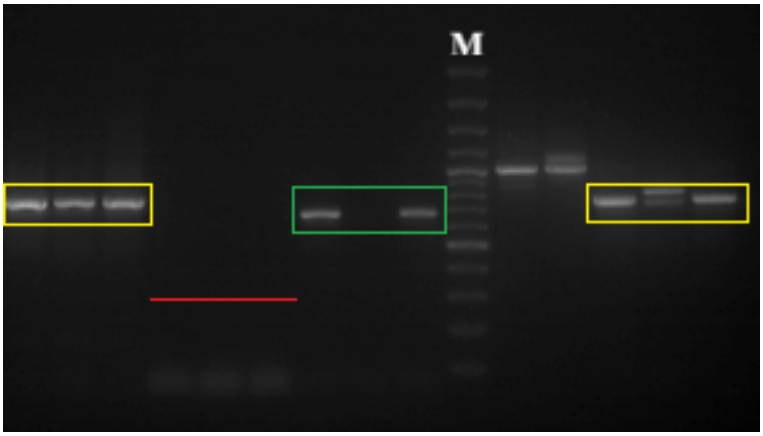


Figure 25: Agarose gel electrophoresis of PCR products obtained with different primer sets. Yellow lanes: amplification with D2–D3 primers; green lanes: amplification with SCAR-JAV; red lanes: amplification with SCAR-INCO primers; Lane M: 100bp DNA ladder

4.3 Sequencing and phylogenetic analysis

Six PCR products amplified from the D2–D3 region of the 28S rDNA were successfully sequenced bidirectionally. The resulting sequences ranged from 720 to 1100 bp in length, with high-quality chromatograms free of ambiguous base calls. BLAST analysis indicated that all sequences shared 96–100% nucleotide identity with reference sequences of the *Meloidogyne incognita* group in GenBank (Figure 27, Table 11). No significant similarity was detected with other *Meloidogyne* species.

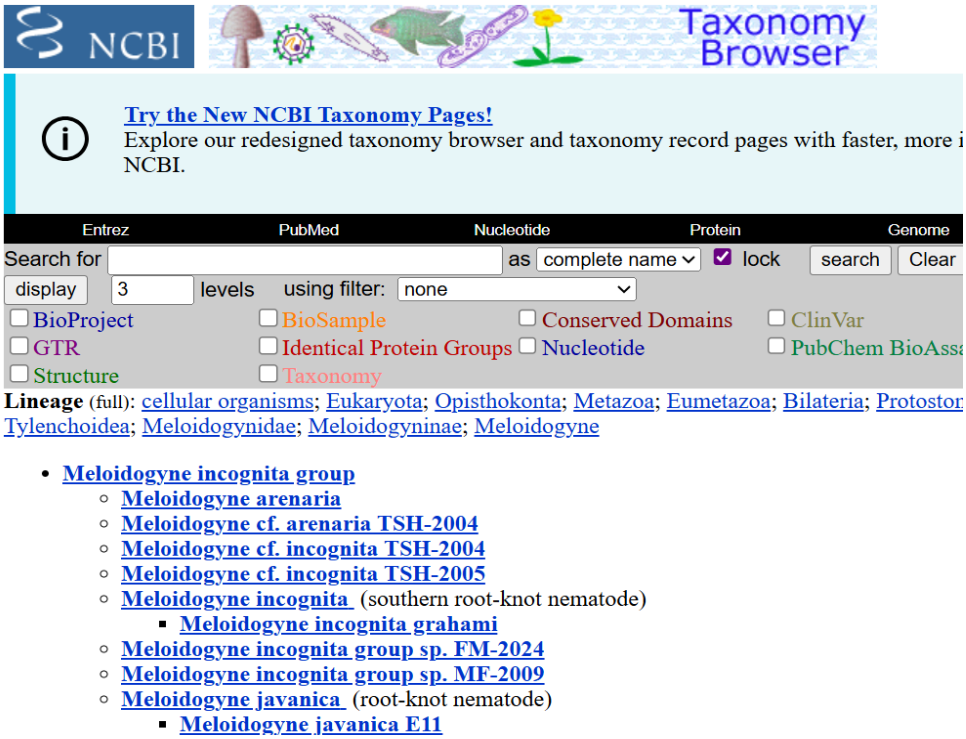


Figure 26: Taxonomic hierarchy of the *Meloidogyne incognita* group as displayed in the NCBI Taxonomy Browser, showing its relationship to closely related Meloidogyne species.

Table 11: BLAST identification of Meloidogyne isolates based on DNA sequences.

Samples	Sequence size (bp)	Similarity percentage	Species *Meloidogyne* identified with Blast	Accession numbers
5	728	99.45%	*M.arenaria*	EU364889.1
			M. incognita	MT159684.1
			M. javanica	JX100423.1
16	733	99.31%	*M.arenaria*	EU364889.1
			M. incognita	MN847618.1
			M. javanica	JX100423.1
18	1300	99.59%	*M. javanica*	OP802558.1
		99.45%	*M. incognita*	MN847618.1
		99.31%	*M. arenaria*	EU364889.1
21	767	96.56%	*M. javanica*	PV341221.1
		96.71%	*M. incognita*	MT159684.1
		96.82%	*M. arenaria*	MK026624.1
22	729	99.58%	*M. javanica*	PV341221.1
		99.17%	*M. incognita*	MT159684.1

→Continued

		99.17%	*M. arenaria*	EU364889.1
26	752	98.19%	*M. javanica*	PV341221.1
		98.06%	*M. incognita*	MT159684.1
		97.93%	*M. arenaria*	EU364889.1

4.4 Discussion of nematological analysis

The primary aim of the nematological analysis in this study was to investigate the occurrence of *Xiphinema index*, the well-known vector of *Grapevine fanleaf virus* (GFLV), in Algerian vineyards. This nematode has been reported as the principal agent responsible for the transmission of GFLV in many viticultural regions worldwide, including Europe, North Africa, and the Near East, where it plays a crucial role in the epidemiology of grapevine viral diseases(Van Ghelder *et al.*, 2015; Nguyen *et al.*, 2019; M'rabet Samaali *et al.*, 2024) . Because of its soil borne persistence and its highly specific transmission of nepoviruses, the presence of *X. index* represents a major phytosanitary threat to vineyards. Therefore, our survey initially targeted the detection and molecular confirmation of this nematode species.

Unexpectedly, no *Xiphinema* specimens were recovered from the analyzed samples. Instead, morphology and molecular identification revealed the presence of root-knot nematodes belonging to the genus *Meloidogyne*. Blast showed 96-99% identity with members of the species belonging to *Meloidogyne incognita* group, which includes *M. arenaria* and *M. javanica*. Nevertheless, the molecular evidence strongly supports the conclusion that members of *M. incognita* group are the main root-knot nematodes infecting grapevines in the surveyed Algerian vineyards. Although the using of D2D3 region does not provide sufficient resolution for the accurate identification of *Meloidogyne* species. More specific markers, such as mitochondrial COII-16S, ITS, and species-specific SCAR primers, are recommended to achieve reliable species discrimination (Fanelli *et al.*, 2017; Troccoli *et al.*, 2024).

Although root-knot nematodes (*Meloidogyne* spp.) are not vectors of *Grapevine fanleaf virus* (GFLV), their detection remains highly significant for viticulture. These nematodes are among the most destructive plant-parasitic nematodes worldwide, owing to their wide host range and ability to cause severe damage. In grapevine cultivation, *Meloidogyne* spp. are recognized as important pests, inducing root galls that impair root function , reduce plant vigor, and ultimately lead to substantial yield losses (Yang *et al.*, 2021; Ndifon, 2024).

Conclusion and perspectives

Conclusion and perspectives

This study aimed to investigate the occurrence and distribution of *Grapevine fanleaf virus* (GFLV) in Algerian vineyards. The research utilized a combination of symptomatology, serological tests, molecular analyses, nematological studies, and High Throughput sequencing (HTS) techniques. Field symptomatology assessments revealed typical GFLV-associated symptoms, including leaf malformations, fan-shaped leaves, mosaic patterns, and shortened internodes, with intensity varying among cultivars and regions. These visual observations provided the evidence of a viral presence in the surveyed vineyards.

Serological detection by DAS-ELISA established an overall GFLV infection rate of approximately 20%, with significant regional and varietal variation. The central region recorded the highest infection frequency (27%), whereas vineyards from the southern region were free of the virus. Cultivar-based evaluation allowed classification into three groups: highly susceptible cultivars (Ahmer Bouameur, Alicante), moderately susceptible cultivars, and those with low or negligible infection rates.

Molecular analysis by using RT-PCR confirmed the results of the serological test. The phylogenetic analysis of Algerian GFLV isolates revealed their close relationship with isolates from other regions, indicating potential pathways for virus spread and adaptation. This information is crucial for understanding the epidemiology of the virus and developing effective management strategies.

A key achievement of this study was the generation of the first complete genome of GFLV in Algeria by using the HTS approach. The resulting data will provide a valuable reference for future molecular and epidemiological studies. In addition to GFLV, other pathogen data were identified using this methods and will be presented separately in another publication. These results underscore the importance of HTS in revealing undetected viral diversity and enhancing traditional diagnostic methods by providing a more thorough comprehension of viral populations, thereby facilitating the development of management strategies in agricultural practices.

The nematological analysis aimed to detect *Xiphinema index* as a potential vector of GFLV. Although, *X. index* , was not found the results revealed the presence of *Meloidogyne incognita*, a nematode species of significant agronomic importance. This finding indicate the diversity of nematode-related threats to viticulture in Algeria and emphasizes the need to consider both virus-vector nematodes and direct root parasites in vineyard health management. Further studies are essential to map the distribution of these root-knot nematodes across various grape-

growing regions, evaluate their impacts on vine productivity, and develop integrated strategies for its control.

In summary, this study represents the first comprehensive characterization of GFLV in Algeria. By integrating field observations with serological, molecular, nematological, HTS, and phylogenetic analyses, it provides a holistic understanding of the virus's prevalence, distribution, and associated vineyard constraints. The findings emphasis the urgent need for sanitary certification of grapevine material, systematic virus monitoring, and integrated management approaches to ensure the sustainability of Algerian viticulture.

Perspectives

Building upon these findings, several future research avenues are proposed:

- **Symptom–virus association:** Establishing a correlation between symptoms severity and viral load, mixed infections, and cultivar susceptibility. Such correlation can offer Insights into more how different plant cultivars respond to viral threats and guide breeders in developing more resilient varieties. Understanding these dynamics is crucial for implementing effective management strategies in agriculture.
- **Vector identification and investigation the role of other nematodes:** Expanding nematode surveys to confirm the presence or absence of *Xiphinema index* and to investigate the role of other nematode species in virus ecology. This comprehensive approach will improve our understanding of nematode interactions and their potential impact on viral spread, ultimately facilitating the development of more effective management strategies. Furthermore, it will provide valuable insights into the ecological dynamics of these organisms within their environments.
- **Resistance and tolerance screening:** Evaluation and cultivating grapevine varieties exhibiting resistance or tolerance to GFLV and nematodes. This approach involves assessing several grapevine cultivars to determine which exhibit favourable characteristics for production in challenging conditions. By selecting and breeding these cultivars, researchers aim to enhance the resilience of grapevines, leading to improved yields and quality in the face of pests and diseases.
- **High throughput sequencing**: The integration of high throughput sequencing (HTS) technologies in future research will be a crucial step toward advancing the molecular understanding of grapevine viruses in Algeria. The complete genome sequencing of

additional GFLV isolates using HTS will allow an in-depth characterization of their genetic variability, evolutionary dynamics, and phylogenetic relationships with global strains. Furthermore, HTS should be applied to conduct comprehensive virome studies in Algerian vineyards to identify the full spectrum of present viruses and viroids, including novel pathogens.

References

References

Aballay, E., Prodan, S., Correa, P., & Allende, J. (2020). Assessment of rhizobacterial consortia to manage plant-parasitic nematodes of grapevine. *Crop Protection, 131*, 105103. https://doi.org/10.1016/j.cropro.2020.105103

Adams, A. N., & Clark, M. F. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology, 34*(3), 475–483. https://doi.org/10.1099/0022-1317-34-3-475

Adams, I. P., Glover, R. H., Monger, W. A., Mumford, R., Jackeviciene, E., Navalinskiene, M., Samuitiene, M., & Boonham, N. (2009). Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. *Molecular Plant Pathology, 10*(4), 537–545. https://doi.org/10.1111/j.1364-3703.2009.00545.x

Agarwal, A., Cunningham, J. P., Valenzuela, I., & Blacket, M. J. (2020). A diagnostic LAMP assay for the destructive grapevine insect pest, phylloxera (*Daktulosphaira vitifoliae*). *Scientific Reports, 10*, 21229. https://doi.org/10.1038/s41598-020-77928-9

Aiter, N., Lehad, A., Haddad, B., Taibi, A., Meziani, S., Rabhi, M., Khelifi, L., & Chaouia, C. (2020). Sanitation of autochthonous grapevine varieties from Algeria by chemotherapy. *Acta Phytopathologica et Entomologica Hungarica, 55*(1), 43–50. https://doi.org/10.1556/038.55.2020.013

Al Rwahnih, M., Alabi, O. J., Hwang, M. S., Tian, T., Mollov, D., & Golino, D. (2021). Characterization of a new nepovirus infecting grapevine. *Plant Disease, 105*(5), 1432–1439. https://doi.org/10.1094/PDIS-08-20-1831-RE

Al Rwahnih, M., Daubert, S., Golino, D., Islas, C., & Rowhani, A. (2015). Comparison of next-generation sequencing versus biological indexing for the optimal detection of viral pathogens in grapevine. *Phytopathology, 105*(6), 758–763. https://doi.org/10.1094/PHYTO-06-14-0165-R

Allaya, M., & Abaab, A. (Eds.). (2006). *Directory of infectious diseases of grapevines and viroses and virus-like diseases of the grapevine: Bibliographic report 1998–2004*. CIHEAM.

Almasi, M. (2015). Establishment and application of a reverse transcription loop-mediated isothermal amplification assay for detection of grapevine fanleaf virus. *Molecular Biology, 5*, Article 1000149. https://doi.org/10.4172/2168-9547.1000149.

Ammad, F., Benchabane, M., & Toumi, M. (2014). Diversity of Fungal Trunk Pathogens Associated with Grapevine Dieback of Grapevine in Algeria. *Jordan Journal of Biological Sciences, 7*(1), 35–39. https://doi.org/10.12816/0008211.

Andret-Link, P., Laporte, C., Valat, L., Ritzenthaler, C., Demangeat, G., Vigne, E., Laval, V., Pfeiffer, P., Stussi-Garaud, C., & Fuchs, M. (2004). GRAPEVINE FANLEAF VIRUS: STILL A MAJOR THREAT TO THE GRAPEVINE INDUSTRY. *Journal of Plant Pathology*, 14.

Andret-Link, P., Schmitt-Keichinger, C., Demangeat, G., Komar, V., & Fuchs, M. (2004). The specific transmission of Grapevine fanleaf virus by its nematode vector Xiphinema index is solely determined by the viral coat protein. *Virology, 320*(1), 12–22. https://doi.org/10.1016/j.virol.2003.11.022

Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & Castillo, P. (2016). Unravelling the biodiversity and molecular phylogeny of needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and description of six new species. *PLOS ONE, 11*(2), e0147689. https://doi.org/10.1371/journal.pone.0147689

Arkam, M., Alves, A., Lopes, A., Čechová, J., Pokluda, R., Eichmeier, A., Zitouni, A., Mahamedi, A. E., & Berraf-Tebbal, A. (2021). Diversity of Botryosphaeriaceae causing grapevine trunk diseases and their spatial distribution under different climatic conditions in Algeria. *European Journal of Plant Pathology*, *161*(4), 933–952. https://doi.org/10.1007/s10658-021-02377-7

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C., & Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. *Frontiers in Plant Science, 7*, Article 382. https://doi.org/10.3389/fpls.2016.00382

Askary, T. H., Khalil, A., Nazir, N., Khan, A. A., & Banday, S. A. (2018). Nematode Parasites of Grapevines. In E. Lichtfouse (Ed.), *Sustainable Agriculture Reviews 31* (Vol. 31, pp. 389–423). Springer International Publishing. https://doi.org/10.1007/978-3-319-94232-2_7

Bachir, A., Selmi, I., Lehad, A., Louanchi, M., & Mahfoudhi, N. (2019). The occurrence of grapevine rugose wood disease in Algeria. *Acta Phytopathologica et Entomologica Hungarica, 54*(2), 221–227. https://doi.org/10.1556/038.54.2019.023

Bagi, F., Barać, G., Iličić, R., Savić, Z., Starovlah, M., Konstantin, Đ., & Popović, T. (2022). Incidence of Grapevine Fanleaf Virus (GFLV) and Grapevine Leafroll-Associated Viruses (GLRaV 1−3) in Vojvodina Province. *Contemporary Agriculture, 71*(1–2), 102–109. https://doi.org/10.2478/contagri-2022-0015

Barba, M., Czosnek, H., & Hadidi, A. (2014). Historical Perspective, Development and Applications of Next-Generation Sequencing in Plant Virology. *Viruses, 6*(1), 106–136. https://doi.org/10.3390/v6010106

Basso, M. F., Fajardo, T. V. M., & Saldarelli, P. (2017). Grapevine virus diseases: Economic impact and current advances in viral prospection and management. *Revista Brasileira de Fruticultura, 39*, e-411. https://doi.org/10.1590/0100-29452017411

Belkacem, N. E. H., Ammad, F., Berdja, R., & Bouziane, M. N. (2025). Susceptibility assessment of native and foreign grapevine varieties and rootstocks toward *Botryosphaeria* dieback: An Algerian comparative case study. *Journal of Plant Diseases and Protection, 132*, 1–10. https://doi.org/10.1007/s41348-025-01059-4

Benjama, A., Boubaker, A., Khlief, H., Krimi, Z., Nesme, X., López, M., & Zoina, A. (2002). Susceptibility of stone-fruit rootstocks, rose and grapevine to *Agrobacterium radiobacter* var. *Tumefaciens* in Arab Mediterranean countries. *EPPO Bulletin, 32*(3), 463–466. https://doi.org/10.1046/j.1365-2338.2002.00589.x

Bissaad, F. Z., Bounaceur, F., & Doumandji-Mitichet, B. (2017). Dynamique spatio-temporelle de *Planococcus ficus* (Signoret, 1875) dans les vignobles de la Mitidja (Algeria). *Lebanese Science Journal, 18*, 26–46. https://doi.org/10.22453/LSJ-018.1.026046

Botineau. (2010). *Botanique systématique et appliquée des plantes à fleurs*. Lavoisier.

Brister, J. R., Ako-adjei, D., Bao, Y., & Blinkova, O. (2015). NCBI Viral Genomes Resource. *Nucleic Acids Research, 43*(D1), D571–D577. https://doi.org/10.1093/nar/gku1207

Bruisson, S., Lebel, S., Walter, B., Prévotat, L., Seddas, S., & Schellenbaum, P. (2017). Comparative detection of a large population of grapevine viruses by TaqMan RT-qPCR and ELISA. *Journal of Virological Methods, 240*, 73–77. https://doi.org/10.1016/j.jviromet.2016.12.003

Buja, I., Sabella, E., Monteduro, A. G., Rizzato, S., Bellis, L. D., Elicio, V., Formica, L., Luvisi, A., & Maruccio, G. (2022). Detection of Ampelovirus and Nepovirus by Lab-on-a-Chip: A Promising Alternative to ELISA Test for Large Scale Health Screening of Grapevine. *Biosensors*, *12*(3), 147. https://doi.org/10.3390/bios12030147

Butiuc-Keul, A., & Coste, A. (2023). Biotechnologies and strategies for grapevine improvement. *Horticulturae, 9*(1), 62. https://doi.org/10.3390/horticulturae9010062

Calzarano, F., Pagnani, G., Pisante, M., Bellocci, M., Cillo, G., Metruccio, E. G., & Di Marco, S. (2021). Factors Involved on Tiger-Stripe Foliar Symptom Expression of Esca of Grapevine. *Plants*, *10*(6), 1041. https://doi.org/10.3390/plants10061041

Canchignia, H., Altimira, F., Montes, C., Sánchez, E., Tapia, E., Miccono, M., Espinoza, D., Aguirre, C., Seeger, M., & Prieto, H. (2017). Candidate nematicidal proteins in a new *Pseudomonas veronii* isolate identified by its antagonistic properties against *Xiphinema index*. *The Journal of General and Applied Microbiology*, *63*(1), 11–21. https://doi.org/10.2323/jgam.2016.07.001

Castillo, G., & Bello-Bedoy, R. (2022). Effect of *Planococcus ficus* (Signoret) on the growth of three wine grape cultivars. *Revista Fitotecnia Mexicana*, *45*(2), 227. https://doi.org/10.35196/rfm.2022.2.227

Čepin, U., Gutiérrez-Aguirre, I., Ravnikar, M., & Pompe-Novak, M. (2016). Frequency of occurrence and genetic variability of grapevine fanleaf virus satellite RNA. *Plant Pathology, 65*, 510–520. https://doi.org/10.1111/ppa.12428

Cigsar, I., Digiaro, M., Gokalp, K., Ghanem-Sabanadzovic, N. A., De Stradis, A., Boscia, D., & Martelli, G. P. (2003). Grapevine deformation virus, a novel nepovirus from turkey. *Journal of Plant Pathology*, *85*(3), 183–191.

National Academies of Sciences, Engineering, and Medicine. (2025). *Advancing vineyard health: Insights and innovations for combating grapevine red blotch and leafroll diseases*. National Academies Press.

Constable, F. E., Connellan, J., Nicholas, P., & Rodoni, B. C. (2012). Comparison of enzyme-linked immunosorbent assays and reverse transcription–polymerase chain reaction for the reliable detection of Australian grapevine viruses in two climates during three growing seasons. *Australian Journal of Grape and Wine Research, 18*, 239–244. https://doi.org/10.1111/j.1755-0238.2012.00188.x

Courty, P.-E., Auclair, A., Hilly, J.-M., Durney, C., Sportes, A., Jacquens, L., Mercier, L., Vigne, E., Wipf, D., Mondy, S., & Trouvelot, S. (2024). Influence of the fanleaf virus on the grapevine root holobiont: the hidden side of a viral decline. *OIV, PO 105*.

Crnogorac, A., Panno, S., Mandic, A., Gašpar, M., Caruso, A. G., Noris, E., Davino, S., & Matic, S. (2021). Survey of five major grapevine viruses infecting Blatina and Žilavka cultivars in Bosnia and Herzegovina. *PLoS ONE*, *16*(1 January). Scopus. https://doi.org/10.1371/journal.pone.0245959.

D'Addabbo, T., Ladurner, E., & Troccoli, A. (2023). Nematicidal activity of a garlic extract formulation against the grapevine nematode *Xiphinema index*. *Plants, 12*(4), 739. https://doi.org/10.3390/plants12040739

Daragó, Á., Szabó, M., Hrács, K., Takács, A., & Nagy, P. (2013). In vitro investigations on the biological control of *Xiphinema index* with *Trichoderma* species. *Helminthologia, 50*(2), 132–137. https://doi.org/10.2478/s11687-013-0121-7

De Luca, F., Fanelli, E., Di Vito, M., Reyes, A., & De Giorgi, C. (2004). Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in populations and species of *Pratylenchus* from the Mediterranean region. *European Journal of Plant Pathology, 110*, 949–957. https://doi.org/10.1007/s10658-004-0813-4

De Souza, J. O., Klaassen, V., Stevens, K., Erickson, T. M., Heinitz, C., & Al Rwahnih, M. (2024). Characterization of genetic diversity in the capsid protein gene of grapevine fleck virus

and development of a new real-time RT-PCR assay. *Viruses, 16*(9), 1457. https://doi.org/10.3390/v16091457

Del Frari, G., Oliveira, H., & Boavida Ferreira, R. (2021). White rot fungi (Hymenochaetales) and esca of grapevine: Insights from recent microbiome studies. *Journal of Fungi, 7*(9), 770. https://doi.org/10.3390/jof7090770

Demangeat, G. (2007). Transmission des nepovirus par les nématodes Longidoridae. *Virologie, 11*, Article 102. https://doi.org/10.1684/vir.2007.0102

Demangeat, G., Voisin, R., Minot, J.-C., Bosselut, N., Fuchs, M., & Esmenjaud, D. (2005). Survival of *Xiphinema index* in vineyard soil and retention of grapevine fanleaf virus over extended time in the absence of host plants. *Phytopathology, 95*(10), 1151–1156. https://doi.org/10.1094/PHYTO-95-1151

Di Serio, F., Izadpanah, K., Hajizadeh, M., & Navarro, B. (2017). Viroids infecting the grapevine. In B. Meng, G. P. Martelli, D. A. Golino, & M. Fuchs (Eds.), *Grapevine viruses: Molecular biology, diagnostics and management* (pp. 373–392). Springer International Publishing.

Diaz-Lara, A., Stevens, K., Aguilar-Molina, V. H., Fernández-Cortés, J. M., Chabacano León, V. M., De Donato, M., Sharma, A., Erickson, T. M., & Al Rwahnih, M. (2023). High-Throughput Sequencing of Grapevine in Mexico Reveals a High Incidence of Viruses including a New Member of the Genus Enamovirus. *Viruses, 15*(7), 1561. https://doi.org/10.3390/v15071561

Digiaro, M., Elbeaino, T., & Martelli, G. P. (2017). Grapevine fanleaf virus and other Old World nepoviruses. In B. Meng, G. P. Martelli, D. A. Golino, & M. Fuchs (Eds.), *Grapevine viruses: Molecular biology, diagnostics and management* (pp. 47–82). Springer International Publishing.

Djennane, S., Prado, E., Dumas, V., Demangeat, G., Gersch, S., Alais, A., Gertz, C., Beuve, M., Lemaire, O., & Merdinoglu, D. (2021). A single resistance factor to solve vineyard degeneration due to grapevine fanleaf virus. *Communications Biology, 4*(1), 637. https://doi.org/10.1038/s42003-021-02164-4

Donda, B. P., Kesoju, S. R., Arnold, K., McRoberts, N., & Naidu, R. A. (2023). Spatio-temporal spread of grapevine leafroll disease in Washington State vineyards. *Plant Disease, 107*(5), 1471–1480. https://doi.org/10.1094/PDIS-04-22-0939-RE

El Aou-ouad, H., Montero, R., Baraza, E., & Bota, J. (2022). Recovering ancient grapevine cultivars in the Balearic Islands: Sanitary status evaluation and virus elimination. *Plants, 11*(13), 1754. https://doi.org/10.3390/plants11131754

El Sayed, T., Saker, K., El Shorbagy, A., Mandour, A., Alajouz, M., & Soliman, A. (2023). Characterization and elimination of grapevine fanleaf virus using thermotherapy in combination with meristem tip culture. *Egyptian Academic Journal of Biological Sciences, C: Physiology and Molecular Biology, 15*(2), 101–117. https://doi.org/10.21608/eajbsc.2023.312619

Erilmez, S., & Kaya, A. (2016). Comparison of DAS-ELISA and RT-PCR methods for the diagnosis of grapevine viruses. *Bitki Koruma Bülteni, 56*, 297–302.

Esmenjaud, D., Demangeat, G., Van Helden, M., & Ollat, N. (2014). Advances in biology, ecology and control of *Xiphinema index*, the nematode vector of grapevine fanleaf virus. *Acta Horticulturae, 1045*, 67–73. https://doi.org/10.17660/ActaHortic.2014.1045.9.

Esmenjaud, D., Kunztmann, P., Schwaerzler, F., Lemaire, O., Ollat, N., & Demangeat, G. (2013). Caractérisation et choix des nématodes vecteurs Xiphinema pour la sélection d'une résistance durable à la maladie du court-noué de la vigne. *Innovations Agronomiques*, *27*, 9–22.

Esmenjaud, D., Banora, M. Y., Julien-Portier, U., Lafargue, M.-D.-D., Tandonnet, J.-P., Rubio, B., Walker, A., Van Ghelder, C., & Ollat, N. (2023). A muscadine locus confers resistance to predominant species of grapevine root-knot nematodes (*Meloidogyne* spp.) including virulent populations. *OENO One*, *57*(3), 219–232. https://doi.org/10.20870/oeno-one.2023.57.3.7443

Evangelou, V., Lytra, I., Krokida, A., Antonatos, S., Georgopoulou, I., Milonas, P., & Papachristos, D. P. (2023). Insights into the Diversity and Population Structure of Predominant Typhlocybinae Species Existing in Vineyards in Greece. *Insects, 14*(11), 894. https://doi.org/10.3390/insects14110894

Everaert, E. A., Viaene, N., Quataert, P., Haegeman, A., & De Jonghe, K. (2024). Towards improved nepovirus detection and identification in *Xiphinema* nematodes. *PhytoFrontiers*. https://doi.org/10.1094/PHYTOFR-03-24-0018-R

Faist, H., Ankenbrand, M. J., Sickel, W., Hentschel, U., keller, A., & Deeken, R. (2023). Opportunistic Bacteria of Grapevine Crown Galls Are Equipped with the Genomic Repertoire for Opine Utilization. *Genome Biol Evol*, *15*(12), 2–17. https://doi.org/10.1093/gbe/evad228.

Fanelli, E., Cotroneo, A., Carisio, L., Troccoli, A., Grosso, S., Boero, C., Capriglia, F., & De Luca, F. (2017). Detection and molecular characterization of the rice root-knot nematode Meloidogyne graminicola in Italy. *European Journal of Plant Pathology*, *149*(2), 467–476. https://doi.org/10.1007/s10658-017-1196-7

FAOSTAT. (n.d.). *Crops and livestock products*. Food and Agriculture Organization of the United Nations. Retrieved August 12, 2024, from https://www.fao.org/faostat/en/#data/QCL/visualize

Fayek, M. A., Jomaa, A. H., & Shalaby, A.-B. A. (2009). Meristem tip culture for in vitro eradication of grapevine leafroll-associated virus-1 (GLRaV-1) and grapevine fanleaf virus (GFLV) from infected Flame Seedless grapevine plantlets. *Iniciación a la Investigación*.

Ferris, H., Zheng, L., & Walker, M. A. (2012). Resistance of grape rootstocks to plant-parasitic nematodes. *Journal of Nematology*, *44*(4), 377–386.

Flegg, J. J. M. (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology*, *60*(3), 429–437. https://doi.org/10.1111/j.1744-7348.1967.tb04497.x.

Fröbel, S., & Zyprian, E. (2019). Colonization of different grapevine tissues by *Plasmopara viticola*: A histological study. *Frontiers in Plant Science*, *10*, 951. https://doi.org/10.3389/fpls.2019.00951

Frolov, I. G., Aubakirova, K. P., Bakytzhanova, Z. N., Rakhatkyzy, A., Yerbolova, L. S., & Galiakparov, N. N. (2025). Phylogenetic Analysis of Grapevine Fanleaf Virus, Grapevine Virus A, and Grapevine Leafroll-Associated Virus 3 in Kazakhstan. *Microorganisms*, *13*(9), 2142. https://doi.org/10.3390/microorganisms13092142

Fuchs, M. (2020). Grapevine viruses: A multitude of diverse species with simple but overall poorly adopted management solutions in the vineyard. *Journal of Plant Pathology, 102*, 643–653. https://doi.org/10.1007/s42161-020-00579-2

Fuchs, M. (2024). Grapevine viruses: Did you say more than a hundred? *Journal of Plant Pathology, 107*, 217–227. https://doi.org/10.1007/s42161-024-01819-5

Fuchs, M., Schmitt-Keichinger, C., & Sanfaçon, H. (2017). A renaissance in nepovirus research provides new insights into their molecular interface with hosts and vectors. In K. Maramorosch, F. A. Murphy, & A. J. Shatkin (Eds.), *Advances in Virus Research* (Vol. 99, pp. 61–105). Elsevier.

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C., & Milgroom, M. G. (2012). Grapevine powdery mildew (*Erysiphe necator*): A fascinating system for the study of the biology, ecology, and epidemiology of an obligate biotroph. *Molecular Plant Pathology, 13*(1), 1–16. https://doi.org/10.1111/j.1364-3703.2011.00728.x

Gaire, F., Schmitt, C., Stussi-Garaud, C., Pinck, L., & Ritzenthaler, C. (1999). Protein 2A of grapevine fanleaf nepovirus is implicated in RNA2 replication and colocalizes to the replication site. *Virology*, *264*(1), 25–36. https://doi.org/10.1006/viro.1999.9927

Gambino, G., Perrone, I., & Gribaudo, I. (2008). A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis, 19*(6), 520–525. https://doi.org/10.1002/pca.1078

Gelvin, S. B. (Ed.). (2018). *Agrobacterium biology: From basic science to biotechnology*. Springer International Publishing.

Gharate, P. S., Somkuwar, R. G., Waghmare, G. M., Gobade, N. J., Thutte, A. S., & Ausari, P. K. (2025). Management of abiotic stress in grapes (Vitis vinifera)—A review. *Current Horticulture*, *13*(3–13). https://doi.org/252010.5958/2455-7560.2025.00033.5

Gholampour, Z., Zakiaghl, M., Asquini, E., Moser, M., Gualandri, V., Mehrvar, M., & Si-Ammour, A. (2024). Application of High-Throughput Sequencing for Comprehensive Virome Profiling in Grapevines Shows Yellows in Iran. *Viruses*, *16*(2), Article 2. https://doi.org/10.3390/v16020204

Giménez-Romero, A., Galván, J., Montesinos, M., Bauzà, J., Godefroid, M., Fereres, A., Ramasco, J. J., Matías, M. A., & Moralejo, E. (2022). Global predictions for the risk of establishment of Pierce's disease of grapevines. *Communications Biology*, *5*(1), 1389. https://doi.org/10.1038/s42003-022-04358-w

Gottula, J., Lapato, D., Cantilina, K., Saito, S., Bartlett, B., & Fuchs, M. (2013). Genetic variability, evolution, and biological effects of *Grapevine fanleaf virus* satellite RNAs. *Phytopathology*, *103*(11), 1180–1187. https://doi.org/10.1094/PHYTO-11-12-0310-R

Guţă, I. C., Buciumeanu, E. C., Tătaru, L. D., & Topală, C. M. (2017). Regeneration of grapevine virus-free plants by in vitro chemotherapy. *Acta Horticulturae, 1188*, 319–322. https://doi.org/10.17660/ActaHortic.2017.1188.42

Habili, N., Wu, Q., Rinaldo, A., & Constable, F. (2023). A chronological study on grapevine leafroll-associated virus 2 in Australia. *Viruses, 15*(5), 1105. https://doi.org/10.3390/v15051105

Hajizadeh, M., Torchetti, E. M., Sokhandan-Bashir, N., Navarro, B., Doulati-Baneh, H., & Di Serio, F. (2015). Grapevine viroids and *Grapevine fanleaf virus* in north-west Iran. *Journal of Plant Pathology, 97*(3), 489–495.

Hao, Z., Fayolle, L., Van Tuinen, D., Chatagnier, O., Li, X., & Vivienne, G.-P. (2012). Local and systemic mycorrhiza-induced protection against the ectoparasitic nematode *Xiphinema index* involves priming of defence gene responses in grapevine. *Journal of Experimental Botany*, *63*(10), 3657–3672. https://doi.org/10.1093/jxb/ers046

Hed, B., & Centinari, M. (2024). Mechanical leaf removal for improved *Botrytis* bunch rot control in *Vitis vinifera* 'Pinot gris' and 'Pinot noir' grapevines in the northeastern United States. *Plant Disease*, *108*(11), 3156–3162. https://doi.org/10.1094/PDIS-02-24-0383-RE

Hemmer, C., Djennane, S., Ackerer, L., Hleibieh, K., Marmonier, A., & Ritzenthaler, C. (2018). Nanobody-mediated resistance to *Grapevine fanleaf virus* in plants. *Plant Biotechnology Journal*, *16*(3), 660–671. https://doi.org/10.1111/pbi.12819

Hily, J.-M., Candresse, T., Garcia, S., Vigne, E., Tannière, M., & Lemaire, O. (2018). High-throughput sequencing and the viromic study of grapevine leaves: From the detection of

grapevine-infecting viruses to the description of a new environmental *Tymovirales* member. *Frontiers in Microbiology, 9*, 1782. https://doi.org/10.3389/fmicb.2018.01782

Isnard, H. (1969). *L'Algérie ou la décolonisation difficile*. *Méditerranée, 10*(2), 1–18. https://doi.org/10.3406/medit.1969.1325

Ito, T., & Nakaune, R. (2016). Molecular characterization of a novel putative ampelovirus tentatively named grapevine leafroll-associated virus 13. *Archives of Virology, 161*(9), 2555–2559. https://doi.org/10.1007/s00705-016-2914-8

Jarrell, K. R., Rebek, E. J., Wayadande, A. C., & Giles, K. L. (2020). Biology, ecology, and management of eastern grape leafhopper (*Hemiptera*: Cicadellidae), a key pest of vineyards in North America. *Journal of Integrated Pest Management, 11*(1), 6. https://doi.org/10.1093/jipm/pmaa003

Ji, W., Han, K., Lu, Y., & Wei, J. (2020). Predicting the potential distribution of the vine mealybug, *Planococcus ficus*, under climate change by MaxEnt. *Crop Protection, 137*, 105268. https://doi.org/10.1016/j.cropro.2020.105268

Jones, J. T., Haegeman, A., Danchin, E. G. J., Gaur, H. S., Helder, J., & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology, 14*(9), 946–961. https://doi.org/10.1111/mpp.12057

Kanapiya, A., Amanbayeva, U., Tulegenova, Z., Abash, A., Zhangazin, S., & Mukiyanova, G. (2024). Recent advances and challenges in plant viral diagnostics. *Frontiers in Plant Science, 15*, 1451790. https://doi.org/10.3389/fpls.2024.1451790

Kearse, M., Sturrock, S., & Meintjes, P. (2012a). The Geneious 6.0.3 read mapper. *Bioinformatics, 28*(12), 1647–1649.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., & Drummond, A. (2012b). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kellner, N., Antal, E., Szabó, A., & Matolcsi, R. (2022). The effect of black rot on grape berry composition. *Acta Alimentaria, 51*(1), 126–133. https://doi.org/10.1556/066.2021.00195

Kenfaoui, J., Radouane, N., Mennani, M., Tahiri, A., El Ghadraoui, L., & Barka, E. A. (2022). A panoramic view on grapevine trunk diseases threats: Case of Eutypa dieback, Botryosphaeria dieback, and Esca disease. *Journal of Fungi, 8*(6), 595. https://doi.org/10.3390/jof8060595

Khan, N., Fahad, S., Naushad, M., & Faisal, S. (2020). Grape production: Critical review in the world. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.3595842

Khan, M. R. (Ed.). (2023). *Novel biological and biotechnological applications in plant nematode management*. Springer Nature Singapore. https://doi.org/10.1007/978-981-99-2893-4

Khassein, A., Suleimanova, G., Malakhova, N., & Kizildeniz, T. (2024). Grapes recovery from *Grapevine fanleaf virus* by chemotherapy using salicylic acid. *Research on Crops, 25*(4), 605–610.

Koledenkova, K., Esmaeel, Q., Jacquard, C., Nowak, J., Clément, C., & Ait Barka, E. (2022). *Plasmopara viticola*, the causal agent of downy mildew of grapevine: From its taxonomy to disease management. *Frontiers in Microbiology, 13*, 889472. https://doi.org/10.3389/fmicb.2022.889472

Komínková, M., Ben Mansour, K., Komínek, P., Brožová, J., & Střalková, R. (2024). Multiple infections with viruses of the family *Tymoviridae* in Czech grapevines. *Viruses, 16*(3), 343. https://doi.org/10.3390/v16030343

Konup, L., Riabyi, M., Nikolaeva, N., Konup, A., Chystiakova, V., & Vlasov, V. (2025). Grapevine fanleaf degeneration disease (GFDD) in vineyards in the Odesa region: Diagnosis and prospects for recovery by heat treatment. *Karantin i Zakhyst Roslyn, 1*, 14–19. https://doi.org/10.36495/2312-0614.2025.1.14-19

Krebelj, A. J., Čepin, U., Ravnikar, M., & Novak, M. P. (2015). Spatio-temporal distribution of *Grapevine fanleaf virus* (GFLV) in grapevine. *European Journal of Plant Pathology, 142*(1), 159–171. https://doi.org/10.1007/s10658-015-0600-4.

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small

RNAs: A generic method for diagnosis, discovery and sequencing of viruses. *Virology*, *388*(1), 1–7. https://doi.org/10.1016/j.virol.2009.03.024

Křižan, B., Ondrušiková, E., Holleinová, V., Moravcová, K., & Bláhová, L. (2009). Elimination of *Grapevine fanleaf virus* in grapevine by in vivo and in vitro thermotherapy. *Horticultural Science*, *36*(3), 105–108. https://doi.org/10.17221/37/2008-HORTSCI

Kubina, J., Hily, J.-M., Mustin, P., Komar, V., Garcia, S., & Vigne, E. (2022). Characterization of *Grapevine fanleaf virus* isolates in 'Chardonnay' vines exhibiting severe and mild symptoms in two vineyards. *Viruses*, *14*(10), 2303. https://doi.org/10.3390/v14102303

Kunova, A., Pizzatti, C., Saracchi, M., Pasquali, M., & Cortesi, P. (2021). Grapevine powdery mildew: Fungicides for its management and advances in molecular detection of markers associated with resistance. *Microorganisms*, *9*(7), 1541. https://doi.org/10.3390/microorganisms9071541.

Kyrkou, I., Pusa, T., Ellegaard-Jensen, L., Sagot, M.-F., & Hansen, L. H. (2018). Pierce's disease of grapevines: A review of control strategies and an outline of an epidemiological model. *Frontiers in Microbiology*, *9*, 2141. https://doi.org/10.3389/fmicb.2018.02141

Levadoux, L., Benabderrabou, A., & Douaouri, B. (1971). *Ampélographie Algérienne Cépage de cuve te de table cultivés en Algérie*. Société Nationale d'édition et de diffusion 3

Li, Z., Dos Santos, R. F., Gao, L., Chang, P., & Wang, X. (2021). Current status and future prospects of grapevine anthracnose caused by *Elsinoe ampelina*: An important disease in humid grape-growing regions. *Molecular Plant Pathology*, *22*(7), 899–910. https://doi.org/10.1111/mpp.13076

Lopez Pinar, A., Rauhut, D., Ruehl, E., & Buettner, A. (2016). Effects of *Botrytis cinerea* and *Erysiphe necator* fungi on the aroma character of grape must: A comparative approach. *Food Chemistry*, *207*, 251–260. https://doi.org/10.1016/j.foodchem.2016.03.110

Lund, K. T., Riaz, S., & Walker, M. A. (2017). Population structure, diversity and reproductive mode of the grape phylloxera (*Daktulosphaira vitifoliae*) across its native range. *PLOS ONE*, *12*(2), e0170678. https://doi.org/10.1371/journal.pone.0170678

MacKenzie, D. J., McLean, M. A., Mukerji, S., & Green, M. (1997). Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription–polymerase chain reaction. *Plant Disease, 81*(2), 222–226. https://doi.org/10.1094/PDIS.1997.81.2.222

Malik, I. M., Tak, H., Lone, G. M., & Dass, W. M. (2022). Phytoparasitic nematodes as the major threat to viticulture. *Environmental and Experimental Biology, 20*(1), 1–10. https://doi.org/10.22364/eeb.20.01

Maliogka, V. I., Martelli, G. P., Fuchs, M., & Katis, N. I. (2015). Control of viruses infecting grapevine. In *Advances in virus research* (Vol. 91, pp. 175–227). Elsevier.

Martelli, G. P. (2014). Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology, 96*(1), 1–136.

Martelli, G. P. (2017). An overview on grapevine viruses, viroids, and the diseases they cause. In B. Meng, G. P. Martelli, D. A. Golino, & M. Fuchs (Eds.), *Grapevine viruses: Molecular biology, diagnostics and management* (pp. 31–46). Springer International Publishing. https://doi.org/10.1007/978-3-319-57706-7_2

Martelli, G. P., & Boudon-Padieu, E. (Eds.). (2006). *Directory of infectious diseases of grapevines and viruses and virus-like diseases of the grapevine: Bibliographic report 1998–2004*. CIHEAM.

Martin, I. R., Vigne, E., Velt, A., Hily, J.-M., Garcia, S., & Schmitt-Keichinger, C. (2021). Severe stunting symptoms upon nepovirus infection are reminiscent of a chronic hypersensitive-like response in a perennial woody fruit crop. *Viruses, 13*(11), 2138. https://doi.org/10.3390/v13112138

Massart, S., Olmos, A., Jijakli, H., & Candresse, T. (2014). Current impact and future directions of high throughput sequencing in plant virus diagnostics. *Virus Research, 188*, 90–96. https://doi.org/10.1016/j.virusres.2014.03.029

Mekuria, T. A., Gutha, L. R., Martin, R. R., & Naidu, R. A. (2009). Genome diversity and intra- and interspecies recombination events in *Grapevine fanleaf virus*. *Phytopathology, 99*(12), 1394–1402. https://doi.org/10.1094/PHYTO-99-12-1394

Meng, B., Martelli, G. P., Golino, D. A., & Fuchs, M. (Eds.). (2017). *Grapevine viruses: Molecular biology, diagnostics and management*. Springer International Publishing. https://doi.org/10.1007/978-3-319-57706-7

Miljanić, V., Rusjan, D., Škvarč, A., Chatelet, P., Štajner, N., & others. (2022). Elimination of eight viruses and two viroids from preclonal candidates of six grapevine varieties (*Vitis vinifera* L.) through in vivo thermotherapy and in vitro meristem tip micrografting. *Plants, 11*(8), 1064. https://doi.org/10.3390/plants11081064

Mokili, J. L., Rohwer, F., & Dutilh, B. E. (2012). Metagenomics and future perspectives in virus discovery. *Current Opinion in Virology*, *2*(1), 63–77. https://doi.org/10.1016/j.coviro.2011.12.004

Morgan, S. W., Read, D. A., Burger, J. T., & Pietersen, G. (2022). Diversity of viroids infecting grapevines in South Africa. *Research square*, 20. https://doi.org/10.1007/s11262-023-01971-7.

M'rabet Samaali, B., Loulou, A., Mougou Hamdane, A., & Kallel, S. (2024). Acquisition and transmission of *Grapevine fanleaf virus* (GFLV) by *Xiphinema index* and *Xiphinema italiae* (Longidoridae). *Journal of Helminthology, 98*, e26. https://doi.org/10.1017/S0022149X24000154

Muntean, M.-D., Drăgulinescu, A.-M., Tomoiagă, L. L., Comșa, M., Răcoare, H.-S., & Chedea, V. S. (2022). Fungal grapevine trunk diseases in Romanian vineyards in the context of the international situation. *Pathogens, 11*(9), 1006. https://doi.org/10.3390/pathogens11091006

Naidu, R. A., Maree, H. J., & Burger, J. T. (2015). Grapevine leafroll disease and associated viruses: A unique pathosystem. *Annual Review of Phytopathology*, *53*, 613–634. https://doi.org/10.1146/annurev-phyto-102313-045946

Naidu, R. A., & Mekuria, T. A. (2010). First report of *Grapevine fleck virus* from Washington vineyards. *Plant Disease, 94*(6), 784. https://doi.org/10.1094/PDIS-94-6-0784A

Navrotskaya, E., Porotikova, E., Yurchenko, E., Galbács, Z. N., Várallyay, E., & Vinogradova, S. (2021). High-throughput sequencing of small RNAs for diagnostics of grapevine viruses and viroids in Russia. *Viruses, 13*(12), 2432. https://doi.org/10.3390/v13122432

Ndifon, E. M. (2024). Nematode populations affect grapevine seedlings: Understanding the host–parasite interaction is critical for preventing negative effects. *Revista de Investigaciones Agropecuarias, 50*(2), 140–147. https://doi.org/10.58149/FEBD-2Q06

Negrel, L., Baltenweck, R., Demangeat, G., Le Bohec-Dorner, F., Rustenholz, C., Velt, A., Gertz, C., Bieler, E., Dürrenberger, M., Gombault, P., Hugueney, P., & Lemaire, O. (2022). Comparative Metabolomic Analysis of Four Fabaceae and Relationship to In Vitro Nematicidal Activity against Xiphinema index. *Molecules*, *27*(10), 3052. https://doi.org/10.3390/molecules27103052

Nguyen, V. C., Villate, L., Gutierrez-Gutierrez, C., Castillo, P., Van Ghelder, C., Plantard, O., & Esmenjaud, D. (2019). Phylogeography of the soil-borne vector nematode Xiphinema index highly suggests Eastern origin and dissemination with domesticated grapevine. *Scientific Reports*, *9*(1), 7313. https://doi.org/10.1038/s41598-019-43812-4

Nuzzo, F., Moine, A., Nerva, L., Pagliarani, C., Perrone, I., Boccacci, P., Gribaudo, I., Chitarra, W., & Gambino, G. (2022). Grapevine virome and production of healthy plants by somatic embryogenesis. *Microbial Biotechnology*, *15*(5), 1357–1373. https://doi.org/10.1111/1751-7915.14011

O'Hearn, J. S., & Walsh, D. B. (2021). Grapevine leafroll-associated virus 3 vectored by *Pseudococcus maritimus* at low population levels. *Journal of Entomological Science, 56*(1), 106–110. https://doi.org/10.18474/0749-8004-56.1.106

Organisation Internationale de la Vigne et du Vin (OIV). (2024). *State of the world vitivinicultural sector in 2024*. OIV.

Olah, R., Turcsan, M., Olah, K., Farkas, E., Deak, T., Jahnke, G., & Sardy, D. A. N. (2022). Somatic Embryogenesis: A Tool for Fast and Reliable Virus and Viroid Elimination for Grapevine and other Plant Species. *Horticulturae*, *8*(6), 508. https://doi.org/10.3390/horticulturae8060508

Oliver, J. E., & Fuchs, M. (2011a). Tolerance and resistance to viruses and their vectors in *Vitis* spp.: A virologist's perspective of the literature. *American Journal of Enology and Viticulture, 62*(4), 438–451. https://doi.org/10.5344/ajev.2011.11036

Oliver, J. E., & Fuchs, M. F. (2011). Fanleaf degeneration/decline disease of grapevines. *Grapes Cornell University*, 1–3.

Oliver, J. E., Vigne, E., & Fuchs, M. (2010). Genetic structure and molecular variability of *Grapevine fanleaf virus* populations. *Virus Research, 152*(1–2), 30–40. https://doi.org/10.1016/j.virusres.2010.05.017

Orfanidou, C. G., Moraki, K., Panailidou, P., Lotos, L., Katsiani, A., Avgelis, A., Katis, N. I., & Maliogka, V. I. (2021). Prevalence and Genetic Diversity of Viruses Associated with Rugose Wood Complex in Greek Vineyards. *Plant Disease*, *105*(11), 3677–3685. https://doi.org/10.1094/PDIS-02-21-0266-RE

Orlov, I., Hemmer, C., Ackerer, L., Lorber, B., Ghannam, A., Poignavent, V., Hleibieh, K., Sauter, C., Schmitt-Keichinger, C., Belval, L., Hily, J.-M., Marmonier, A., Komar, V., Gersch, S., Schellenberger, P., Bron, P., Vigne, E., Muyldermans, S., Lemaire, O., … Klaholz, B. P. (2020). Structural basis of nanobody recognition of grapevine fanleaf virus and of virus resistance loss. *Proceedings of the National Academy of Sciences*, *117*(20), 10848–10855. https://doi.org/10.1073/pnas.1913681117

Panattoni, A., Luvisi, A., & Triolo, E. (2013). Elimination of viruses in plants: Twenty years of progress. *Spanish Journal of Agricultural Research, 11*(1), 173–188. https://doi.org/10.5424/sjar/2013111-3201

Panattoni, A., & Triolo, E. (2010). Susceptibility of grapevine viruses to thermotherapy on in vitro collection of Kober 5BB. *Scientia Horticulturae, 125*(1), 63–67. https://doi.org/10.1016/j.scienta.2010.03.001

Panno, S., Caruso, A. G., Bertacca, S., Pisciotta, A., Di Lorenzo, R., & Davino, S. (2021). Genetic structure and molecular variability of *Grapevine fanleaf virus* in Sicily. *Agriculture, 11*(6), 496. https://doi.org/10.3390/agriculture11060496

Pantaleoni, R. A., Cesaroni, C., Cossu, C. S., Deliperi, S., Fadda, leonarda, Fois, X., Lentini, A., Loi, A., Loru, lora, Molinu, A., Nuvoli, M. T., Ramassini, W., Sassu, A., Serra, G., & Verdinelli, M. (2012). Impact of alien insect pests on Sardinian landscape and culture. *Biodiversity Journal*, *3*(4), 297–310.

Paul, N. C., Khapte, P. S., Ponnaganti, N., Changan, S. S., Chavan, S. B., & Reddy, K. S. (2025). Grapevine (*Vitis vinifera*) yield prediction using an optimized weighted ensemble machine learning approach. *Smart Agricultural Technology, 12*, 101151. https://doi.org/10.1016/j.atech.2025.101151

Powell, K. S., Cooper, P. D., & Forneck, A. (2013). The biology, physiology and host–plant interactions of grape phylloxera (*Daktulosphaira vitifoliae*). In *Advances in insect physiology* (Vol. 45, pp. 159–218). Elsevier. https://doi.org/10.1016/B978-0-12-417042-2.00004-7

Prazaru, S. C., Dal Mas, G., Padoin, M., Rizzardo, D., Meggio, F., & Duso, C. (2023). Effect of leaf removal and insecticide applications on population densities of leafhoppers and mites associated with grapevines. *Insects, 14*(10), 791. https://doi.org/10.3390/insects14100791

Prokić, A., Dreo, T., & Obradović, A. (2018). Bacterial blight of grapevine: *Xylophilus ampelinus*. *Biljni Lekar, 46*(6), 674–680.

Poojari, S., Moreau, D. L., Kahl, D., Ritchie, M., Ali, S., & Úrbez-Torres, J. R. (2020). Disease incidence and genetic variability of economically important grapevine viruses in Nova Scotia. *Canadian Journal of Plant Pathology*, *42*(4), 584–594. https://doi.org/10.1080/07060661.2020.1730443

Rahali, M., Migliaro, D., Layadi, Z., Bertazzon, N., Angelini, E., & Crespan, M. (2019). Genetic identification, origin and sanitary status of grapevine cultivars (*Vitis vinifera* L.) grown in Babar, Algeria. *Vitis – Journal of Grapevine Research, 58*, 153–158. https://doi.org/10.5073/VITIS.2019.58.153-158

Reglinski, T., Elmer, P. A. G., Taylor, J. T., Wood, P. N., & Hoyte, S. M. (2010). Inhibition of *Botrytis cinerea* growth and suppression of botrytis bunch rot in grapes using chitosan. *Plant Pathology, 59*(5), 882–890. https://doi.org/10.1111/j.1365-3059.2010.02312.x

Rienth, M., Vigneron, N., Walker, R. P., Castellarin, S. D., Sweetman, C., Burbidge, C. A., Bonghi, C., Famiani, F., & Darriet, P. (2021). Modifications of Grapevine Berry Composition Induced by Main Viral and Fungal Pathogens in a Climate Change Scenario. *Frontiers in Plant Science, 12*, 717223. https://doi.org/10.3389/fpls.2021.717223

Ritzenthaler, C. (2011). Parallels and distinctions in the direct cell-to-cell spread of plant and animal viruses. *Current Opinion in Virology*, *1*(5), 403–409. https://doi.org/10.1016/j.coviro.2011.09.006

Ritzenthaler, C., & Hofmann, C. (2007). Tubule-guided movement of plant viruses. In E. Waigmann & M. Heinlein (Eds.), *Viral transport in plants* (pp. 63–83). Springer. https://doi.org/10.1007/978-3-540-68799-1_4

Ritzenthaler, C., Laporte, C., Gaire, F., Dunoyer, P., Schmitt, C., Duval, S., Piéquet, A., Loudes, A. M., Rohfritsch, O., Stussi-Garaud, C., & Pfeiffer, P. (2002). Grapevine Fanleaf Virus Replication Occurs on Endoplasmic Reticulum-Derived Membranes. *Journal of Virology*, *76*(17), 8808–8819. https://doi.org/10.1128/JVI.76.17.8808-8819.2002

Sabella, E., Pierro, R., Luvisi, A., Panattoni, A., D'Onofrio, C., Scalabrelli, G., Nutricati, E., Aprile, A., De Bellis, L., & Materazzi, A. (2018). Phylogenetic analysis of viruses in Tuscan Vitis vinifera sylvestris (Gmeli) Hegi. *PLOS ONE*, *13*(7), e0200875. https://doi.org/10.1371/journal.pone.0200875

Automatic citation updates are disabled. To see the bibliography, click Refresh in the Zotero tab.Savoi, S., Eitle, M. W., Berger, H., Curto, M., Meimberg, H., Griesser, M., & Forneck, A. (2020). Comparative Transcriptome Analysis of Two Root-Feeding Grape Phylloxera (D. vitifoliae) Lineages Feeding on a Rootstock and V. vinifera. *Insects*, *11*(10), 691. https://doi.org/10.3390/insects11100691

Schellenberger, P., Andret-Link, P., Schmitt-Keichinger, C., Bergdoll, M., Marmonier, A., Vigne, E., Lemaire, O., Fuchs, M., Demangeat, G., & Ritzenthaler, C. (2010). A Stretch of 11 Amino Acids in the βB-βC Loop of the Coat Protein of *Grapevine Fanleaf Virus* Is Essential for Transmission by the Nematode *Xiphinema index*. *Journal of Virology*, *84*(16), 7924–7933. https://doi.org/10.1128/JVI.00757-10

Schmitt-Keichinger, C., Hemmer, C., Berthold, F., & Ritzenthaler, C. (2017a). Molecular, cellular, and structural biology of *Grapevine fanleaf virus*. In B. Meng, G. P. Martelli, D. A. Golino, & M. Fuchs (Eds.), *Grapevine viruses: Molecular biology, diagnostics and management* (pp. 83–107). Springer International Publishing. https://doi.org/10.1007/978-3-319-57706-7_5

Schmitt-Keichinger, C., Hemmer, C., Berthold, F., & Ritzenthaler, C. (2017b). Molecular, cellular, and structural biology of *Grapevine fanleaf virus*. In B. Meng, G. P. Martelli, D. A. Golino, & M. Fuchs (Eds.), *Grapevine viruses: Molecular biology, diagnostics and management* (pp. 83–107). Springer International Publishing. https://doi.org/10.1007/978-3-319-57706-7_5

Schulze-Sylvester, M., Corronca, J. A., & Paris, C. I. (2021). Vine mealybugs disrupt biomass allocation in grapevine. *OENO One, 55*(1), 93–103. https://doi.org/10.20870/oeno-one.2021.55.1.4458

Setiono, F. J., Chatterjee, D., Fuchs, M., Perry, K. L., & Thompson, J. R. (2018). The distribution and detection of grapevine red blotch virus in its host depend on time of sampling and tissue type. *Plant Disease, 102*(11), 2187–2193. https://doi.org/10.1094/PDIS-03-18-0450-RE

Shatnawi, M., Anfoka, G., Shibli, R., Al-Mazra'Awi, M., Shahrour, W., & Arebiat, A. (2011). Clonal propagation and cryogenic storage of virus-free grapevine (*Vitis vinifera* L.) via meristem culture. *Turkish Journal of Agriculture and Forestry, 35*(2), 173–184. https://doi.org/10.3906/tar-0912-519

Singh, S., Awasthi, L. P., Jangre, A., & Nirmalkar, V. K. (2020). Transmission of plant viruses through soil-inhabiting nematode vectors. In *Applied plant virology* (pp. 291–300). Elsevier. https://doi.org/10.1016/B978-0-12-818654-2.00016-3

Smaha, D., Mokrini, F., Khayi, S., Iraqi, D., Lahlali, R., & Dababat, A. A. (2023). Research note: Nematode-associated constraints in cropping systems. *Nematropica, 53*(1), 67-69

Soltani, N., Stevens, K. A., Klaassen, V., Hwang, M.-S., Golino, D. A., & Al Rwahnih, M. (2021). Quality assessment and validation of high-throughput sequencing for grapevine virus diagnostics. *Viruses, 13*(6), 1130. https://doi.org/10.3390/v13061130

Somkuwar, R.G., Samarth, R.R., Sharma, A.K. (2024). Grape. In: Rajasekharan, P.E., Rao, V.R. (eds) Fruit and Nut Crops. Handbooks of Crop Diversity: Conservation and Use of Plant Genetic Resources. Springer, Singapore. https://doi.org/10.1007/978-981-99-5348-6_2

Song, Y., Hanner, R. H., & Meng, B. (2021). Probing into the effects of grapevine leafroll-associated viruses on the physiology, fruit quality and gene expression of grapes. *Viruses, 13*(4), 593. https://doi.org/10.3390/v13040593

Stevens, K. A., & Al Rwahnih, M. (2024). High-throughput sequencing for the detection of viruses in grapevine: Performance analysis and best practices. *Viruses, 16*(12), 1957. https://doi.org/10.3390/v16121957

Szabó, M., Csikász-Krizsics, A., Dula, T., Farkas, E., Roznik, D., Kozma, P., & Deák, T. (2023). Black Rot of Grapes (Guignardia bidwellii)—A Comprehensive Overview. *Horticulturae*, *9*(2), 130. https://doi.org/10.3390/horticulturae9020130

Szegedi, E., & Civerolo, E. L. (2011). Bacterial diseases of grapevine. *International Journal of Horticultural Science, 17*, 956. https://doi.org/10.31421/IJHS/17/3/956

Tahirine, M., Louanchi, M., & Aitouada, M. (2020). Actualisation à la révélation de deux virus responsables à la maladie du court noué de la vigne du genre Vitis dans la région Centre et Ouest de l'Algérie par voie sérologique et biochimique. *Journal Algérien des Régions Arides*, *14*(1), 150–158.

Tamborindeguy, C., Hata, F. T., Molina, R. D. O., & Nunes, W. M. D. C. (2023). A New Perspective on the Co-Transmission of Plant Pathogens by Hemipterans. *Microorganisms, 11*(1), 156. https://doi.org/10.3390/microorganisms11010156

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thind, T. S., Arora, J. K., Mohan, C., & Raj, P. (2004). Epidemiology of powdery mildew, downy mildew, and anthracnose diseases of grapevine. In S. A. M. H. Naqvi (Ed.), *Diseases of Fruits and Vegetables, Volume I* (pp. 621–638). Dordrecht: Kluwer Academic Publishers.

Automatic citation updates are disabled. To see the bibliography, click Refresh in the Zotero tab.Szabó, M., Csikász-Krizsics, A., Dula, T., Farkas, E., Roznik, D., Kozma, P., & Deák, T. (2023). Black Rot of Grapes (Guignardia bidwellii)—A Comprehensive Overview. *Horticulturae*, *9*(2), 130. https://doi.org/10.3390/horticulturae9020130

Van Ghelder, C., Reid, A., Kenyon, D., & Esmenjaud, D. (2015). Development of a real-time PCR method for the detection of the dagger nematodes *Xiphinema index*, *X. diversicaudatum*, *X. vuittenezi*, and *X. italiae*, and for the quantification of *X. index* numbers. *Plant Pathology, 64*(2), 489–500. https://doi.org/10.1111/ppa.12269

Van Zyl, S., Vivier, M. A., & Walker, M. A. (2016). *Xiphinema index* and its relationship to grapevines: A review. *South African Journal of Enology & Viticulture, 33*. https://doi.org/10.21548/33-1-1302

Vigne, E., Garcia, S., Komar, V., Lemaire, O., & Hily, J.-M. (2018). Comparison of serological and molecular methods with high-throughput sequencing for the detection and quantification of Grapevine fanleaf virus in vineyard samples. *Frontiers in Microbiology, 9,* 2726. https://doi.org/10.3389/fmicb.2018.02726

Vigne, E., Marmonier, A., & Fuchs, M. (2008). Multiple interspecies recombination events within RNA2 of Grapevine fanleaf virus and Arabis mosaic virus. *Archives of Virology, 153,* 1771–1776. https://doi.org/10.1007/s00705-008-0182-y

Villate, L., Morin, E., Demangeat, G., Van Helden, M., & Esmenjaud, D. (2012). Control of *Xiphinema index* populations by fallow plants under greenhouse and field conditions. *Phytopathology, 102,* 627–634. https://doi.org/10.1094/PHYTO-01-12-0007

Weiland, C. M., Superior, E. P., Cantos, M., Troncoso, A., & Perez-Camacho, F. (2003). Regeneration of virus-free plants by in vitro chemotherapy of GFLV (Grapevine Fanleaf Virus) infected explants of *Vitis vinifera* cv. 'Zalema.' *International Symposium on Grapevine Growing, Commerce and Research, 652,* 463–466.

Wernet, V., & Fischer, R. (2023). Establishment of *Arthrobotrys flagrans* as biocontrol agent against the root pathogenic nematode *Xiphinema index*. *Environmental Microbiology, 25,* 283–293. https://doi.org/10.1111/1462-2920.16282

Yang, Y., Hu, X., Liu, P., Chen, L., Peng, H., Wang, Q., & Zhang, Q. (2021). A new root-knot nematode, Meloidogyne vitis sp. nov. (Nematoda: Meloidogynidae), parasitizing grape in Yunnan. *PLOS ONE, 16*(2), e0245201. https://doi.org/10.1371/journal.pone.0245201

Zherdev, A., Vinogradova, S., Byzova, N., Porotikova, E., Kamionskaya, A., & Dzantiev, B. (2018). Methods for the diagnosis of grapevine viral infections: A review. *Agriculture, 8,* 195. https://doi.org/10.3390/agriculture8120195

Zhou, J., Fan, X., Dong, Y., Zhang, Z. P., Ren, F., & Hu, G. (2015). Detection and genetic variation analysis of Grapevine fanleaf virus (GFLV) isolates in China. *Archives of Virology, 160,* 2661–2667. https://doi.org/10.1007/s00705-015-2566-0

Appendices

Appendix 1:

Cultivar list authorized for production and marketing in Algeria according to official journal (February 2011).

Table grape varieties.

Adari	Perlette
Ahmeur bou ameur	Reine des vignes
Alphonse la vallee	Servant blanc
Bezoul el khadem	Valensi = Mokrani = panse de Provence
Cardinal	Farana
Chaouch blanc	Black pearl
Chaouch rose	Centennial
Chasselas	Argentina
Dabouki	King's ruby
Dattier de Beyrouth	Aledo
Gros noir des Beni Abbes	Nerona
Guerbez = gros vert = saint jeannet	Bronx
Italia	Emerald
Madeleine du Sahel	Christmas rose
Muscat d'Alexandrie	Pasiga
Muscat de Hambourg	Alvina
Ohanes= UVA de almeria	Dona Maria
Panse prÈcoce = sicilien	Matilde
Perle de ksaba	Datal
Danam	

Dried grape varieties

1. Sultanine
2. Muscat d'Alexandrie
3. Corinthe noire
4. King's ruby
5. Centennial

Wine grape varieties

Black or red grape	**White grape**
Alicante bouschet	Chardonnay
Aramon gris	Chenin Blanc
Aramon noir	Clairette
Cabernet franc	Farana
Cabernet sauvignon	Grenache blanc
Carignan	Macabeu = Macabeo
Cinsault	Merseguerra = Listan = palomino
Grenache franc	Muscat d'Alexandrie
Grenache rose	Sauvignon
Grenache velu	Tizourine bou afrara = s. d'AlgÈrie
Merlot	Ugni blanc = el maoui
Morastel = gros matterou	Valenci blanc
Pinot noir	Pinot Blanc
Syrah	
Tipasi = toustrai = plant romain	
Grenache gris	
Grenache noir	

Appendix 2: List of Grapevine Cultivars, Sampling Regions, and Number of Samples collected.

Cultivars	Region	wilaya	cite	Number of sample
Ladhari	west	Mostaganem	Abdelmalek Ramdane	10
		Mostaganem	Hadjadj (Bosquet)	10
Carignon	west	Mostaganem	Abdelmalek Ramdane	10
		Mascara	Sidi Daho Ben Zarfa	10
Cinsault	west	Mostaganem	Hadjadj (Bosquet)	20
		Ain Temouchent	El Amira	20
	west	Mascara	Sidi Daho Ben Zarfa	10
		Mascara	Mascara	10
Gros noir	west	Mostaganem	Hadjadj (Bosquet)	10
	Center	Boumerdes	Si Mustafa	9
		Blida	Tassala El merdja	10
Alicante	West	Ain Temouchent	El Amira	10
Mersguerra	West	Ain Temouchent	El Amira	10
Cardinal	West	Ain Temouchent	El Amira	10
	Center	Medea	Benchicaou	2
		Boumerdes	Si Mustafa	10
			Bouedj Menail	10
		Tizi Ouzou	Draa El Mizan	1
			Tadmit	4
Dattier	West	Ain Temouchent	El Amira	10
		Telemcen	El Fehoul	10
		Sidi-Bel-Abbès	Sfisef	10
	Center	Medea	ITAFV	5
		Blida	Mouzaia	10
Muscat	West	Telemcen	Bensekrane	10
		Ain Temouchent	El Amira	10
Valensi	West	Mascara	Sidi Daho Ben Zarfa	20
Ahmer Bou Ameur	Center	Medea	Benchicaou V3	18
			ITAFV	5
			Benchicaou V2	11
Michel Palieri	Center	Medea	Benchicaou V2	10
Muscat Italy	Center	Medea	Benchicaou V1	10
		Boumerdes	Leghata	8
		Blida	Tassala El merdja	10
		Bejaia	Souk letnin	7
Seibel	Center	Boumerdes	Leghata	8
		Tizi Ouzou	Azzefoun Mellata	10
			Azzefoun Timelouka	10
	East	Bejaia	Souk letnin	7
Issers	Center	Boumerdes	Si Mustafa	4
Red Glob	Center	Blida	Tessala El Merdja	10
		Tizi Ouzou	Azzefoun Timelouka	10
	East	Bejaia	Souk letnin	7
Sebseb	South	Ghardaia	Sebseb	2
Metlili			Metlili	3
			Nouvelle Metlili	2
Vigne commun	South	Tamanrasset	Tazrouk	5

Appendix 3 : Detection of GFLV by DAS-ELISA

The incidence of *Grapevine fanleaf virus* (GFLV) in Algeria was assessed using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). All collected samples were tested with the GFLV Complete 480 kit (Bioreba AG, Switzerland), following the manufacturer's protocol.

1. Antigen extraction

- The phloem of grapevine shoots was manually detached with a scalpel to obtain 2 g of tissue and ground in a mortar with 10ml of extraction buffer (10×).
- The resulting plant extract was transferred into numbered Eppendorf tubes and stored at −20 °C until use.

2. Coating

- Microplates were sensitised with antibody diluted in sensitisation buffer (1/1000) at 100 µL per well and incubated for 3 h at 37 °C.
- After incubation, plates were washed three times (5 min each) with washing buffer and then dried.

3. Antigen addition

- Wells were filled with phloem extract containing the antigen according to a plate diagram (100 µL/well).
- Each plate included two negative and two positive control wells.
- Plates were incubated overnight at 4 °C.

4. Conjugate

- Plates were washed three times as described in section 2.
- Alkaline phosphatase-conjugated serum (diluted in conjugation buffer, 100 µL/well) was added.
- Incubation followed for 2 h at 37 °C.

5. Substrate reaction and measurement

- After three additional washes, freshly prepared p-nitrophenylphosphate in substrate buffer (2 mg/20 mL) was added to each well.
- Plates were incubated at 37 °C for 1 h.
- Photometric readings were taken at 405 nm after 1 h and 2 h using a microtiter plate reader.

Appendix 4: Purification of PCR Products by Gel Extraction

Amplification of PCR products using the NucleoSpin® Gel & PCR Clean-up Mini Kit (MACHEREY-NAGEL), following the following steps:

- **Electrophoresis in agarose gel**

Electrophoresis was performed on a 1.5% agarose gel for samples that were positive by PCR, alongside a 100 bp molecular weight marker. The electrophoresis was carried out at 100 V until optimal separation of the target fragments was achieved. The DNA bands were visualized under UV illumination, and the bands corresponding to the target fragment were carefully excised with a sterile scalpel. The excised gel slices were then placed in clean microcentrifuge tubes, and Buffer NTI was added to each tube. The samples were incubated for 10 min at 50°C with brief vortexing performed every 2-3 min.

- **DNA binding to Silica**

The dissolved gel solution was transferred into a silica-based spin column, and centrifugation was performed for 30 s at 11,000 × g. The flow-through was discarded and the column was placed back into the collection tube.

- **Wash silica membrane**

The column was washed by adding 700 µL of Wash Buffer NT3 to the NucleoSpin column. Centrifugation was performed for 30 s at 11,000×g, and the flow-through was discarded.

- **Drying silica membrane**

The column was centrifuged for 1 min at 11,000×g to remove residual wash buffer completely.

- **DNA Elution**

DNA elution was performed by applying NE elution buffer (15-30 µL) to the center of the silica membrane. The column was incubated at room temperature for 1 min and then centrifuged for 1min at 11,000×g. The DNA concentration and purity were assessed using a NanoDrop spectrophotometer.

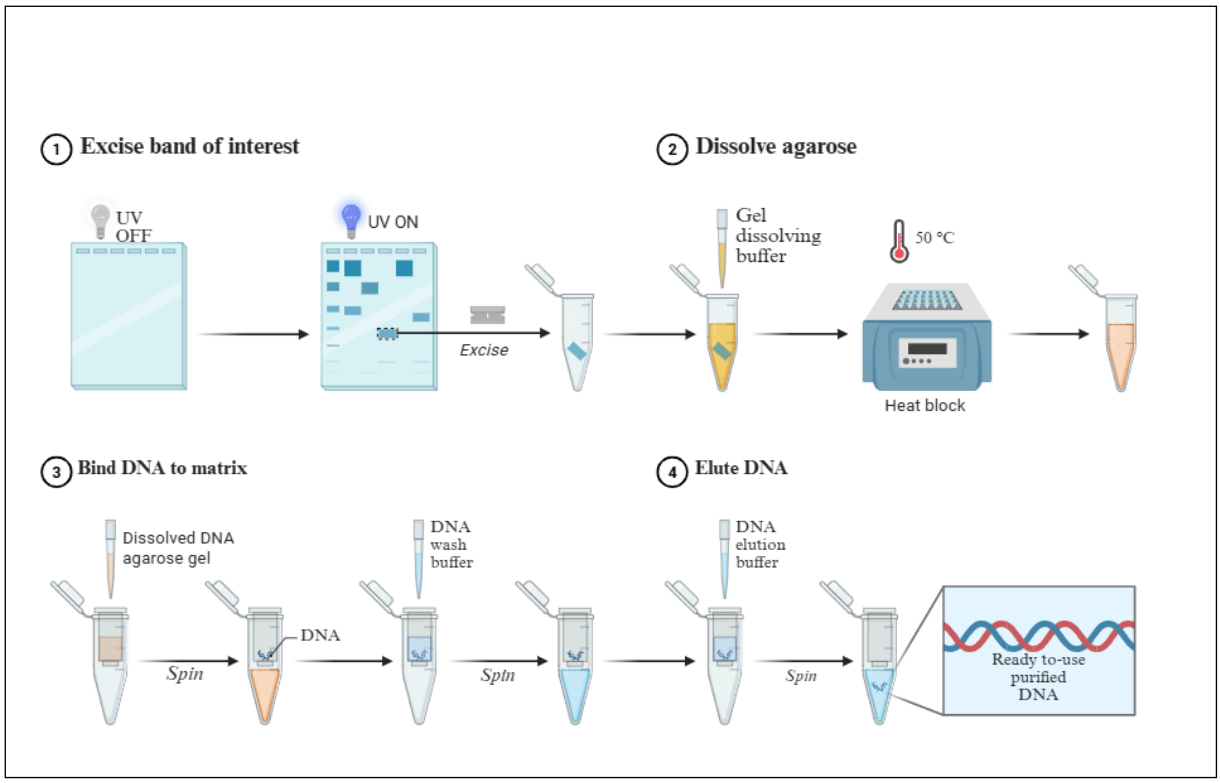


Figure 28: DNA Gel Extraction and Purification Process created by BioRender.com

Appendix 5: Cloning of PCR Products into pGEM®-T Easy Vector

- **Ligation**

The PCR product was cloned into pGEM-T Easy vector System II (Promega, Madison, USA) following the manufacturer's instruction. The ligation reaction was prepared in a final volume of 10 µL, containing 1 µL of pGEM-T Easy vector (50 ng), 1 µL of T4 DNA ligase (3U/µl), 5 µL of 2X Rapid Ligation Buffer, and 3µl of the PCR-purified PCR product. It was then mixed gently by pipetting and incubated overnight at 4°C.

- **Transformation**

Transformation was performed by introducing the ligation mixture into competent *E. coli* JM109 cells using a heat shock method, which facilitates the uptake of the plasmid.
Tubes containing competent cells were retrieved from the freezer at -70°C and placed on ice for 5 minutes. Subsequently, 40 µL of cells were aliquoted into 2 ml tubes, followed by the addition of 2 µL of ligation mixture. The mixture was gently flicked to ensure proper mixing and then incubated on ice for 20 minutes.

The cells were subjected to a heat shock in a 42°C water bath for 45-50 seconds and then immediately transferred to an ice bath for 2 minutes. Subsequently, 950 µL of pre-warmed SOC medium was added to each tubes. The tubes were incubated at 37°C for 1.5 hours with shaking at 150 rpm. Following incubation, the cells were centrifuged at 1000 x g for 10 minutes. Approximately 700 µL of the supernatant was discarded, and the cell pellet was resuspended in the remaining medium.

- **Plating**

60µl from each transformation tubes were plated onto LB (Luria Bertani) agar plates supplemented with ampicillin, IPTG and X-Gal. The plates were then incubated at overnight 37°C.

- **Colony Selection and Processing**

After incubation, colonies were screened based on α-complementation. White colonies indicated successful insertion of the PCR product into the vector, while blue colonies did not contain an insert. Six colonies were selected from each plate. Each selected colony was transferred to a 1.5 mL tube containing 20 µL of sterile distilled water and mixed thoroughly. A small aliquot of each suspension was spotted onto a pre-numbered LB-ampicillin plate for

archiving. The remaining cell suspension was then subjected to lysis by incubation at 95°C for 10 minutes, followed by immediate placement on ice for 5 minutes. This lysate was used as template for colony PCR.

- **Colony PCR**

5 µL of each cell lysate was added to a PCR master mix**.** The final 25 µL reaction contained: 5 µL of 5X PCR buffer, 1.5 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTPs (10 mM), 0.2 µL of *Taq* DNA polymerase (5 U/µL), 0.5 µL of each primer (T7 and SP6, 10 µM), and 10.8 µL of nuclease-free water**.** The PCR conditions were as follows: initial denaturation at 94°C for 3 minutes**;** followed by 29 cycles of denaturation at 94°C for 30 seconds**,** annealing at 50°C for 30 seconds**,** and extension at 72°C for 40 seconds; with a final extension at 72°C for 7 minutes**.** The PCR products were analyzed by electrophoresis on a 1.5% agarose gel in TBE buffer (Figure 18)**.**

Appendix 6: Plasmid DNA Isolation by Miniprep (QIAprep® Spin Kit)

Plasmid DNA was isolated from bacterial cultures using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol.

Single colonies were inoculated into LB medium containing ampicillin and grown overnight at 37°C with shaking. 1-5 mL of the overnight culture was centrifuged at >10,000 × g for 3 minutes to pellet the cells. The pellet was resuspended in 250 µL of Buffer P1 (resuspension buffer). 250 µL of Buffer P2 (lysis buffer) was added, and the tube was gently inverted 4-6 times until the solution became clear. 350 µL of Buffer N3 (neutralization buffer) was added immediately, and the tube was mixed thoroughly by inversion. The lysate was centrifuged at maximum speed for 10 minutes to precipitate genomic DNA and proteins.

The supernatant was applied to a QIAprep spin column. The column was centrifuged for 30-60 seconds, and the flow-through was discarded. The column was washed by adding 500 µL of Buffer PB (wash buffer) and centrifuging. This was followed by a second wash with 750 µL of Buffer PE (wash buffer with ethanol). After the second wash, the column was centrifuged for an additional 1 minute to remove residual ethanol.

The plasmid DNA was eluted by adding 50 µL of Buffer EB (elution buffer) or nuclease-free water to the center of the column membrane, incubating for 1 minute, and then centrifuging for 1 minute.

The DNA concentration and purity were assessed using a NanoDrop spectrophotometer. The purified plasmid DNA was ready for sequencing.

Appendix 7 : Viruses identified globally in grapevines to date with the name of those identified by high-throughput sequencing in bold (Fuchs.,2025)

Family	Genus	Species	Virus name/abbreviation	Vector	Disease
Alphaflexiviridae	*Potexvirus*	*Potexvirus ecspotati*	Potato virus X (PVX)	None	Unknown
	Potexvirus, subgenus Mandarivirus	*Mandarivirus citriflavivenae*	Citrus yellowing vein clearing virus (CYVCV)	Aphis	Leaf necrosis
Amesuviridae	*Temfrudevirus*	*Temfrudevirus temperatum*	Temperature fruit decay-associated virus (TFDaV)		
Betaflexiviridae	*n/a*	*n/a*	Grapevine Kizil Sapak virus (GKSV)	Unknown	Unknown
	Foveavirus	*Foveavirus rupestris*	Grapevine rupestris stem pittingassociated virus (GRSPaV)	None	Rugose wood
		Foveavirus tafvirus	Grapevine virus T (GVT)	None	unknown
		Foveavirus alphavitis	Grapevine foveavirus A (GFVA)	None	unknown
	Trichovirus	*Trichovirus necroacini*	Grapevine berry inner necrosis virus (GINV)	Eriophyid mite	Berry inner necrosis
		Trichovirus pinovitis	Grapevine Pinot gris virus (GPGV)	Eriophyid mite	Leaf mottling/ deformation
	Vitivirus	*Vitivirus alphavitis*	Grapevine virus A (GVA)	Mealybugs/soft scales	Rugose wood
		Vitivirus betavitis	Grapevine virus B (GVB)	Mealybugs/soft scales	Corky bark
		Vitivirus deltavitis	Grapevine virus D (GVD)	unknown	unknown
		Vitivirus epsilonvitis	Grapevine virus E (GVE)	Mealybugs	Unknown
		Vitivirus phivitis	Grapevine virus F (GVF)	Unknown	Unknown
		Vitivirus gammavitis	Grapevine virus G (GVG)	Mealybugs	Unknown
		Vitivirus etavitis	Grapevine virus H (GVH)	Mealybugs	Unknown
		Vitivirus iotavitis	Grapevine virus I (GVI)	Unknown	Unknown
		Vitivirus jeitavitis	Grapevine virus J (GVJ)	Unknown	Unknown
		Vitivirus lambdavitis	Grapevine virus L (GVL)	Unknown	Unknown

		n/a	Grapevine virus N (GVN)	Unknown	Unknown
		n/a	Grapevine virus O (GVO)	Unknown	Unknown
		n/a	Grapevine virus P (GVP)	Unknown	Unknown
Bromoviridae	*Alfamovirus*	*Alfamovirus AMV*	Alfalfa mosaic virus (AMV)	Aphids	Yellow mosaic
	Anulavirus	*Anulavirus GLPV*	Grapevine line pattern virus (GLPV)	None	Line pattern
	Cucumovirus	*Cucumovirus CMV*	Cucumber mosaic virus (CMV)	Aphids	Unknown
	Ilarvirus	*Ilarvirus SnlV1*	Solanum nigrum ilarvirus 1 (SnlV1)	Unknown	Unknown
		n/a	Grapevine virus S (GVS)	None	Unknown
Caulimoviridae	*Badnavirus*	*Badnavirus venavirus*	Grapevine vein clearing virus (GVCV)	Aphids	Vein clearing
		Badnavius vitis	Grapevine badnavirus 1 (GBV1)	Mealybugs	Unknwon
		Badnavirus decoloratiovitis	Grapevine Roditis leaf discoloration-associated virus (GRLDaV)	Mealybugs	Roditis discoloration
	Ruflodivirus	*n/a*	Grapevine-associated caulimovirus (GaCMV)	Unknown	Unknown
		n/a	Grapevine pararetrovirus (GPRV)	Unknown	Unknown
Closteroviridae	*Ampelovirus*	*Ampelovirus univitis*	Grapevine leafroll-associated virus 1 (GLRaV1)	Mealybugs/soft scales	Leafroll
		Ampelovirus trivitis	Grapevine leafroll-associated virus 3 (GLRaV3)	Mealybugs/soft scales	Leafroll
		Ampelovirus tetravitis	Grapevine leafroll-associated virus 4 (GLRaV4)	Mealybugs/soft scales	Leafroll
		Ampelovirus tredecimvitis	Grapevine leafroll-associated virus 13 (GLRaV13)	Unknown	Leafroll
		Ampelovirus pruni	Plum bark necrosis stem pittingassociated virus (PBNSPaV)	Unknown	Unknown
	Closterovirus	*Closterovirus vitis*	Grapevine leafroll-associated virus 2 (GLRaV2)	Unknown	Leafroll/ Incompatibility
	Velarivirus	*Velarivirus septemvitis*	Grapevine leafroll-associated virus 7 (GLRaV7)	Unknown	Unknown
Endornaviridae	*Alphaendornavirus*	*Alphaendornavirus vitis*	Grapevine endophyte endornavirus (GEEV)	None	Unknown

		n/a	Grapevine endornavirus 1 (GEV1)		
		n/a	Grapevine endornavirus 2 (GEV2)		
Fimoviridae	*Emaravirus*	*Emaravirus vitis*	Vitis emaravirus (VEV)	Unknown	Unknown
Rhabdoviridae	*Varicosavirus*	*Varicosavirus vitis*	Vitis varicosavirus (VVV)	Unknown	Unknown
Geminiviridae	*Begomovirus*	*n/a*	Grapevine begomovirus A (GBVA)	Whiteflies	Unknown
		Grablovirus vitis	Grapevine red blotch virus (GRBV)	Treehopper	Red blotch
		Grablovirus silvestris	Wild Vitis latent virus 1 (WVV1)	Unknown	Unknown
	Maldovirus	*Maldovirus vitis*	Grapevine geminivirus A (GGVA)	Unknown	Unknown
Mayoviridae	*Idaeovirus*	*Idaeovirus rubi*	Raspberry bushy dwarf virus (RBDV)	None	Yellow line pattern
n/a	*Virtovirus*	*n/a*	Grapevine virus satellite (GV-Sat)	Unknown	Unknown
Nanoviridae	*n/a*	*n/a*	Grapevine-associated nano-like virus (GaNlV)	Unknown	Unknown
Partitiviridae	*Alphapartitivirus*	*n/a*	Grapevine alphapartitivirus (GAPV)	Unknown	Unknown
	Deltapartitivirus	*n/a*	Vitis cryptic virus (VCV)	None	Unknown
Phenuiviridae	*Rubodvirus*	*Rubodvirus armeniaense*	Grapevine Garan dmak virus (GGDV)	Unknown	Unknown
		Rubodvirus argentinaense	Grapevine Muscat rose virus (GMRV)	Unknown	Unknown
Potyviridae	*Potyvirus*	*Potyvirus phaseovulgaris*	Bean common mosaic virus (BCMV)	Aphids	Unknown
		Potyvirus yituberosi	Potato virus Y (PVY)	Aphids	Unknown
		n/a	Grapevine-associated poty-like virus (GaPlV)	Unknown	Unknown
Secoviridae	*Cheravirus*	*Cheravirus mali*	Apple latent spherical virus (ALSV)	Unknown	Unknown
	Fabavirus	*Fabavirus alphaviciae*	Broad bean wilt virus 1 (BBMV1)	Aphids	Unknown
		Fabavirus vitis	Grapevine fabavirus (GFabV)	Unknown	Unknown
		Fabavirus betavitis	Grapevine secovirus (GSV)	Unknown	Unknown
	Nepovirus	*Nepovirus italiaense*	Artichoke Italian latent virus (AILV)	Unknown	Degeneration
		Nepovirus arabis	Arabis mosaic virus (ArMV)	Dagger nematode	Degeneration

		Nepovirus myrtilli	Blueberry leaf mottle virus (BBLMV)	Unknown	Unknown
		Nepovirus avii	Cherry leafroll virus (CLRV)	Unknown	Degeneration
		Nepovirus anatoliense	Grapevine Anatolian ringspot virus (GARSV)	Unknown	Degeneration
		Nepovirus bulgariense	Grapevine Bulgarian latent virus (GBLV)	Unknown	Degeneration
		Nepovirus deformationis	Grapevine deformation virus (GDeV)	Unknown	Degeneration
		Nepovirus chromusivum	Grapevine chrome mosaic virus GCMV)	Unknown	Degeneration
		Nepovirus foliumflabelli	Grapevine fanleaf virus (GFLV)	Dagger nematode	Degeneration
		Nepovirus tunisiaense	Grapevine Tunisian ringspot virus (GTRV)	Unknown	Degeneration
		Nepovirus alphavitis	Grapevine nepovirus A (GNVA)	Unknown	Unknown
		Nepovirus persicae	Peach rosette mosaic virus (PRSM)	Dagger nematode	Degeneration
		Nepovirus rubi	Raspberry ringspot virus (RpRSV)	Dagger nematode	Degeneration
		Nepovirus nicotianae	Tobacco ringspot virus (TRSV)	Dagger nematode	Degeneration
		Nepovirus lycopersici	Tomato ringspot virus (ToRSV)	Dagger nematode	Degeneration
		Nepovirus nigranuli	Tomato black ring virus (TBRV)	Dagger nematode	Degeneration
	Stralarivirus	*Stralarivirus fragariae*	Strawberry latent ringspot virus (SLRSV)	Dagger nematode	Degeneration
	Waikavirus	*n/a*	Grapevine stunt virus (GSV)	Leafhopper	Short internodes/ poor fruit set
Sedoreoviridae	*unassigned*	*n/a*	Grapevine Cabernet Sauvignon reovirus (GCSV)	Plant/Leaf hoppers	Unknown
Solemoviridae	*Enamovirus*	*Enamovirus GEV*	Grapevine enamovirus 1 (GEV1)	Aphids	Unknown

	Polerovirus	*Polevorirus GPOV*	Grapevine polerovirus 1 (GpoV1)	Unknown	Unknown
		n/a	Grapevine Ajinashika virus (GagV)	Unknown	Ajinashika
	Sobemovirus	*Sobemovirus SOMV*	Sowbane mosaic virus (SoMV)	Leafhoppers	Unknown
Tombusviridae	*Alphacarmovirus*	*Alphacarmovirus dianthi*	Carnation mottle virus (CarMV)	None	Unknown
	Betanecrovirus	*Betanecrovirus nicotianae*	Tobacco necrosis virus D (TNV-D)	None	Unknown
	Tombusvirus	*Tombusvirus algeriaense*	Grapevine Algerian latent virus (GALV)	None	Unknown
		Tombusvirus petuniae	Petunia asteroid mosaic virus (PAMV)	None	Unknown
	Umbravirus	*n/a*	Grapevine umbra-like virus (GULV)	Unknown	Unknown
Tospoviridae	*Orthotospovirus*	*Orthotospovirus tomatomaculae*	Tomato spotted wilt virus (TSWV)	Thrips	Unknown
Tymoviridae	*Marafivirus*	*Marafivirus asteroides*	Grapevine asteroid mosaic-associated virus (GAMaV)	None	Asteroid mosaic
		n/a	Grapevine rupestris vein feathering virus (GRVFV)	None	Vein feathering
		Marafivirus syrahense	Grapevine Syrah virus 1 (GsyV1)	None	Unknown
		n/a	Grapevine-associated marafivirus (GaMV)	None	Unknown
	Maculavirus	*Maculavirus vitis*	Grapevine fleck virus (GFkV)	None	Fleck
		n/a	Grapevine redglobe virus (GRGV)	None	Unknown
Virgaviridae	*Tobamovirus*	*n/a*	Grapevine virga-like virus (GVLV)	None	Unknown
		Tobamovirus tabaci	Tobacco mosaic virus (TMV)	None	Unknown
		Tobamovirus tomatotessellati	Tomato mosaic virus (ToMV)	None	Unknown
		Tobamovirus viridimaculae	Cucumber green mottle virus (CGMMV)	None	Unknown
		n/a	Grapevine labile rod-shaped virus (GLRSV)	None	Unknown

Liste of publications

Laidoudi, N. E., Alisawi, O., Yahiaoui, B., Djenaoui, A., Mahdid, I., Bachir, A., De Luca, F., Fanelli, E., Minafra, A., Mahfoudhi, N. & Lehad, A. (2025). Occurrence of Grapevine fanleaf virus in Algerian vineyards, and complete genome sequencing. Phytopathologia Mediterranea 64(2): 219-228. doi: 10.36253/phyto-15993

Liste of communications

Laidoudi Nour Elhouda, Yahiaoui Bilal, Lehad Arezki, Djenaoui Anfel, Mahdid Imane, Prevalence and genetic diversity of Grapevine Fanleaf virus in algerian vinyards. Poster presentation. 14th Arab Congress of Plant Protection (ACPP 2025). 3-6 November 2025. Algiers Algeria.

Laidoudi Nour Elhouda, Yahiaoui Bilal, Lehad Arezki, Djenaoui Anfel, Mahdid Imane, Neggaz Rahil, Epidemiological study of grapevine fanleaf virus in Algeria. Poster presentation. 1er Symposium International de la Protection des Végétaux de l'ENSA. 21-23 November 2023 Algiers, Algeria.

Laidoudi Nour Elhouda, Yahiaoui Bilal, Lehad Arezki, Djenaoui Anfel, Mahdid Imane, Sanitary Status of Grapevine Cultivars Grown in Southern Algeria. Poster presentation. 1er **S**éminaire **N**ational sur l'**E**nvironnement **S**aharien" SNES'2024 ". 16-17 October 2024. Adrar Algeria.

Laidoudi Nour Elhouda, Yahiaoui Bilal, Lehad Arezki, Djenaoui Anfel, Mahdid Imane, Neggaz Rahil. Exploitation and valorisation of grapevine as natural resource in Algeria. Poster presentation. National seminar on phytotherpy and pharmacognosy (NSPP). 14-15 March 2023; Setif Algeria

Laidoudi Nour Elhouda, Yahiaoui Bilal, Lehad Arezki, Djenaoui Anfel, Mahdid Imane, Neggaz Rahil. Valorisation des ressources naturelles cas de cépages des vignes en Algérie. Poster presentation. 1er séminaire national sur la biodiversité de la Faune et la Flore en Algérie (SNBFFA-1). 29-30 November 2022. Constantine Algeria.