UNIVERSITAT POLITÈCNICA DE VALÈNCIA

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Preservation and Improvement of Valencian Agro-diversity University Research Institute (COMAV)

Pathogenicity and resistance mechanisms of blue lupin (Lupinus angustifolius) accessions upon infection with Fusarium oxysporum f. sp. lupini

Master's Thesis

Erasmus Mundus Master Programme in Plant Breeding - emPLANT +

AUTHOR: Rayo Flores, Camila Andrea

Tutor: Gramazio, Pietro

External cotutor: Svoboda, Thomas

ACADEMIC YEAR: 2023/2024

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Pathogenicity and resistance mechanisms of blue lupins (*Lupinus angustifolius*) accessions upon infection with *Fusarium oxysporum* f. sp. *lupini*

Master Thesis

Erasmus Mundus Master Programme in Plant Breeding - emPLANT +

AUTHOR: Rayo Flores, Camila Andrea
SUPERVISOR: Thomas Svoboda
TUTOR: Gramazio, Pietro
ACADEMIC YEAR: 2023/2024
VIENNA, AUSTRIA
JULY 2024

Master thesis

Pathogenicity and resistance mechanisms of blue lupin (*Lupinus angustifolius*) accessions upon infection with *Fusarium oxysporum* f. sp. *lupini*

submitted by

Camila RAYO

in the framework of the international Master programme

Erasmus Mundus Master's Program in Plant Breeding (emPLANT+)

in partial fulfilment of the requirements for the academic degree

Master of Science

Vienna, July 2024

Supervisor
DDipl.Ing. Dr.nat.techn. Thomas Svoboda MSc.
Institute of Microbial Genetics (IMiG)
Dept. of Applied Genetics and Cell Biology (DAGZ)
University of Natural Resources and Life Sciences, Vienna (BOKU)

Tutor
Dr. Pietro Gramazio
Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV)
Dept. of Biotechnology
Universitat Politècnica de València, Valencia (UPV)

AFFIDAVIT

I hereby declare that I have authored this dissertation independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature, as well as those which were generated using artificial intelligence tools, are duly identified, and cited, and the precise references included. Any contribution from colleagues is explicitly stated in the authorship statement of the published papers.

I further declare that this dissertation has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree.

I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, 07.2024

Camila Andrea RAYO FLORES

RESUMEN

El lupino azul (*Lupinus angustifolius*) es un cultivo subestimado pero valioso por su alto contenido de proteínas, adaptabilidad ambiental y fijación simbiótica de nitrógeno; sin embargo su productividad se ve afectada por enfermedades fúngicas, siendo la marchitez, causada por *Fusarium oxysporum f.* sp*. lupini* (Fol), una de las amenazas más significativas para el cultivo de lupino en Europa, ya que disminuye considerablemente el rendimiento del cultivo. Este estudio evaluó 20 accesiones no caracterizadas de *L. angustifolius* en dos rondas de cribados experimentales mediante PCR en tiempo real (qPCR) para determinar su resistencia contra Fol. Plántulas de 3 días fueron infectadas con una concentración de $5x10^4$ esporas*mL^{-1}; a los 4 días las radiculas se liofilizaron para la posterior extracción de ADN y análisis mediante qPCR, calculando un ratio basado en el valor del ciclo de cuantificación (Cq) para cada accesión (Cq Lupino/Cq Fusarium) con la finalidad de discriminar la susceptibilidad de cada una de las accessiones frente a Fol. Bajo esta premisa, las accesiones L26 y L49 fueron identificadas como las más resistente y susceptible, respectivamente. Análisis posteriores a nivel de ADN genómico y de RNA-Seq permitirán la identificación de genes de resistencia y susceptibilidad, ampliando el entendimiento de las interacciones Fol-Lupino, así como las rutas metabólicas implicadas en el mecanismo de defensa. Los conocimientos obtenidos de este estudio no solo proponen el uso del método de cribado con qPCR como una herramienta de detección temprana de infección de Fol en lupinos para determinar niveles de susceptibilidad, sino que también proporcionan información valiosa para desarrollar estrategias de mejora que mitiguen enfermedades relacionadas con *Fusarium* y, en última instancia, mejoren la resiliencia y productividad de los cultivos de lupino y otras leguminosas.

Palabras clave: *Lupinus angustifolius*, Fusarium, interacciones hospedero-patógeno, mecanismo de resistencia, genómica, ARN-seq.

ABSTRACT

The blue lupin (*Lupinus angustifolius*) is an undervalued but valuable crop due to its high protein content, environmental adaptability, and symbiotic nitrogen fixation. However, its productivity is affected by fungal diseases, with Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lupini* (Fol), being one of the most significant threats to lupin cultivation in Europe, as it considerably reduces crop yield. This study evaluated 20 yet uncharacterized accessions of *L. angustifolius* in two rounds of experimental screenings using real-time PCR (qPCR) to determine their resistance to Fol. Three-day-old seedlings were infected with a concentration of $5x10^4$ spores*mL^{-1}; after 4 days, the radicles were lyophilized for subsequent DNA extraction and qPCR analysis, calculating a ratio based on the quantification cycle (Cq) value for each accession (Cq Lupin/Cq Fusarium) to discriminate the susceptibility of each accession to Fol. Under this premise, accessions L26 and L49 were identified as the most resistant and the most susceptible, respectively. Subsequent genomic DNA and RNA-Seq analyses will allow the identification of resistance and susceptibility genes, enhancing the understanding of Fol-lupin interactions and the metabolic pathways involved in the defense mechanism. The insights gained from this study not only propose the use of the qPCR screening method as a tool for early detection of Fol infection in lupins to determine susceptibility levels but also provide valuable information for developing improvement strategies to mitigate Fusarium-related diseases and ultimately enhance the resilience and productivity of lupin and other legume crops.

Keywords: *Lupinus angustifolius*, Fusarium, host-pathogen interactions, resistance mechanism, genomics, RNA-seq.

ACKNOWLEDGEMENTS

I would like to express my gratitude to everyone who contributed to the completion of this thesis. First of all, I want to thank my supervisor, Dr. Thomas Svoboda, for giving me the possibility to do my master's thesis and for all the guidance, feedback, and continuous encouragement during this journey. I would like to extend this gratitude to Dr. Joseph Strauss for allowing me to use the facilities of the Department of Applied Genetics and Cell Biology (DAGZ) at BOKU University. Thanks also to Dr. Fred Eickmeyer from ESKUSA for providing the plant material to develop this master thesis.

My gratitude stands with BOKU University and the Polytechnic University of Valencia (UPV) for providing the resources and facilities to develop my thesis project. I am grateful to the Erasmus emPLANT + Program for the financial support during these two years and for helping me pursue my aspirations in science.

This experience would not have been the same without all the friends I made at AG Strauss; thank you for being so kind and friendly to me since day one and for giving me valuable advice on my experiments, but most importantly, for being there to listen, support and make me laugh. Thanks, Flo, Matti, Franz, and Paulina, especially for being such good friends.

Quisiera agradecer de todo corazón a mi supervisor en la UPV, Pietro, siempre atento, empático y supportive. Gracias por todo el apoyo emocional y técnico durante todo este proceso. A mis amigas Zully, Natalí, Mechi, Kritika, Patri, Hayu, Pau, Mimi por siempre escuchar, dar buenos consejos y, sobre todo, ser mi soporte durante esta etapa tan dura. Quererlas es poco.

A mi familia, decirles gracias sería quedarme corta. Extrañarlas por estos dos años no ha sido para nada fácil. Mami, mamita C, tía Sandra, tía Vero, gracias por siempre ayudarme a perseguir mis sueños, por creer en mí incluso en los momentos que yo no lo hacía. Las amo. Se que papá, papito y mamatía estarían tan felices.

Gracias a toda mi familia y amigos que estuvieron apoyándome todo este tiempo, en especial a Mónica, Jose por hacerme parte de su familia y a mi hermanita Yadhi, te debo muchísimo tiempo.

Zu guter Letzt möchte ich meinem besten Freund und Partner danken, meinem Schatzi, Kili. Danke für all die glücklichen und nicht so glücklichen Momente, dafür, dass du mir zugehört hast, dich um mich gekümmert hast, als ich krank war. Seit Anfang an bringst du mich zum Lachen, und wenn ich traurig war oder einen schlechten Tag hatte, hast du mir Mango aus Peru gebracht. Das weiß ich sehr zu schätzen. Ohne deine Liebe und ständige Unterstützung hätte ich es nicht geschafft. Trotz aller Schwierigkeiten ist das Leben wunderbar, seitdem ich dich kenne. ild.

Hacer esta maestría fue una de las experiencias más desafiantes que he atravesado, con muchas dudas y lágrimas, pero me ha demostrado la fortaleza que tengo, que soy capaz de hacer todo lo que me proponga y que no hay sueños grandes cuando el esfuerzo es mayor.

TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS

Fusarium oxysporum f. sp. lupini (Fol)
Biological nitrogen fixation (BNF)
First screening (S1)
Second screening (S2)
Differentially expressed genes (DEG)

RELACIÓN CON LOS OBJETIVOS DE DESARROLLO SOSTENIBLE DE LA AGENDA 2030

Objetivos de Desarrollo Sostenibles	Alto	Medio	Bajo	No Procede
ODS 1. **Fin de la pobreza**				X
ODS 2. **Hambre cero**	X			
ODS 3. **Salud y bienestar**	X			
ODS 4. **Educación de calidad**				X
ODS 5. **Igualdad de género**				X
ODS 6. **Agua limpia y saneamiento**				X
ODS 7. **Energía asequible y no contaminante**				X
ODS 8. **Trabajo decente y crecimiento económico**				X
ODS 9. **Industria, innovación e infraestructuras**			X	
ODS 10. **Reducción de las desigualdades**				X
ODS 11. **Ciudades y comunidades sostenibles**				X
ODS 12. **Producción y consumo responsables**		X		
ODS 13. **Acción por el clima**			X	
ODS 14. **Vida submarina**				X
ODS 15. **Vida de ecosistemas terrestres**	X			
ODS 16. **Paz, justicia e instituciones sólidas**				X
ODS 17. **Alianzas para lograr objetivos**			X	

Este estudio se alinea estrechamente con varios Objetivos de Desarrollo Sostenible (ODS), particularmente con el Hambre Cero (ODS 2), Salud y Bienestar (ODS 3), y Vida de Ecosistemas Terrestres (ODS 15). Al identificar accesiones de lupino azul (*Lupinus angustifolius*) con potencial resistencia a *Fusarium oxysporum* f. sp. *lupini* (Fol), un importante patógeno fúngico, el estudio impacta directamente en los rendimientos de los cultivos y la seguridad alimentaria. El desarrollo de variedades de lupino resistentes a Fol, a través de programas de mejoramiento, contribuye a una producción estable y aumentada de una legumbre rica en proteínas, esencial para la nutrición humana y animal. Los lupinos son leguminosas ricas en proteínas y nutrientes esenciales y juegan un papel vital en la salud humana en la mitigación de enfermedades como la anemia, particularmente en países en vías de desarrollo. Además, la capacidad de los lupinos para adaptarse a diversas condiciones ambientales, mejorar la calidad del suelo y fijar nitrógeno apoya prácticas agrícolas sostenibles que protegen la biodiversidad y mantienen ecosistemas saludables.

Adicionalmente, el estudio apoya el Consumo y Producción Responsables (ODS 12) al promover prácticas de producción sostenible de lupinos, que requieren menos insumos de nutrientes y reducen la necesidad de fungicidas químicos. El uso de técnicas avanzadas como qPCR y análisis de transcriptomas para evaluar la resistencia de los lupinos impulsa la innovación en la investigación agrícola, alineándose con Industria, Innovación e Infraestructura (ODS 9). El desarrollo de cultivos resistentes a enfermedades también contribuye a la resiliencia agrícola y la mitigación del cambio climático (ODS 13). Si bien los esfuerzos colaborativos y el intercambio de conocimientos (ODS 17) son inherentes a la investigación científica, el enfoque principal sigue siendo la mejora de los resultados agrícolas y la sostenibilidad.

I. INTRODUCTION

1. THE CROP: *Lupinus angustifolius*

The genus *Lupinus* has been tracked in agricultural practices for over 4000 years. Domestication of *Lupinus angustifolius* for green manure production began in the Baltic region in the 1860s, and subsequently in Germany. However, their development into agricultural crops occurred in Europe and Australia, with numerous varieties cultivated mainly in Germany and Australia from the 1930s to the 1970s. This species was initially native from the Mediterranean regions of Europe, Africa and Asia, but widely distributed around the world (Kurlovich, 2002; IUCN, 2014; Rojas-Sandoval, 2022).

Lupinus angustifolius, commonly known as blue lupins or narrow-leafed lupin or Australian sweet lupin, is a fast-growing annual grain legume species from the Fabaceae family. This herbaceous plant can reach heights of 0.3 to 1.5 meters, featuring hairy stems and palmately compound leaves with five to nine linear-narrow leaflets. The flowers, arranged in terminal racemes, stand out for their characteristic blue-ish color, although some varieties produce white flowers. The fruit is a legume pod measuring five to seven centimeters long and containing five to six hard-coated seeds of 7-8mm long. The seeds show variability in coloration, ranging from white to brown or mottled, and they are capable of remaining viable in the soil for up to 20 years. Figure 1 shows the lupin flowers and seeds. (FAO, 2017; Heuzé et al., 2019; Rojas-Sandoval, 2022).

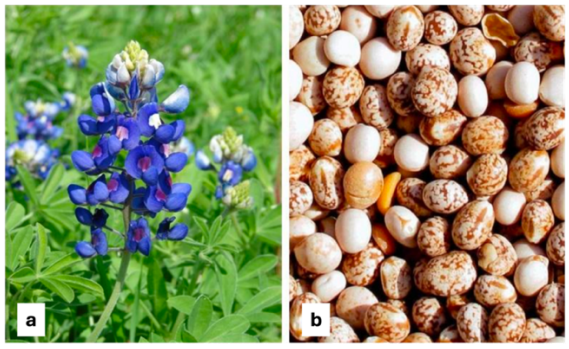

Figure 1. *a: Lupin flowers, b: Lupin seeds (Photography by Diaz, 2015). Retrieved from https://researchgate.net. Licensed for non-commercial reuse*

Lupinus angustifolius is a diploid species with 40 chromosomes (2n = 40). Like most species within the genus, blue lupins are self-compatible, reproducing almost exclusively through self-pollination and reaching maturity in 105-150 days. This species is well adapted to a wide range of environmental conditions, highlighting its tolerance to low temperatures during the vegetative state. In Australia, blue lupins are sown between late April and harvested in October, thriving with annual rainfall of around 500 mm (Australian Government, 2013; Pulse Crop Database, 2024; Roman et al., 2023; White et al., 2008)

Grain legumes play an important role in human and animal nutrition, as they represent valuable sources of protein and essential nutrients. Lupins stand out among legumes due to its capacity to produce high proportions of protein. According to the Australian Government

(2013), the amount of protein of *L. angustifolius* can be up to 32%, while the crude fiber and fat 15% and 6% respectively. Figure 2 shows a comparison of crude protein content among *Pisum sativum*, *Vicia faba*, and *L. angustifolius*, in which blue lupins have the highest protein content.

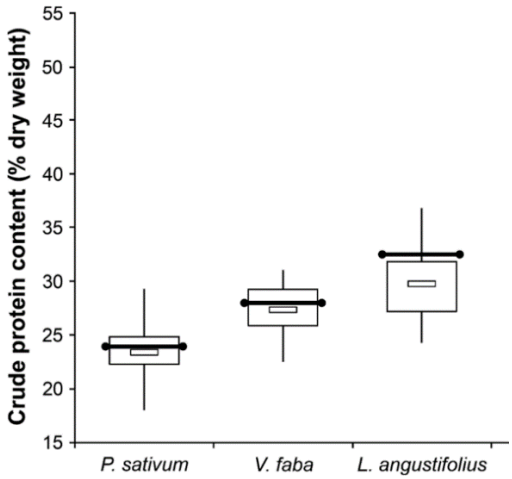

Figure 2. *Seed crude protein content of three legumes (Schumacher et al., 2011)*

1.1. Agronomic importance and environmental impact

Lupins are known for their ability to adapt to a wide range of environmental conditions, making them a valuable crop for protein production. One of the primary beneficiaries of high-protein lupin production is the livestock farming industry, which uses lupins as an alternative to oilseed-based meals. They can be used in various forms, such as concentrate or as forage (Borreani et al., 2009; Notz & Reckling, 2022; Schrenk et al., 2019; Sedláková et al., 2016). As food crop, lupin consumption has been historically concentrated in the Mediterranean region and Andean highlands of South America. Lawrance (2007), estimated that only 4% of lupin production was grown for human consumption.

In addition to their nutritional benefits, lupins are well-suited to thrive in poor and unproductive soils having an important role as soil enhancer due to its deep taproot which improve soil structure and drainage (Heuzé et al., 2019). Furthermore, lupin production requires lower nutrient inputs, particularly phosphorus (P), compared to other major crops like wheat and canola, as studies have shown that to achieve 90% of maximum grain yield, lupin needed approximately 67% less P than wheat and 75% less P than canola (Bolland & Brennan, 2008)

Like all legumes, lupins possess the ability for biological nitrogen fixation (BNF) through the formation of root nodules that establish a symbiotic relationship with compatible soil bacteria from the genus *Bradyrhizobium* (Lucas et al., 2015). Jansen (2006) reported that, besides promoting biodiversity, lupins can fix between fix 300-400 kg N/ha, in Europe and Australia. Moreover, BNF has a positive impact in crop rotation systems. For decades, Western Australia has practiced a wheat:lupin rotation, utilizing lupin residues for sustainable wheat production and livestock grazing (Abraham et al., 2019; Gresta et al., 2017). Lupins, like *L.*

albus, can be used as winter crops for phytoremediation in contaminated soils, improving soil quality through increased live bacteria, bioavailable metal fractions, and green manure, when rotated with metal-accumulating crops like industrial hemp (Fumagalli et al., 2014).

Apart from the use as fodder and food crop, several lupin species hold significant ornamental value for the horticultural industry due to their visually attractive and vibrant color of its flower spikes (Rojas-Sandoval, 2022).

1.2. Lupin production

Among the 10 grain legume crops acknowledged by the Food and Agriculture Organization of the United Nations (FAO), lupins constitute about 1% of the total production, and are frequently grown in areas where other pulses like beans, chickpeas, cowpeas, and peas are not typically cultivated (Gresta et al., 2017). Worldwide, *L. albus, L. angustifolius, L. luteus and L. mutabilis* are the four lupin species that have gained agricultural importance being cultivated on a commercial scale for food and feed purposes (Schrenk et al., 2019).

According to the most recent data from FAOSTAT, the global lupin production totals 1.65 million tons, being *L. angustifolius* the most widely cultivated species, accounting for more than 1.3 million tons (79%). Moreover, lupin production in Europe represents 34.7% of the global total (Figure 3), standing at approximately 0.57 million tons (FAOSTAT, 2023; Gresta et al., 2017).

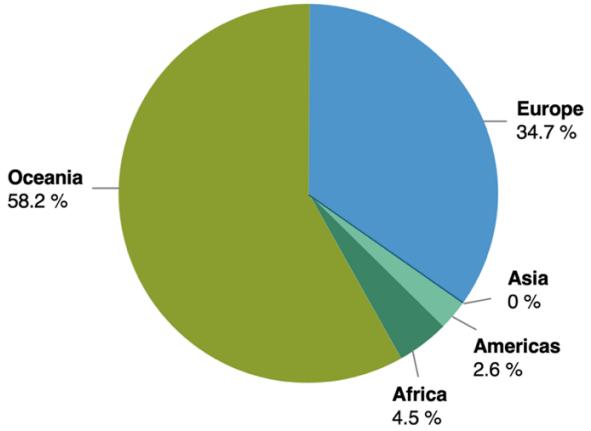

Figure 3. *Worldwide distribution of lupin production (Source: FAOSTAT, 2023)*

Australia has dominated lupin production over the last few decades, being able to almost doubled Europe's harvested area and production levels for this crop. However, European lupin production has also been on an upward trajectory, as evident from Figure 4, exhibiting a growing trend. As of 2022, lupins are cultivated across approximately 315,851 hectares in Europe. Poland, the Russian Federation, Germany, and Greece are the major lupin-producing countries, with each cultivating more than 10,000 hectares (FAOSTAT, 2023).

As shown in Figure 5, within the European Union, Poland leads the lupin production, primarily cultivating blue lupin (*L. angustifolius*) and yellow lupin (*L. luteus*), followed by Germany, where the blue lupin is the predominant species grown, both under conventional and organic farming systems (Notz & Reckling, 2022; Schrenk et al., 2019).

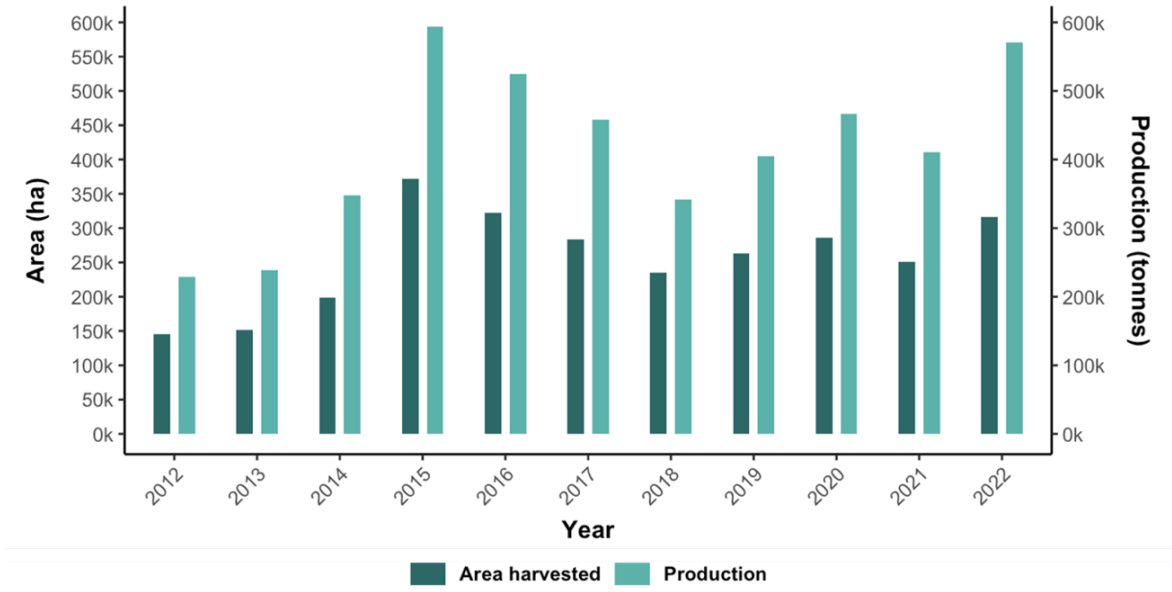

Figure 4. *Lupin cultivated areas and production in Europe (Source: FAOSTAT, 2023)*

Europe's dependence on imported soybeans could be mitigated by utilizing native European legumes, such as white, yellow, and blue lupins, as viable alternatives, thanks to their high-quality protein content, potential health benefits, sustainable production practices, and consumer acceptability (Lucas et al., 2015).

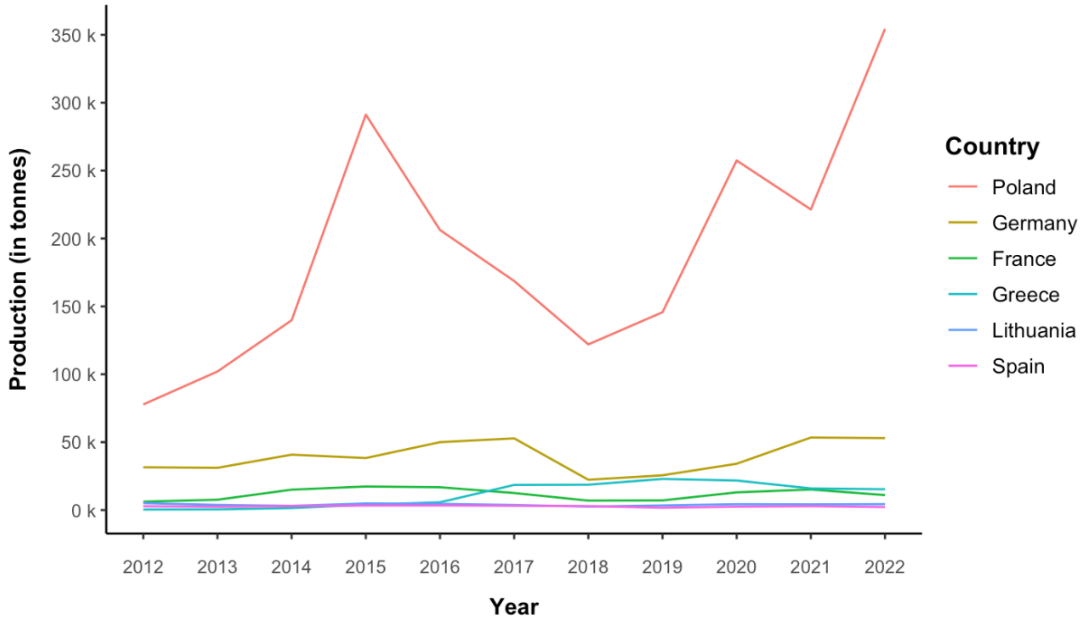

Figure 5. *Lupin production in EU countries in the period 2012-2022 (Source: FAOSTAT, 2023)*

1.3. Current challenges and perspectives of lupin cultivation

The widespread cultivation of *L. angustifolius* faces mainly three major challenges: low and unstable yields, the presence of bitter and toxic alkaloids, and susceptibility to diseases and pests. While in Australia, the highest yields are achieved by winter-hardy autumn-sown

varieties, which can yield 2.5-4.0 t/ha under good conditions, being comparable to the typical yield range for soybeans of 3-4.5 t/ha, the situation is different in Europe (Kingwell, 2003). In European countries such as Poland and Germany, lupin yields typically do not exceed 1.5 t/ha. Consequently, breeding efforts have focused on improving yield stability as a priority (IAFE-NRI, 2021; Schrenk et al., 2019).

Alkaloid content has been a significant concern due to their toxicity, preventing direct consumption of fresh lupins as food. However breeding programs have successfully developed varieties with low alkaloid (QAs) content, known as "sweet lupins," with levels generally below 500 mg/kg (Roman et al., 2023). The availability of genetic and genomic resources now offers opportunities to ensure alkaloid levels remain below industry limits, improving the quality of this high-protein grain legume. Moreover, novel techniques like marker-assisted selection and genomic tools are expected to be used to maintain and further reduce alkaloids levels (Abraham et al., 2019; Frick et al., 2017).

Fungal diseases represent the most significant biotic threat to lupin cultivation and productivity, being anthracnose, caused by *Colletotrichum lupini*, the most important. This disease affects all *Lupinus* species worldwide. Anthracnose symptoms include twisting stems, lesions with orange conidia, seeds becoming brown and wrinkled, and seedlings experiencing necrosis, potentially causing significant yield losses. Despite recent progress in genomics, the limited gene pool for resistance poses a risk of pathogens becoming resistant (Bebeli et al., 2020; Talhinhas et al., 2016). In comparison to other cultivated lupins, *L. angustifolius* is proved to be more resistant than *L. mutabilis*, *L. albus* and *L. luteus* (Gresta et al., 2017).

Furthermore, Fusarium wilt and root rot (*Fusarium spp.*) are considered one of the main seed-borne fungal diseases affecting lupins. *F. avenaceum*, *F. acuminatum*, *F. redolens*, *F. solani*, and *F. oxysporum* have been identified as aggressive pathogens, with severe infections leading to substantial yield reduction. Symptoms include root rot, wilting, chlorosis followed by necrosis, potentially causing plant death (Holtz et al., 2013).

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a rising threat to lupins, especially dense crops in Western Australia and Western Europe. It infects reproductive structures like pods, causing up to 25% yield loss, and basal stems, with long-lived sclerotia in the soil contaminating future crops. Integrated management and ongoing research on key influencing factors is crucial for effective mitigation (DPIRD, 2023).

On the other hand, pleiochaeta root rot and brown spot, caused by *Pleiochaeta setosa*, can lead to dramatic yield reductions in lupins, with no known resistance sources. In narrow crop rotations, this disease has become a problem causing yield losses, while in wider rotations, its impact is generally limited to slight yield reductions during ripening (Bebeli et al., 2020; Gresta et al., 2017). There are still several challenges to lupin breeding, including the improvement of resistance to fungal pathogens as well as both quantitative and qualitative traits.

2. THE PATHOGEN IN OUR STUDY: *Fusarium* – A major player in plant pathology

The genus *Fusarium* belongs to the phylum Ascomycota which includes a diverse group of filamentous fungi that can be found in soil and plant debris. These fungi are of significant importance in human, animal, and, especially, plant pathology due to their ability to cause a wide range of diseases in economically important crops worldwide. In agriculture, *Fusarium* is capable of infecting various plant parts, including roots, stems, leaves, and reproductive structures, leading to quality reduction and significant yield losses. The impact of Fusarium wilt on food security and agricultural productivity has positioned this genus as a key focus of plant pathology research and disease management strategies (Askun, 2018).

A notable characteristic of *Fusarium* species is the emergence of host-specific forms known as *formae speciales* (f.sp.), which exhibit a high degree of host adaptation, further complicating disease management by overcoming resistance mechanisms in host plants and rapidly adapt to new host genotypes (Babadoost, 2018). Up to 2018, Edel-Hermann & Lecomte (2019) reported 201 *formae speciales* from which 106 were considered well documented. It is important to understand the genetic diversity, host specificity, and pathogenicity mechanisms of these fungi in order to develop successful disease control strategies, such as breeding for resistance, adopting cultural practices (Ekwomadu & Mwanza, 2023).

Within the *Fusarium* genus *F. oxysporum* is one of the most important and highly variable species. Its substantial populations are widely distributed, ranging from native plant communities to cultivated areas, where it can aggressively colonize the root cortex. *F. oxysporum* has attracted considerable attention due to its wide range of hosts, including dicotyledonous and monocotyledonous plants, leading to economic losses from its pathogenic strains (Edel-Hermann & Lecomte, 2019; Lindbeck, 2009).

2.1. *Fusarium oxysporum* f.sp. *lupini* (Fol) as a plant pathogen

One notable *forma specialis* within the *F. oxysporum* complex is *F. oxysporum* f.sp. *lupini*, a significant pathogen of lupins (*Lupinus spp.*). The *forma specialis* is responsible for causing Fusarium wilt in lupins, leading to substantial economic losses (Formela-Luboińska et al., 2020; Zian et al., 2013). Armstrong and Armstrong (1964) identified three distinct races of Fol on European isolates, each exhibiting specificity towards different lupin species. Race 1 infected *L. luteus* (yellow lupin) and some varieties of *L. albus* (white lupin), however, did not cause disease in *L. angustifolius* (blue lupin). Race 2 was pathogenic to yellow lupin and all varieties of white lupin, while remaining non-pathogenic to blue lupin. Race 3, however, could harm both *L. angustifolius* and *L. albus* but was non-pathogenic to *L. luteus* (Lindbeck, 2009).

2.2. Infection Biology – Life Cycle and Characteristics

F. oxysporum f.sp. *lupini* uses a two-phase attack strategy and is hemibiotrophic. At first, it exhibits a biotrophic phase, inhibiting the host's immune system as invasive hyphae move through the plant's tissues. Subsequently, it transitions to a necrotrophic phase, releasing

toxins and enzymes to eliminate the host cells, allowing the fungus to obtain nutrients from the dead tissues (Formela-Luboińska et al., 2020).

The fungi invade the host roots, moving through the cortex and entering the xylem vessels. Afterwards, it spreads systemically by the vascular system. The mycelium branches within the xylem, producing microconidia that move upwards until germinating and penetrating nearby vessels. As the infection progresses, vessel blockage weakens the plant, resulting in wilting, leaf death, and potentially the death of the entire plant. The fungus abundantly sporulates on the surface, and the spores are dispersed by wind, water, or movement of soil/plant debris (Lindbeck, 2009).

The resilience of *F. oxysporum* is attributed to its ability to survive as mycelium and chlamydospores in soil, seeds, and infected crop debris. Chlamydospores can remain dormant or live saprophytically in the absence of a host plant (Figure 6). Warm and arid soil environments, with temperatures ranging between 22°C and 25°C, provide ideal conditions for the disease to thrive (Singh et al., 2007).

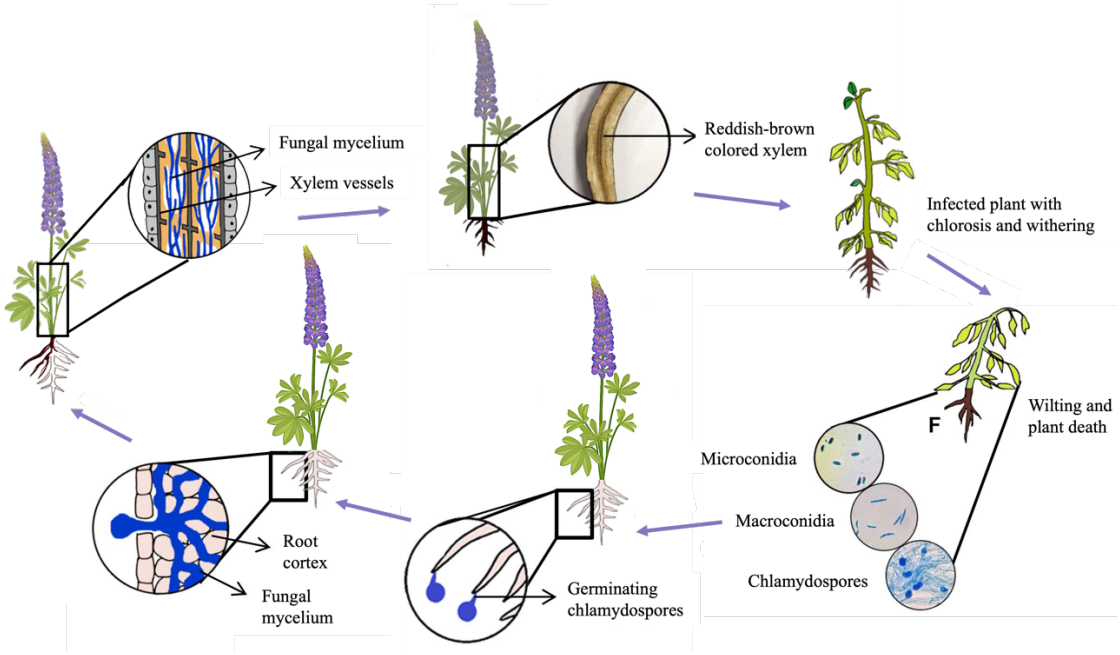

Figure 6. *Disease cycle of Fusarium oxysporum. Adapted from Jangir et al., 2021*

3. THE INTERACTION: *F. oxysporum* f.sp. *lupini* (Fol) - *L. angustifolius*

The infection of this pathogen (Fol) first manifests during the vegetative phase with leaf darkening, progressing to more severe wilt symptoms during flowering or budding. As the disease advances, leaves dry up and quickly lose their leaves, while infected roots may show few symptoms, except for a brown area under the epidermis (Figure 7). Ultimately, the disease leads to plant death, especially in drainage areas (Lindbeck, 2009). Fol is a major disease-causing agent, leading to considerable decreases in seed yield and quality, and has been a significant breeding target in Germany, where attention has been given to developing resistance against Fusarium wilt (Gresta et al., 2017).

Figure 7. *Fusarium wilt disease in lupins.* ***a****: Fusarium inducing yellowing and stunting symptoms in adult plants.* ***b****: Fusarium causing defoliation in lupins at later growth stages.* ***c****: Infected plants exhibit discoloration and spore masses on root surfaces.* ***d****: Fol symptoms on roots. Source a, b & c: Hwang et al., 2014, d: CABI, 2022*

3.1. Fusarium wilt disease control

Accurate pathogen identification is essential for mitigating Fusarium wilt in lupins. Lindbeck (2009) describes three Fol detection methods: firstly, morphological identification of *F. oxysporum*, which confirms its presence but not the *formae speciales*. On the other hand, the host-based method will identify the *formae speciales* in which a positive Fol test indicates susceptibility in lupins but resistance in other pulse crops. Finally, PCR-based identification method, which was only available for *F. oxysporum* f. sp. *ciceris*. However, Zian et al., (2013) describe RAPD-PCR analysis and the use of RAPD-markers to determine the genetic relationships on resistance among lupin cultivars.

Fusarium wilt management in lupins requires an integrated approach involving crop rotation with non-host species, soil amendment with organic matter, such as wheat or barley straw, to promote antagonistic soil microbes and the use of clean seeds (Gresta et al., 2017). Seed treatments and foliar fungicides can partially control the disease by reducing seed-borne inoculum but are ineffective against late-season infections and unable to eradicate the pathogen, making their routine use cost-ineffective for complete disease management (Lindbeck, 2009). Moreover, cultural practices like deep ploughing and removal of infected crop debris can reduce inoculum levels (Haware, 1998). Soil solarization can control the disease, also improving plant growth and yield, but is impractical for large-scale farming operations (Chauhan et al. 1988).

Although there are currently no commercial biological control agents that control Fol, there are biocontrol agents that could be potentially effective against Fusarium wilt. Mohamed et al. (2012) reported that treating lupins with biocontrol agents like *Pseudomonas fluorescens* and *P. putida* can induce pathogenesis-related (PR) protein production in seedlings upon Fol infection, representing a promising strategy for developing induced tolerance against Fusarium wilt. Moreover, Abd El-Rahman et al., (2012) identified *P. fluorescens* and potassium chloride (KCl) as effective biotic and abiotic seed treatments against Fusarium wilt in lupins, enhancing defense-related enzymes, phenolics, flavonoids, and crop yield compared to untreated controls. These findings could offer an eco-friendly and complementary approach for managing soil-borne pathogens as *F. oxysporum*.

Wahid (2006) demonstrated the efficacy of *Trichoderma pseudokoningii* and *Bacillus subtilis* as seed treatments in inhibiting the growth of Fol. A combination of these two biocontrol agents provided better control of Fusarium wilt in lupins compared to their individual applications or a fungicide treatment. Additionally, research by Shaban & Zian (2011) revealed that biofumigation using mustard and canola seed amendments effectively controlled Fol.

Understanding the pathogen's mechanisms of pathogenicity, host specificity, and virulence factors is crucial for developing successful disease management strategies integrated with agricultural practices. However, these integrated approaches are generally less effective than using resistant crop varieties.

3.2. Resistant breeding for *L. angustifolius* against Fol

Developing lupin varieties resistant to Fusarium wilt is essential to prevent yield losses, ensuring sustainable production, and meeting the growing demand for plant-based proteins. Breeding for genetic resistance offers an effective long-term strategy against this persistent pathogen, as resistant varieties are considered the most efficient for disease management and eradication. Resistant cultivars can reduce dependence on chemical control, leading to more sustainable and eco-friendly lupin production systems while maintaining yield, quality, and economic viability (Abraham et al., 2019; Kupstou et al., 2002).

This research aims to provide relevant insights for developing breeding strategies aimed at enhancing Fusarium wilt resistance in *L. angustifolius*, as well as contribute to the improvement of crop resilience and productivity in blue lupins.

II. OBJECTIVES

This master thesis is conducted in the frame of the Legume Generation Project “Boosting innovation in breeding for the next generation of legume crops for Europe” which aims to boost the breeding and competitiveness of major food and feed legume crops in Europe, including lupins. The focus of the project is on accelerating the production of novel germplasm and developing improved cultivars directly addresses the need for high-yielding, resilient protein crop varieties (European Commission, 2023). The global shift towards plant-based proteins aligns with cultivating lupins as a valuable protein crop meeting human and animal feed demands. Its low-input requirements and potential market for lupin-based products promotes its sustainable production as an alternative protein source (Lucas et al., 2015; Roman et al., 2023).

This study aims to unravel the resistance mechanisms of blue lupins upon infection with *Fusarium oxysporum* f. sp. *lupini* and its pathogenicity. To achieve this objective, screening analyses were conducted on 20 yet uncharacterized *Lupinus angustifolius* accessions to assess resistant levels by utilizing genomic analyses. This research will:

- Address key aspects of the pathogen-host interaction by assessing Lupin/Fusarium DNA ratios as potential indicators for resistance, contributing to the development of diagnostic tools for resistance screening.
- Provide insights through transcriptomic analysis on highly resistant and susceptible *L. angustifolius* accessions by comparing the differences between the genomes.

The long-term objective of the major project is to identify and investigate specific genes and elucidate the molecular pathways associated with resistance to Fusarium wilt in blue lupin, thereby providing valuable insights for breeding programs to develop more resilient cultivars

III. MATERIALS AND METHODS

1. LUPIN GERMINATION AND GROWTH

1.1. Plant material: *L. angustifolius* accessions

The lupin seed accessions were provided by ESKUSA GmbH, a company based in Bavaria (Germany), that initiated a blue lupin breeding program in 2015. In the same year, they took part in the LupiZAV project funded by the *Bundesanstalt für Landwirtschaft und Ernährung* (BLE/BMEL). As part of this project, the N.I. Vavilov Institute of Plant Industry in St. Petersburg provided ESKUSA with 81 accessions of *L. angustifolius*. These accessions were propagated for maintenance on a light sandy soil (approximately 30 soil points, pH 6.3) at 319 m.a.s.l. By the end of 2017, only 74 accessions had sufficiently large individual plant progeny (Eickmeyer, 2023; Zeise et al., 2018). The LupiZAV project aimed to assess and record pH tolerance, phenotypic characteristics, and agronomic traits, with the most important traits listed in Table 1.

Table 1. *Traits recorded over the LupiZAV project period and their characteristics*

Trait	Characteristic/classification
Flower color	Blue, violet, pink, white
Maturity	Early, mid-early, mid-late, late
Leaf color	very light yellow-green, very dark green
Height	Tall, small
Growth type	Determinate, indeterminate (branched)
Start of branching	At the base, from the middle
Branch slope	Horizontal, perpendicular
Stem inclination	High, low
Burst resistance of Pod	Low, medium
Seed color	Anthracite, mottled (multicolor), white
Yield potential	High, low
Wilting symptoms	Conspicuous, inconspicuous
Anthracnose symptoms	Conspicuous, inconspicuous
pH tolerance	Low, medium
Varieties used as reference	Azure, Boregin, Boruta

Table adapted from Zeise et al., 2018

As part of the Legume Generation project (www.legumegeneration.eu), the German company ESKUSA GmbH provided seeds from 20 uncharacterized *L. angustifolius* accessions listed in Table 2, in which the previous annotations from some accessions can be found (Eickmeyer, 2023; Zeise et al., 2018). The seeds from each accession are shown in Figure 8. The plant material was received, and experiments were conducted in the Department of Applied genetics and Cell Biology (DAGZ) at the Institute of Microbial Genetics (IMiG) department at BOKU, Tulln (Austria).

Table 2. *Accessions of L. angustifolius used in this study*

ID Accession	Seed color	Previous annotations (Retrived from Eickmeyer, 2023; Zeise et al., 2018)
LuB 3	Mottled	-
LuB 4	Mottled	-
LuB 18-1	Mottled	-
LuB 19	Mottled	-
LuB 23	Mottled	-
LuB 26	Mottled	High protein value Tall-growing and stable
LuB 28	Mottled	-
LuB 30	Mottled	-
LuB 37-1	Mottled	-
LuB 46	Mottled	Perform better at soil pH 8 Lime-tolerant
LuB 47	White	-
LuB 49	Mottled	-
LuB 54	White	Seed quality May exhibit reduced susceptibility to anthracnose
LuB 55-1	White	
LuB 57	Mottled	High protein value
LuB 58	White	May exhibit reduced susceptibility to anthracnose
LuB 64	White	May exhibit reduced susceptibility to verticillium wilt
LuB 66	White	Seed quality
LuB 80	Mottled	High protein value Tall-growing and stable
LuB 81	White	-

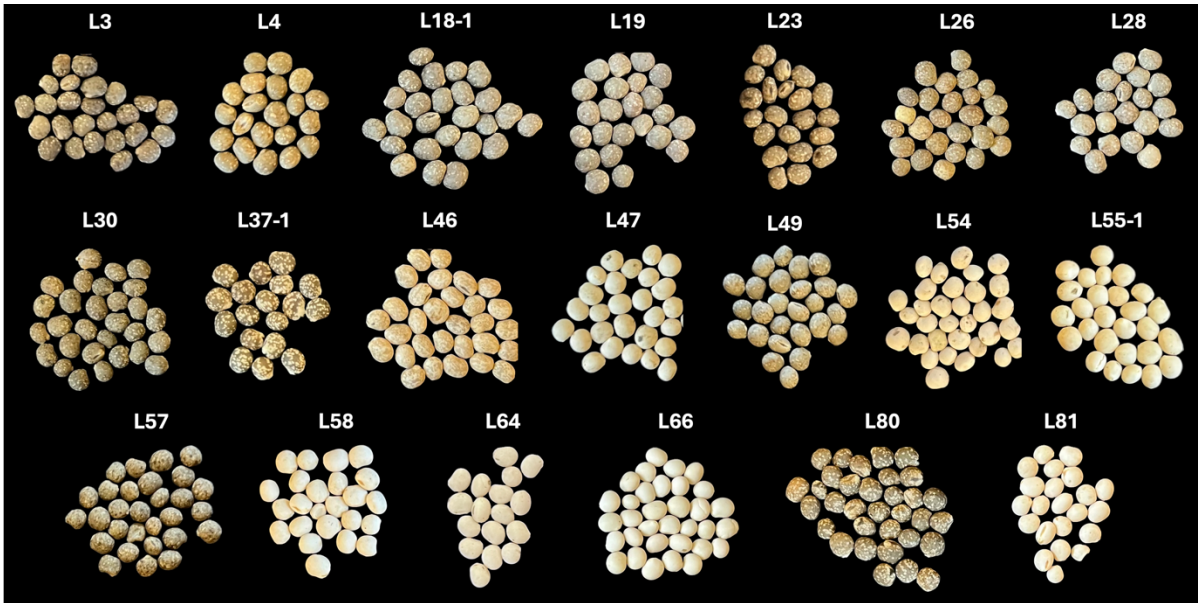

Figure 8. *Visual appearance of the seeds from the 20 blue lupin accessions*

To simplify experimentation, all accessions will be referred to using a standardized naming convention. Each accession will be identified by the letter "L" followed by its corresponding number.

1.2. Lupin germination and growth

1.2.1. Sterilization of lupin seeds

Lupin seeds from each cultivar were sterilized in 50 mL Falcon™ tubes. 10 seeds were rinsed with 25 mL of 75% ethanol (EtOH) for one minute, followed by immersion in a 10% sodium hypochlorite (NaClO) solution for 15 minutes with gentle circular movements. After discarding NaClO, the seeds were washed four times with distilled water to remove residual NaClO. The sterilized seeds are then used for germination.

1.2.2. Germination of lupin seeds

Sterile filter paper was placed in petri dishes (90mm x 15mm) and soaked with 8 mL of sterile water. 10 sterilized lupin seeds were placed on the filter paper, and the dishes were sealed with parafilm. The plates were then incubated in a plant chamber for three days at 20°C with a 16-hour light and 8-hour dark rhythm, maintaining 60% humidity, to facilitate germination.

2. FUSARIUM CULTIVATION AND GROWTH

2.1. Fol strain collection

Fusarium oxysporum f. sp. *lupini* Snyder et Hansen (ATCC 18776) strain was obtained from the American Type Culture Collection (ATCC). Newly generated spores were rapidly stored as spore suspensions at -80°C in 25% glycerol. This step is crucial since *F. oxysporum* can easily either change or lose its virulence after several propagations on plates.

2.2. Cultivation of Fol

2.2.1. Preparation of V8 media

The main component utilized in various media for cultivating plant fungal pathogens is the commercially accessible V8 juice (Campbell Soup Co.). Studies have indicated that *Fusarium* strains exhibit optimal growth in V8 medium, indicating its suitability for their growth (Choi et al., 2009; Mezzomo et al., 2018; Osman et al., 2020). Table 3 shows the reagents used for the preparation of this medium.

To prepare the V8 media, 160 mL of V8 juice was combined with 16g of agar-agar and 2.4 g of $CaCO_3$, adjusting the pH to 7.2 (Koblitz et al., 2023). Subsequently, distilled water was added to the solution to reach a final volume of 800 mL, ensuring thorough mixing using a magnetic stirrer. The resulting liquid medium was transferred into a flask, securely capped, and autoclaved to achieve sterility and eliminate potential contaminants. The sterilized media was poured into 90 mm sterilized Petri dishes and covered with lids under laminar flow conditions. The plates cooled down for 30 minutes before being inverted and placed in the laminar flow cabinet for 48h at room temperature.

Table 3. *Composition for Fusarium V8 media*

V8 Juice Media (JM)	
V8® juice	160 mL/800 mL
$CaCO_3$	2,4 g/800 mL
Agar-agar	16 g/800 mL

2.2.2. Sporulation and incubation conditions

The freezer culture, previously mentioned, was activated using a sterilized inoculation loop and evenly distributed onto the surface of the V8 agar sterilized Petri dishes. Following inoculation, the Petri dishes were placed in a growth chamber set at 20°C with a 12-hour light/12-hour dark cycle for four days, to facilitate the growth and development of the Fol culture (Figure 9).

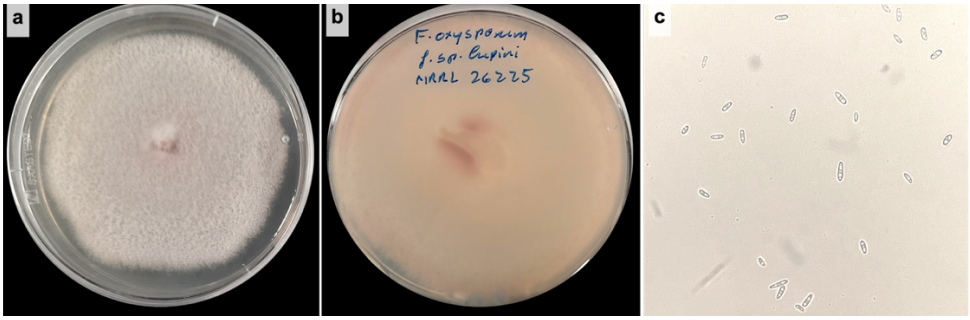

Figure 9. *Fusarium oxysporum f.sp. lupini.* ***a.*** *Plate inoculated with Fol (front).* ***b.*** *Plate inoculated with Fol (reverse).* ***c.*** *Fol spores observed under a Zeiss microscope at 40x/0.65 magnification*

3. SEEDLING INFECTION WITH FOL

3.1. Fol spore harvesting

The grown mycelium was removed by washing it with 2 mL of sterile water using a sterile Drigalski glass spatula. The suspended mycelium was then filtered using 1 mL tips with glass wool to separate the spores from agar and mycelial debris, and subsequently collected in 1,5 mL reaction tubes. Dilutions of 1:10, 1:100 and 1:1000 were prepared from the harvested solution. The dilutions were observed under a microscope using a Neubauer-improved chamber to determine the spore concentration. A concentration of $5x10^4$ spores*mL^{-1} was prepared for the infection of lupin seeds in 1,5 mL reaction tubes.

The spore concentration and incubation period were determined based on preliminary experimental trials conducted by a former member of the team (Elias Messner), which involved trying different spore concentrations as well as incubation period.

3.2. Lupin infection with Fol strain ATCC 18776

Two rounds of screening were performed for the 20 accessions. For the first screening, three seedlings were infected with Fol solution, while one seedling was designated as a negative

control (non-infected). For the second screening, the number of seedlings infected increased to six maintaining one seedling as control.

Three-day-old lupin seedlings were used for the infection assay, following established techniques similar to those described in previous studies (Bani et al., 2018; Rispail & Rubiales, 2015). The seedlings were carefully removed from the petri dishes and their roots immersed in a $5x10^4$ spores*mL^{-1} solution for thirty minutes, mimicking the modified root dipping method by Bani et al. (2012). Subsequently, the seedlings were placed into 25x200 mm DURAN® test tubes containing 20 mL of 1% water bacteriological-agar (Carl ROTH®) and incubated for four days under controlled conditions in a plant chamber set at 20°C with a 16-hour day and 8-hour dark cycle and 60% humidity.

After the incubation period, the samples were harvested by carefully cutting the roots below the cotyledon into small pieces. The fragments were then collected in 1.5 mL reaction tubes. To dry the samples, the tubes were subjected to rapid freezing in liquid nitrogen for 20 minutes followed by lyophilizing for at least 48 hours using an Alpha 2-4 LSCbasic Christ® freeze-dryer. This methodology ensures the integrity of the samples and facilitates subsequent molecular analyses.

4. SCREENING ANALYSIS FOR RESISTANCE

4.1. Genomic DNA extraction

Genomic DNA (gDNA) extraction from lupin was performed following the Cenis-Prep Tudzynkis protocol by Cenis (1992). Composition of the buffers and reagents are listed in Table 4. Prior to the extraction, the lyophilized roots were ground into a fine powder. Next, approximately 30 mg of the powdered material was transferred to a new tube. 600 µL Cenis lysis buffer was added for resuspension, followed by thorough vortexing. Subsequently, 400 µL of 5 M potassium acetate (KAc) was added to precipitate proteins. The tubes were then incubated at -20 °C for 10 minutes before being centrifuged for 30 minutes at 4°C and maximum speed to separate the precipitate.

Table 4. *Composition of buffer and reagents for DNA extraction*

Cenis Lysis Buffer	
200 mM TRIS-HCl pH 8.5	80 mL of 1M stock/400 mL
250 mM NaCl	5,84 g/400 mL
25 mM EDTA	2,92 g/400 mL
0.5% SDS	20 mL of 10% stock/400 mL
Solution is filled up to 400mL with ddH_2O and sterilized by autoclaving.	
Reagents	
5M potassium acetate (KAc)	196.3 g KAc/400 mL
2-propanol	
70% Ethanol (EtOH)	

The supernatant containing the gDNA was recovered in a new 1,5 mL reaction tube and precipitated with 600 µL of 2-propanol. The samples were then incubated at -20 °C for 10 minutes to facilitate precipitation. Afterwards, the precipitated DNA was centrifuged at 4°C and maximum speed for 18 minutes. The supernatant was discarded, and the remaining DNA pellet was washed with 300 µL of 70% ethanol (EtOH), followed by a last centrifugation at 4°C and maximum speed for 10 minutes. Subsequently, the DNA pellet was dried in SpeedVac™ for 15 minutes at 60°C. Afterwards, DNA was resuspended in 100 µL of sterile water and incubated at 65°C to inactivate DNase present in the sample. The resulting gDNA was stored at 4°C until further use.

4.2. Quantitative real-time PCR (qPCR)

The quantitative polymerase chain reaction (qPCR) is a molecular method employed to selectively amplify and quantify specific DNA sequences or regions in real-time. It utilizes fluorescence to accurately measure the amount of DNA formed at each cycle during PCR. Ideally, the amount of DNA doubles with each cycle if the primer efficiency is 100%, resulting in a lower quantification cycle (Cq) value as the quantity of initial target DNA increases.

As the qPCR is highly sensitive, the DNA template, previously described, was diluted 1:3 with Nuclease-free water to ensure that is free from contaminants that may inhibit the reaction. For the detection method in qPCR, SYBR® fluorescence is employed as the intercalating dye. SYBR®Green, emits a fluorescent signal upon binding with double-stranded DNA, allowing for real-time measurement of DNA amplification during each cycle (Adams, 2020; Steward, 2024).

The quantification cycle (Cq), or also known as threshold cycle (Ct), is an important parameter in qPCR analysis, representing the cycle number at which the fluorescence signal generated by the amplification of DNA exceeds a predefined threshold (See Figure 10). This threshold is set above the baseline fluorescence level and indicates the point where sufficient amplicons have accumulated (New England Biolabs, n.d.).

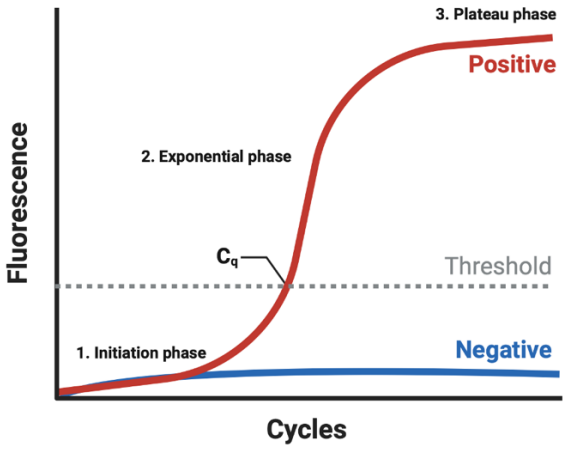

Figure 10. *Identification of the quantification cycles (Cq value) for qPCR diagnosis.*

4.2.1. Diagnostic qPCR

Diagnostic qPCR was performed following the Luna® Universal qPCR Master Mix Protocol (M3003) and run in a BioRad® CFX384™ Real-Time System. Table 5 shows the specific primers "PDF2", and "Foxy" selected for *L. angustifolius* and *F. oxysporum* respectively.

Table 5. *Primers used for the qPCR assessment*

Target	Primer	Primer direction	Primer sequence (5' → 3')	Tm°C[a]	FL[b] (bp)
Lupinus angustifolius	PDF2	Forward	TGTGTTGCTTCAACCATTGGAAA	59.8	109
		Reverse	ATCTTGTTCCCTCATCTGAGCA	59.2	
Fusarium oxysporum	Foxy	Forward	CCGTCGATAGGAGTTCCGTC	56.9	80
		Reverse	TCGAACCGACCATCTCCAAG	56.7	

[a]Tm: melting temperature; [b]FL: fragment length, For *L. angustifolius* (Taylor et al., 2016) primers. For *F. oxysporum* primers (Rocha et al., 2023).

To prepare the master mix for each primer, each amount of the Master mix specified in Table 6 was multiplied by the number of samples, gently vortexed, and stored in a 1.5 mL reaction tube. Then, 7.5 µL of the Master mix was pipetted into each well of a 384-well plate, followed by 2.5 µL of the DNA template, resulting in a final volume of 10 µL per well. Once pipetting was finished, the plate was sealed with an optically transparent film and centrifuged in the Peqlab C1000 Perfect Spin PCR Plate Spinner for 30 seconds to remove bubbles and collect liquid. Afterwards, the plate was inserted into the qPCR machine. Refer to Figure 11 for the cycling instructions for the diagnosis. The qPCR results were analyzed by the CFX Manager™ software Version 3.1.

Table 6. *Pipetting instructions for diagnostic qPCR*

Master mix	Amount for each primer pair
Luna® Universal qPCR Master Mix	5 µL
Primer Forward (1:10)	0,25 µL
Primer Reverse (1:10)	0,25 µL
Nuclease-free water	2 µL

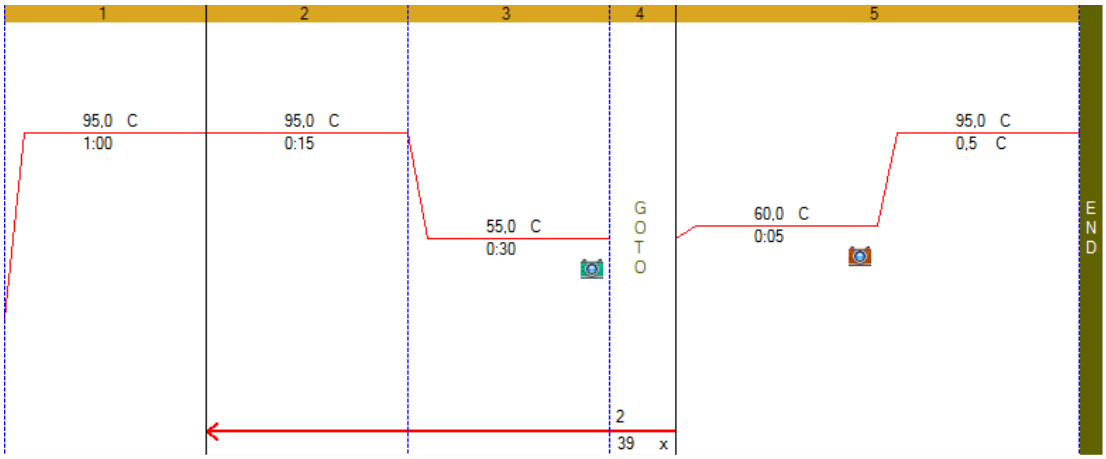

Figure 11. *Cycling instructions for diagnostic qPCR. 1: Initial denaturation, 2: Denaturation, 3: Annealing/Extension (isothermal amplification), 4: Repeat from step 2. 5: Melting curve analysis (stepwise increase in temperature with fluorescence detection every 0.5°C) (CFX Manager™ software Version 3.1.)*

4.2.2. Experimental design for qPCR assays

The experimental design for the qPCR consisted of two screenings. For the first screening, each DNA template was analyzed in duplicate, with three biological repeats and two technical replicates, resulting in a 3x2 arrangement for each pair of primers (Foxy and PDF2). For the second screening, six biological repeats and two technical replicates were assessed, yielding a 6x2 arrangement for both primers. To ensure the reliability and validity of the qPCR results, a negative control from a non-infected plant was included, along with two additional no template controls (NTC), which could be an aliquot of the Master mix or Nuclease-free water. NTC reactions are needed, as they identify and reduce non-template amplification. The experiment was designed following the recommendations of the MIQE Guidelines (Bustin et al., 2009; Steward, 2024).

	1	2	3	4	5	6
A	**U-1** Foxy L23	**U-1** Foxy L23		**U-15** PDF2 L23	**U-15** PDF2 L23	
B	**U-2** Foxy L23	**U-2** Foxy L23		**U-16** PDF2 L23	**U-16** PDF2 L23	
C	**U-3** Foxy L23	**U-3** Foxy L23		**U-17** PDF2 L23	**U-17** PDF2 L23	
D	**U-7** Foxy L23w	**U-7** Foxy L23w		**U-21** PDF2 L23w	**U-21** PDF2 L23w	
E	**T-1** Foxy	**T-1** Foxy		**T-2** PDF2	**T-2** PDF2	

Figure 12. *qPCR diagnosis arrangement in the CFX Manager™ software*

4.2.3. qPCR setup

Figure 12 illustrates the qPCR setup for the assays using the CFX Manager™ software, with accession L23 as an example for screening 1. For sample identification, columns 1-2 are designated for Fol, and columns 4-5 for *L. angustifolius*, with "Foxy" and "PDF2 as primers respectively. The notation "w" represents the negative control for non-infected samples. The light blue checkboxes contain the sample samples, while the yellow ones represent the NTC (no template control).

4.2.4. Establishment of ratio for susceptibility assessment

In this study, qPCR was used to detect Fusarium on infected lupin seeds and quantify the copy number of specific DNA, which will be compared to the lupin DNA copy numbers of each of the 20 accessions.

The Cq value was used to determine the relative abundance of the target DNA between different samples, providing valuable insights into gene expression levels within a given sample (Adams, 2020). As for this research, Cq values from lupin and Fusarium DNA expressed the abundance of the fungus, which were taken to establish a ratio that can shed light on the susceptibility-resistance of the samples tested.

The ratio was obtained by dividing the Cq values of lupin and Fusarium DNA, as shown in Equation 1. Lower Cq values indicate a higher initial amount of target DNA, while higher Cq values indicate a lower amount. Thus, less fungal DNA results in a higher Cq value for the fungus and a lower ratio, suggesting higher resistance. Conversely, more fungal DNA results in a lower Cq value and a higher ratio, indicating greater susceptibility. In summary, a higher ratio value indicates greater susceptibility, while a lower ratio value indicates higher resistance.

$$Ratio = \frac{Cq\ value\ Lupin\ DNA}{Cq\ value\ Fusarium\ DNA} \quad \textbf{(1)}$$

5. RNA-EXTRACTION FOR SEQUENCING

High-throughput RNA sequencing (RNA-seq) is a powerful and accurate technique used to provide insights of the transcriptomes of the organisms which are important for gene expression studies. During the last years, this technique has been often used for analyzing differential gene expression (DEG), being able to generate qualitative and quantitative data (Stark et al., 2019; Wang et al., 2009).

To investigate potential variations in the pathogenicity mechanism of FoI, from the initial stages until the establishment of infection, in both susceptible and resistant Lupin accessions, we conducted an RNA-seq experiment to compare the transcriptomes of inoculated samples.

5.1. RNA extraction

The lyophilized roots were first finely ground into a powder before extraction and placed in 2 mL collection tubes. The composition of the reagents is listed in Table 7. It is important to note that maintaining the samples on ice during every step of this process is essential to prevent RNA degradation.

Under fume hood conditions, 800 µL of TRIzol™ was added to the root powder and vortexed for five seconds. Then, 200 µL of chloroform was added, vortexed for five seconds, and the mixture was centrifuged at max speed for 10 minutes. The supernatant was recovered (approximately 250-300 µL), to which 500 µL of isopropanol (-20°C) was added. The mixture was vortexed, incubated on ice for 15 minutes, and then centrifuged at max speed for 10 minutes at 4°C.

The supernatant was discarded, and the pellet was washed with 200 µL of 55% ethanol (EtOH), followed by centrifugation at max speed for 10 minutes at 4°C. After thoroughly discarding the supernatant, the pellet was put into a SpeedVac™ for 10 minutes at 30°C. The dried pellet was dissolved in 50 µL of RNase-free water. After another centrifugation for 10 minutes at 4°C and max speed, 40 µL of the supernatant was taken, and any "jelly" present was discarded. Finally, the pellet was resuspended in 200 µL of DEPC water and the total RNA was measured on NanoDrop 2000c (Thermo Fisher Scientific).

Table 7. *Composition of reagents for RNA extraction*

Reagents	
TRIZol™	800 µL
Chloroform	200 µL
Isopropanol	500 µL
Ethanol (55%)	200 µL
Additives	
RNase-free water	50 µL
DEPC (Diethylpyrocarbonate) water	200 µL

For the sequencing sampling, the infection assay followed the parameters described earlier, with a variation in the incubation period. For the assessment, two harvesting time-points of 48 and 96 hours post inoculation (hpi) were selected for both, infected and control samples. The experimental setup included 10 biological repeats for each selected accession and time point. Specifically, five samples were infected, while the remaining five served as controls. From these repeats, four samples (two infected, two control) per time point and accession were used for RNA extraction, and the remaining samples were stored as backups for contingency purposes (See Figure 13).

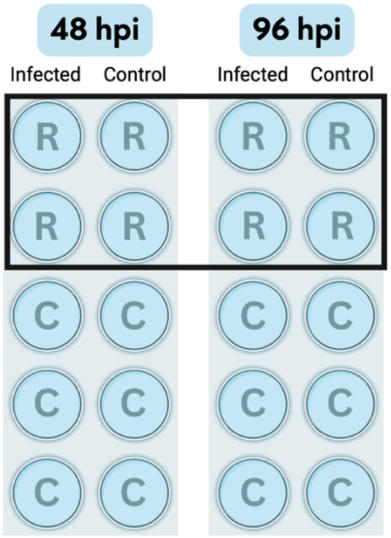

Figure 13. *Experimental design for RNA-seq assay for one accession. R: Sample for RNA extraction, C: Contingency sample*

5.2. Sample arrangement for Sequencing

The RNA control (non-infected) samples of each cultivar for each time point were pooled in just one 1,5 mL collection tube. The samples were carefully packed and kept at -80°C. In addition to the RNA samples, DNA samples of non-infected plants were sent to Procomcure Biotech GmbH for further sequencing analysis as showed in Figure 14.

For a complete schematic overview of the methodology see Figure 15.

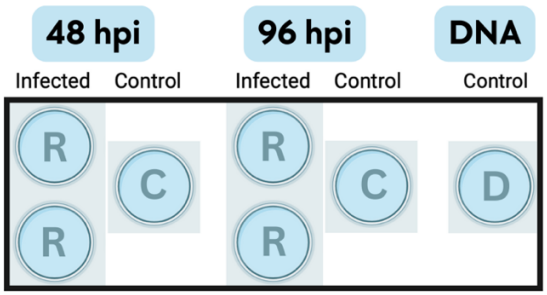

Figure 14. *Samples per accession sent for sequencing*

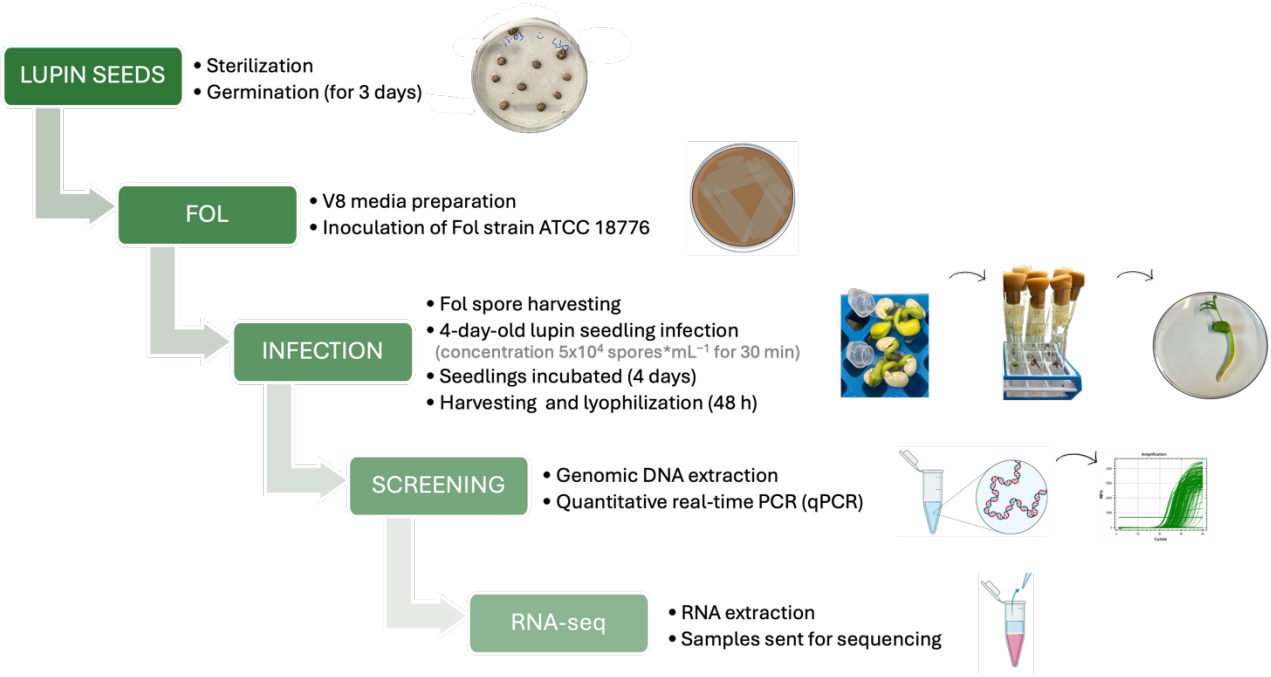

Figure 15. *Schematic overview: Screening workflow*

6. STATISTICAL ANALYSIS

To assess significant differences between infected lupin seedlings, statistical analysis was performed using quantitative PCR (qPCR) data. The quantification cycle (Cq) values obtained from qPCR were exported from the CFX Manager software (Version 3.1). To calculate the Cq Lupin/Cq Fusarium ratios, Microsoft Excel (Version 16.83) was used. Additionally, R Studio (Version 4.2.2, 2022) was employed to perform ANOVA and t-tests to determine significant differences.

IV. RESULTS

The 20 accessions were assessed through two rounds of screenings under identical conditions. The first round consisted of three biological repeats, while the second round had six biological repeats. This screening process aimed to assess the susceptibility behavior of each accession, ultimately identifying those that may exhibit potential resistance and susceptibility.

1. RATIO FOR SUSCEPTIBILITY ASSESSMENT

The raw data obtained from the qPCR analysis were exported to Excel files using the CFX Manager™ software. The specificity of the qPCR reactions was confirmed through melting curve analysis, as shown in Figure 16, where distinct dissociation profiles and melting peaks of the amplified DNA fragments are displayed.

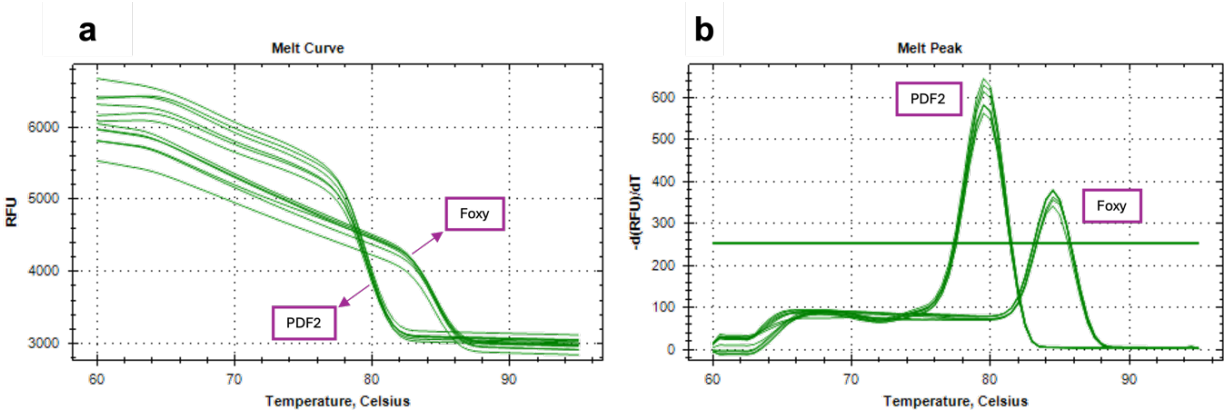

Figure 16. *Melting curve analysis of qPCR products.* ***a****: Relative Fluorescence Unit (RFU) vs. temperature.* ***b****: Derivative plot indicating the melting peaks*

Table 8 shows the results from the setup illustrated in Figure 12 for accession L23, representing one biological repeat and two technical repetitions. The software outputs data columns ranging from well (A1, A2) to Cq standard deviation. The ratio calculation is performed by dividing the mean Cq value of Lupin by the mean Cq value of Fusarium, as described previously (Equation 1). This method was used to calculate the ratios for each biological repeat across all 20 accessions in both screenings (S1 and S2). See Appendix 1 for the data of S1 and S2. It is important to state that the limit of detection was established at Cq value 35, values bigger were considered as undetectable (Weßling & Panstruga, 2012).

Table 8. *Example of the data obtained from the qPCR analysis*

Well	Fluor	Target	Content	Sample	Cq value	Cq Mean	Cq Std. Dev	Cq Lupin/Cq Fusarium (ratio)
A1	SYBR	Foxy	Unkn-1	L23	27.29	27.35	0.086	0.888013826
A2		Foxy	Unkn-1		27.41			
A3		PDF2	Unkn-15		24.15	24.29	0.198	
A4		PDF2	Unkn-15		24.43			

2. NORMALITY TEST

To determine if the data from the two screenings comes from a normally distributed population, the Shapiro-Wilk normality test was performed. The null hypothesis (H0) assumes that the data is normally distributed, while the alternative hypothesis (H1) assumes non-normality. The results in Table 9 show that the W values for both screenings are close to 1, indicating normality. Additionally, the p-values for both screenings are greater than 0.05, failing to reject the null hypothesis, suggesting that the data is normally distributed.

Table 9. *Shapiro-Wilk Normality Test for screening 1 and screening 2*

First screening	Second screening
W = 0.98819 p-value = 0.8302	W = 0.98841 p-value = 0.4031

Figure 17 illustrates the Quantile-Quantile (Q-Q) plots which shows the normal distribution of the dataset. Both Q-Q plots, for screening 1 (S1) and screening 2 (S2), shows that the data points stand closely to the red reference line. The slight deviations at the upper-lower tails of the distribution are relatively minor and do not significantly detract from the overall normality.

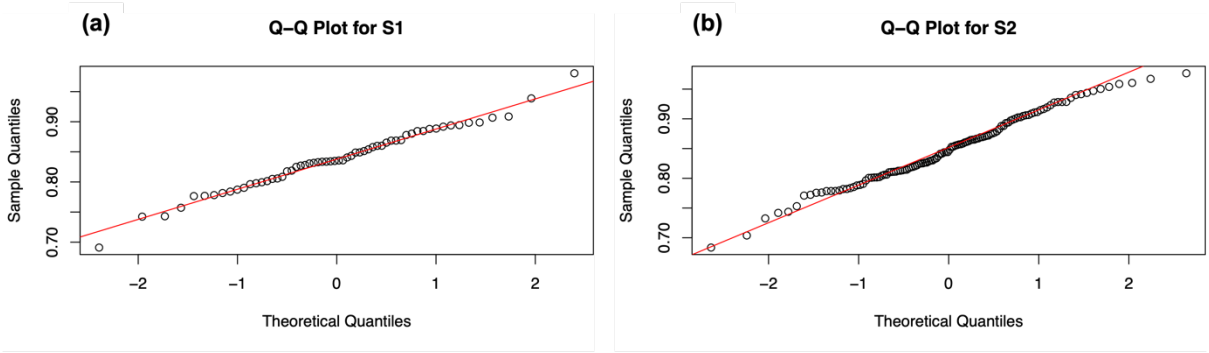

Figure 17. *Normal Q–Q plot. (a) Q-Q plot for screening 1 (b) Q-Q plot for screening 2*

3. ASSESSMENT OF SCREENING 1

After confirming normality, we analyzed the average ratios from each accession in the first screening. Figure 18 displays the average ratios from the three biological repeats for each accession, along with error bars. The results indicate that accession "L64" appears to be the most resistant, with the lowest mean value. On the other hand, accession "L23" has the highest mean value, suggesting it is the most susceptible.

4. ASSESSMENT OF SCREENING 2

For the second screening, we increased the number of biological repeats to six, doubling the number from the first screening to enhance data reliability. Figure 19 displays the average ratios and error bars. The results show that accession "L26" appears to be the most resistant, with the lowest mean ratio. Conversely, accession "L49" has the highest ratio, indicating higher susceptibility.

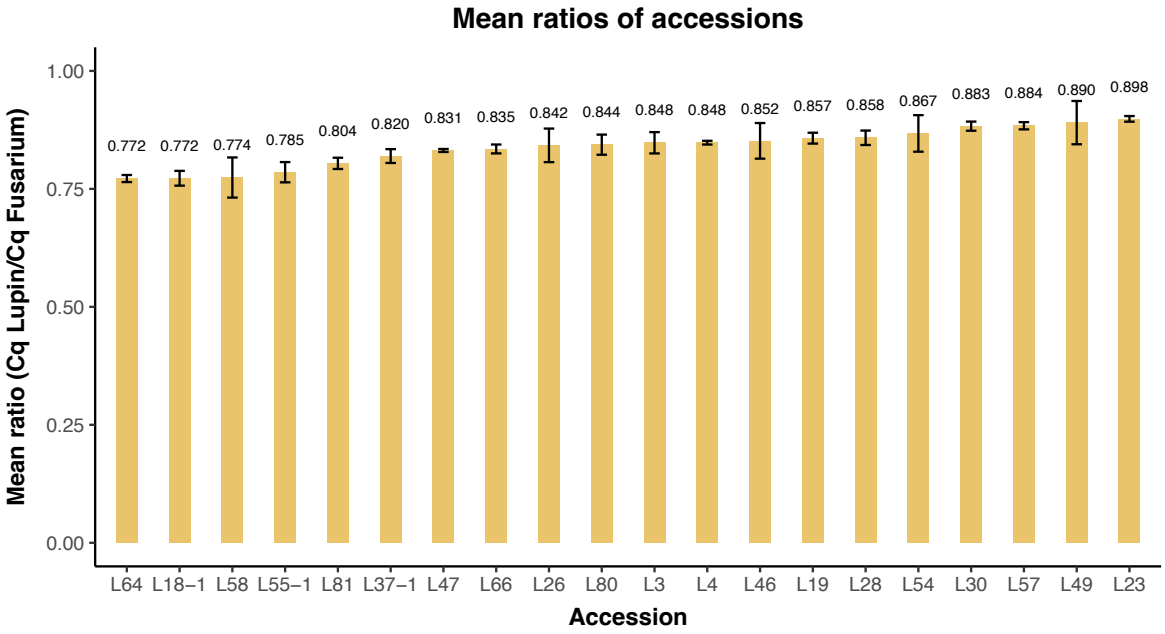

Figure 18. *First screening: Mean ratios of accessions*

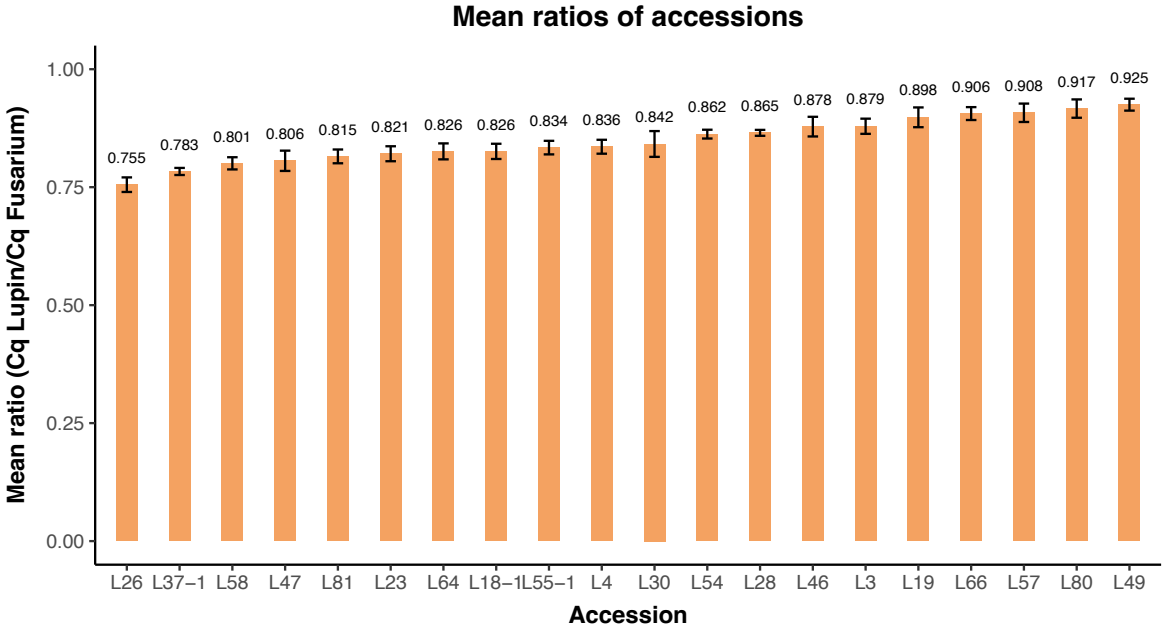

Figure 19. *Second screening: Mean ratios of accessions*

5. CONSISTENCY ASSESSMENT BETWEEN THE SCREENINGS

To assess the consistency of ratios between the first and second screening for each accession, t-test was performed. Initially, it was checked if the variances were equal or unequal using a F-test to compare two variances (from screening 1 and screening 2). The result, shown in Table 10, indicated a p-value of 0.2049, suggesting no significant difference in variances. This allowed us to assume equal variance and perform a Student's t-test.

Table 10. *F-test between screening 1 and screening 2*

Statistic	Value
F	0.74277
Degrees of Freedom (num df)	59
Degrees of Freedom (denom df)	119
p-value	0.2049
95% Confidence interval	0.4841611 - 1.1785509
Ratio of variances	0.7427674

Given the normality of the data, the Student's t-test and the Wilcoxon test were used to compare the means of the two screenings for each accession. This aimed to evaluate parametric (t-test) and non-parametric (Wilcoxon) methods. Table 11 displays the p-values and the adjusted p-values for each test. The adjusted p-values were used to control for false discovery rates when performing multiple comparisons, reducing the likelihood of type I errors (false positives) using the Benjamini-Hochberg method. Significance levels were marked as follows: p-value < 0.001 (***), p < 0.01 (**), p < 0.05 (*), or if not significant (n.s.).

Table 11. *Assessment of the consistency of the data between S1 and S2*

Accession	Student's t-test				Wilcoxon test (Mann-Whitney U)			
	p-value		adjusted p-value		p-value		adjusted p-value	
L19	0.1339	n.s.	0.3015	n.s.	0.2619	n.s.	0.5238	n.s.
L23	0.0035	**	0.0357	*	0.0238	*	0.1905	n.s.
L26	0.1176	n.s.	0.3015	n.s.	0.0476	*	0.1905	n.s.
L3	0.3181	n.s.	0.4893	n.s.	0.5476	n.s.	0.7302	n.s.
L30	0.2033	n.s.	0.4065	n.s.	0.5476	n.s.	0.7302	n.s.
L37-1	0.1121	n.s.	0.3015	n.s.	0.0952	n.s.	0.2381	n.s.
L47	0.2927	n.s.	0.4893	n.s.	0.3810	n.s.	0.6926	n.s.
L49	0.5350	n.s.	0.6704	n.s.	0.5476	n.s.	0.7302	n.s.
L54	0.9093	n.s.	0.9093	n.s.	0.9048	n.s.	0.9524	n.s.
L66	0.0036	**	0.0357	*	0.0476	*	0.1905	n.s.
L18-1	0.0562	n.s.	0.2246	n.s.	0.0476	*	0.1905	n.s.
L28	0.7077	n.s.	0.7450	n.s.	0.9048	n.s.	0.9524	n.s.
L4	0.4540	n.s.	0.6486	n.s.	0.9048	n.s.	0.9524	n.s.
L46	0.5786	n.s.	0.6704	n.s.	0.9048	n.s.	0.9524	n.s.
L55-1	0.1357	n.s.	0.3015	n.s.	0.0952	n.s.	0.2381	n.s.
L57	0.2961	n.s.	0.4893	n.s.	0.2619	n.s.	0.5238	n.s.
L58	0.6034	n.s.	0.6704	n.s.	0.5476	n.s.	0.7302	n.s.
L64	0.0242	*	0.1613	n.s.	0.0476	*	0.1905	n.s.
L80	0.0504	n.s.	0.2246	n.s.	0.0952	n.s.	0.2381	n.s.
L81	0.5737	n.s.	0.6704	n.s.	1.0000	n.s.	1.0000	n.s.

From Table 11, we can see that only three of the 20 accessions showed significant differences in their ratios between the two screenings. These were accessions L23, L64, and L66. The

significance of the differences for accessions L23 and L66 is stronger, as indicated by the highly significant p-values in the Student's t-test. In contrast, most other accessions did not exhibit significant differences in either the t-test or the Wilcoxon test, suggesting consistency in the ratios across the two screenings for these accessions.

This finding implies that for accessions L23, L66, and L64, the variation between the first and second screening may be due to experimental or biological factors that warrant further investigation. For the remaining accessions, the consistent ratios across the two screenings suggest reliable and reproducible measurements.

6. DISTRIBUTION OF RATIOS BY ACCESSIONS FOR S2

After verifying the consistency of the data, it was decided to conduct further analysis of the second screening to gain insight into the potential discrimination between susceptible and resistant accessions.

6.1. Analysis of Variance (ANOVA)

An ANOVA test was conducted to compare the ratio means of 20 accessions from the second screening. Table 12 shows a p-value of 3.78e-13, indicating a significant difference among accessions. In addition, homogeneity of variances was tested using a Levene's test (Table 13) confirmed homogeneity of variances with a p-value > 0.05.

Table 12. *Analysis of variance (ANOVA)*

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Accession	19	0.2543	0.01338	8.159	3.78e-13 ***
Residuals	100	0.1640	0.00164		

Table 13. *Levene's test for homogeneity of variance*

Df	F-value	Pr(>F)
19	0.717	0.2543

6.2. Tukey test

The Tukey HSD test was performed to group similar accessions. Table 14 shows differences between the accessions. L49 has the highest mean ratio (0.9248434), suggesting high susceptibility, while L26 has the lowest mean ratio (0.7552795), suggesting the lowest susceptibility. Accessions sharing a letter in their grouping are not significantly different from each other, indicating a continuum of susceptibility and resistance among the accessions. Although there are accessions that tend towards higher susceptibility (L49, L80) and those that tend towards lower susceptibility (L26, L37-1), there are no sharply distinct groups. This continuum suggests that the more resistant accessions could be useful for breeding programs aimed at improving resistance.

Table 14. *Tukey's HSD Post-Hoc test*

Accession	Mean ratio	Group
L49	0.9248434	a
L80	0.9165485	ab
L57	0.9076477	abc
L66	0.9059792	abcd
L19	0.8980591	abcde
L3	0.8790928	abcdef
L46	0.8783004	abcdef
L28	0.8651441	abcdefg
L54	0.8624271	abcdefg
L30	0.8415637	abcdefg
L4	0.8356871	bcdefgh
L55-1	0.8338659	bcdefgh
L18-1	0.8258690	cdefgh
L64	0.8258651	cdefgh
L23	0.8208633	defgh
L81	0.8152451	efgh
L47	0.8059179	fgh
L58	0.8005306	fgh
L37-1	0.7832876	gh
L26	0.7552795	h

The boxplot in Figure 20 illustrates the distribution of the ratio (Cq Lupin/Cq Fusarium) for all the accessions. Accessions with lower median ratios, such as L26 and L37-1, may be prioritized for further investigation and potential use in breeding programs aimed at enhancing Fusarium resistance.

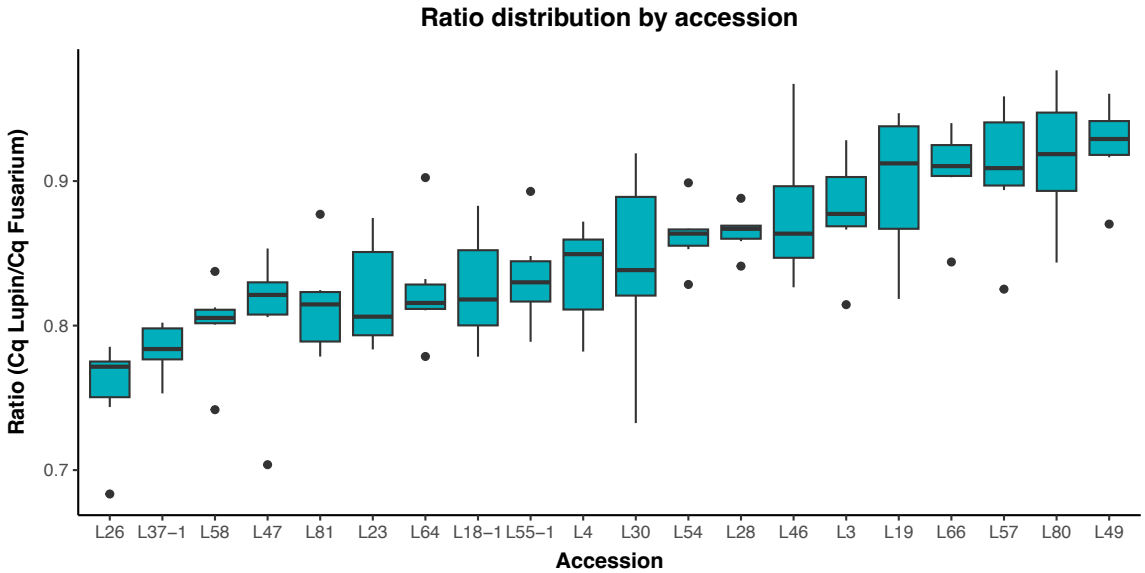

Figure 20. *Ratio distribution by accession*

7. SELECTION OF ACCESSIONS FOR RNA-SEQUENCE ANALYSIS

After assessing the mean values from the second screening, a clearer overview of the potential susceptibility of each of the 20 accessions was obtained. According to the results from the Tukey test, L26 can be categorized as the most resistant accession and L49 as the most susceptible. Both of these accessions are of particular interest for understanding the resistance mechanisms of blue lupin to FoI infection. Additionally, accessions L57 and L80 are notable due to their high protein value (See Table 2).

The boxplot in Figure 21 demonstrates significant difference in the ratio values between accession L26 and the other three accessions (L49, L57, and L80). L26 stands out with the lowest median ratio and a narrow interquartile range (IQR), indicating that it can be the most resistant to Fusarium infection among the compared accessions. The statistical significance annotations further support this conclusion, showing that the differences are not due to random variation but represent genuine differences in resistance levels.

This visual representation and statistical analysis provide strong evidence to prioritize accession L26 for breeding programs aimed at enhancing Fusarium resistance in lupins. The significant differences identified between L26, and the other accessions highlight the importance of selecting the most resistant lines for further development.

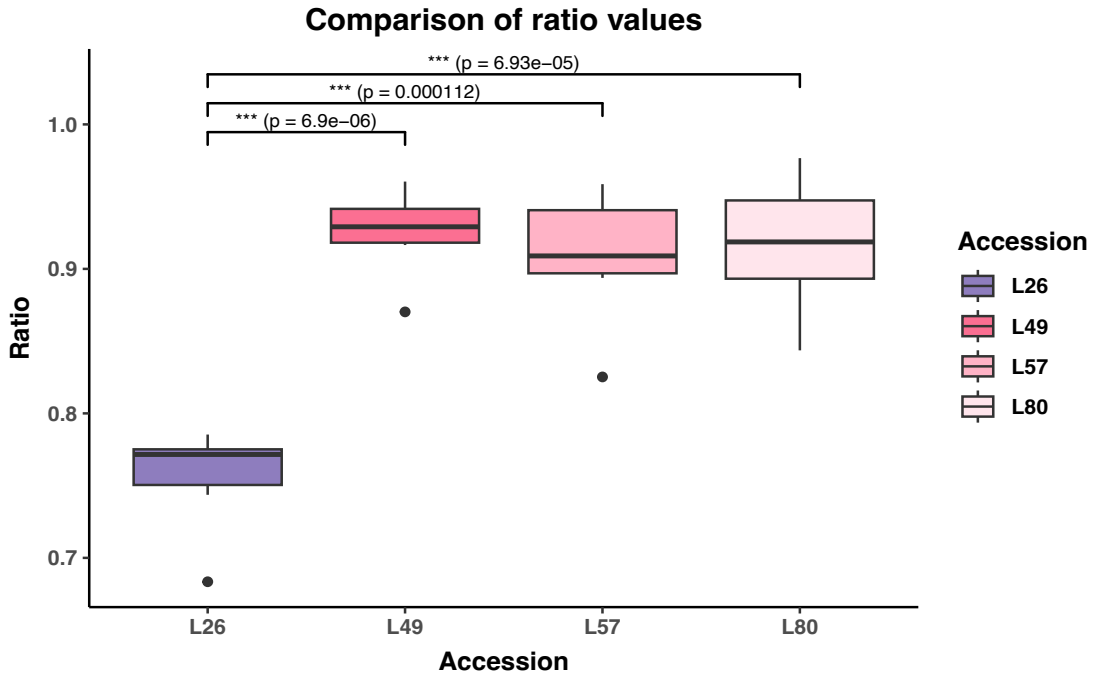

Figure 21. *Comparison of Ratio Values between resistant cultivar L26 and susceptible L49, L57 and L80*

8. RNA-SEQUENCE

Despite the successful extraction and quality testing of DNA from the four selected accessions (L26, L49, L57, L80), the RNA samples from these accessions did not pass the quality tests. RNA was extracted at two different time points, 24- and 48-hours post-inoculation (hpi), to capture dynamic changes in gene expression related to susceptibility and resistance. These time points were chosen based on previous studies highlighting their importance in identifying early-stage dynamics during pathogen infection and host colonization. For instance, Boba et al. (2021) and Thatcher et al. (2016) demonstrated the significance of multiple time points to understand the temporal regulation of fundamental biological processes and the activation of plant defense mechanisms.

Our approach aimed to gain insights into the temporal regulation of pathogen infections and plant defense mechanisms, and to identify key genes or pathways involved in these processes. Due to the RNA quality issue, the RNA-seq experiment needs to be repeated to proceed with the comparative genomics analysis. This additional work is scheduled to occur after the completion of this master thesis, owing to the tight schedule.

V. DISCUSSION

Lupins, a valuable legume crop with high protein content and nitrogen-fixing ability, have been limited by disease constraints. As an emerging crop with promising potential in agriculture, feeding, and medicine, it is essential to have tools to prevent and mitigate potential threats, such as diseases, to ensure their widespread cultivation and utilization. Developing disease-resistant crop varieties is a crucial strategy for improving food security, reducing environmental impacts, and ensuring the long-term sustainability of agricultural production as it reduces the dependence on chemical products (Giménez-Ibánez, 2020).

This study aimed to uncover resistance mechanisms in blue lupins against Fusarium wilt and assess its pathogenicity. Two screenings were conducted on 20 uncharacterized *L. angustifolius* accessions to identify potential resistant accessions. Germplasm banks are a vital source of genetic resources, offering a broad range of genetic diversity that is essential for identifying and incorporating resistance genes into crop breeding programs (Mondal et al., 2023). This is particularly important for lupin farming, as Fusarium wilt poses a significant challenge to lupin growth and can result in substantial yield decreases and lower crop quality.

Evaluating diverse lupin germplasms across contrasting environments has identified valuable sources of Fusarium resistance. Kurlovich et al. (1995) screened accessions in different regions, revealing environment-dependent susceptibility and developing a transgressive resistant segregant of blue lupin (cv. Frost x cv. Apendrilon). Raza et al. (2000) found partial Fusarium wilt resistance in Egyptian white lupin landraces and cultivars under field and greenhouse conditions, consistent across environments. Similarly, Kupstou et al. (2002) and Kuptsov (2000) identified resistant blue lupin accessions such as Crystal, Mitan, Rose, E104, E105, E106, and Tanjil, as well as cultivars Apendrilon, K-1462, and K-2750. Additionally, Georgieva et al. (2018) evaluated 23 white lupine cultivars under natural field infestation, finding nine cultivars resistant to Fol. These findings underscore the importance of exploiting genetic diversity from various environments to develop high-yielding, broadly adapted, and disease-resistant lupin cultivars.

The 20 blue lupin accessions were germinated, infected, and assessed under controlled conditions, as outlined in the Materials and Methods section, to minimize technical bias and ensure replicability. Real-time PCR (qPCR) was then used to quantify Fusarium DNA in the infected seeds. Schena et al. (2013) emphasized that proper DNA extraction and validated primers are crucial for accurate qPCR results. For this study, the Cenis-Prep Tudzynkis protocol was used for DNA extraction, and primers PDF2 and Foxy were chosen based on Rocha et al. (2023) and Taylor et al. (2016). Primer efficiency and the standard curve were previously validated by Elias Messner (see Appendix 2).

From the data obtained from the qPCR analysis, the quantification cycle (Cq) is the parameter to determine the abundance of DNA between Fusarium and Lupin, Cq values of Fusarium and Lupin were used to establish a ratio that can provide insights into the susceptibility and resistance of the tested samples (See Equation 1). This ratio ranging from 0 to 1, in which a lower Cq ratio of pathogen to host indicates higher resistance, while a higher ratio suggests

lower resistance, providing a quantitative measure of relative susceptibility in plant-pathogen systems. The ratios obtained from the processed data of the Cq value Lupin/Fusarium and mean value from both screenings can be found in Appendix 1.

The use of qPCR is a powerful tool to quantify the amount of plant and fungal DNA after infection and determine the progress of infection/level of resistance. Pfaffl (2001) proposed a mathematical model for relative quantification in real-time RT-PCR, later applied by Gao et al. (2004) to quantify the DNA of the soybean pathogen *Fusarium solani f. sp. glycines* relative to its host. Weßling & Panstruga (2012) developed qPCR assays for powdery mildew in Arabidopsis, demonstrating that qPCR allows simultaneous DNA extraction and analysis. These studies highlight the utility of qPCR for providing insights into resistance, tolerance, and disease progression in plant-pathogen interactions.

Quantitative PCR (qPCR) assays detect and quantify low levels of fungal pathogens in crops even amidst abundant host DNA. Malvick & Impullitti (2007) used qPCR to detect *Phialophora gregata* in soybeans weeks before symptoms appeared, while Abdullah et al. (2018) developed a duplex qPCR assay for co-infections by wheat pathogens *Pyrenophora tritici-repentis* and *Parastagonospora nodorum*. These studies highlight how qPCR-based techniques provide a robust and reliable method to assess fungal infections, overcoming the limitations of traditional visual symptom scoring approaches.

Quantifying fungal DNA with qPCR offers a reliable method for assessing Fusarium crown rot (FCR) resistance in cereals, surpassing traditional visual symptom scoring. Studies by Hogg et al. (2007), Knight et al. (2012) and Milgate et al. (2023) show that qPCR effectively measures FCR severity and correlates it with yield loss, enabling the identification of tolerance and partial resistance. Visual assessments can reflect pathogen colonization but weaken over time. Ozdemir et al. (2020) demonstrated that implementing qPCR in breeding programs provides a less subjective and more accurate technique for selecting FCR resistance, enhancing the reliability of resistance selection.

Two screenings were conducted to assess the behavior of the 20 accessions upon infection with Fol. The S1, with three biological repeats, provided preliminary insights, while the S2, with six repeats per accession, ensured reproducibility. Both screenings showed normally distributed data. A t-test revealed no significant differences in most accessions, confirming data reliability, except for L23, L66, and L64 which showed significant variation. According to Dewey et al. (2022), variation in biological studies arises from endogenous (genetic/phenotypic) or exogenous (environmental, experimental) factors. Controlling genetic background or sample size minimizes endogenous variation, while standardizing experimental conditions and increasing replicates regulates exogenous variation. Addressing these factors is crucial for obtaining reliable and precise results in experimental biology.

In this study, we evaluated 20 uncharacterized blue lupin accessions to identify potentially resistant candidates of *L. angustifolius* in frame of the Project “Boosting innovation in breeding for the next generation of legume crops for Europe”. Based on Table 14 and Figure 20, these accessions segregated into two groups: potentially susceptible (L49 to L30) and potentially

resistant (L37-1 to L26). Notably, accessions L26 and L37-1 exhibited the lowest qPCR ratios, suggesting they may possess resistant genes. Further investigation, particularly comparing L26 and L37-1 with susceptible accessions like L49 and L80, could elucidate the defense mechanisms against Fol infection in lupins.

Armstrong and Armstrong (1964) identified three distinct races (1, 2, and 3) of *F. oxysporum* f. sp. *lupini* (Fol) in European isolates, each exhibiting specificity towards different lupin species. Rataj-Guranowska et al. (1984) specifically studied Fol races 2 and 3 employing crossed immunoelectrophoresis to better describe the differences between the races, which are morphologically identical but differ in virulence. A race-specific antigen for race 3 is possibly related to the virulence of the fungus. In addition, it is also noted that the pathogenicity of Fusarium is controlled polygenically. Later, Lindbeck (2009) found that race 3 was potentially pathogenic to *L. angustifolius* and *L. albus* but non-pathogenic to *L. luteus*.

Screening segregating populations and conducting genetic studies can elucidate the genetic control and inheritance patterns of Fusarium resistance. Early studies by Lambert (1955) and Kurlovich et al. (1995) supported the polygenic nature of *Fusarium*, showing that resistance is controlled by multiple genes influenced by environmental conditions. Subsequent genetic analysis identified two dominant Fusarium resistance genes in *L. angustifolius*. Crossing susceptible parents with different resistance genes allowed gene pyramiding, restoring resistance in hybrid progeny and increasing the productivity potential of resistant cultivars (Kuptsov, 2000).

Genetic studies have revealed that resistant lupin genotypes possess two dominant, non-allelic resistance genes, *RFO1* and *RFO2*, while susceptible genotypes either lack both or carry only one in a heterozygous state (Jørnsgård et al., 2007). Further research on the resistance genes *RFO1* and *RFO2* against *F. oxysporum* in *Arabidopsis thaliana* has shown that their expression in susceptible ecotypes restricts pathogen growth in the roots. These genes uniquely protect *Arabidopsis* against various *formae speciales* of the pathogen. *RFO1* encodes a wall-associated kinase-like protein (*WAKL22*), and *RFO2* encodes a receptor-like protein. However, the transcriptional responses during *F. oxysporum* infection remain poorly understood, with results indicating only weak gene induction and suppression of many genes, including the Ethylene Response Factor72 (*ERF72*) gene, which plays a role in suppressing programmed cell death and contributes to increased resistance (Y. C. Chen et al., 2014).

Continuous screening and genetic diversity analysis remain crucial for identifying new resistance sources and preventing resistance breakdown, as this genetic information is crucial for informing breeding strategies to develop more resilient and disease-resistant lupin crops

Regarding the defense mechanism of lupins upon Fol infection, Van Andel (1956) observed that Fol develops in the xylem vessels of root and stem, and the lack of organic substrates seems to impede or limit the growth of the fungus. Salleh & Owen (1984) compared the xylematic fluids from lupin cultivars Neuland and Primorskij, susceptible and resistant, respectively, and they Neuland's xylematic fluids promoted germination of conidia and growth of the germ-tube length

of the fungus compared to xylem fluids from resistant cultivars. This suggests that amino acids may play a role in the resistance or susceptibility of white lupin cultivars to Fusarium wilt.

Infection of yellow lupine embryo axes with Fol triggers oxidative stress, indicated by increased hydrogen peroxide (H_2O_2) and free radicals. The lupine responds with a twofold increase in catalase (CAT) activity, superoxide dismutase (SOD) activity, and enhanced H_2O_2 activity (Morkunas et al., 2004). In a later study, Morkunas et al. (2005) showed that exogenous sucrose stimulates the accumulation of isoflavonoids, particularly genistein, and increases endogenous sucrose, suggesting that sucrose provides carbon skeletons for isoflavonoid synthesis via the Phenylalanine Ammonia-Lyase (PAL) pathway, crucial for defense (Figure 22). Moreover, Morkunas & Gmerek (2007) found increased lignin content and antioxidant enzyme activity in the presence of sucrose, indicating that sucrose enhances defense mechanisms like lignification and the oxidation of toxic compounds, thereby strengthening cell walls and limiting pathogen spread.

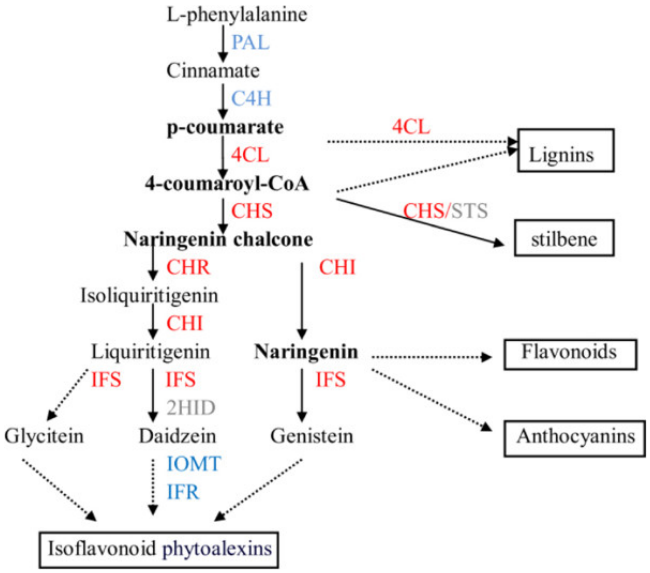

Figure 22. *The isoflavonoid phytoalexin synthesis pathway in soybeans (Chu et al., 2014)*

The cross-talk studies by Morkunas et al. (2011, 2013) found that sucrose strongly stimulated the expression of flavonoid biosynthetic genes and increased chalcone isomerase activity, leading to elevated accumulation of flavonoids, particularly in inoculated embryo axes cultured with sucrose. Additionally, exogenous nitric oxide (NO) and sucrose together stimulates the accumulation of genistein glucoside, increased free isoflavone aglycones. Furthermore, the interaction sucrose-NO increases (PAL) activity, and antioxidant capacity, boosting the lupine defense against the fungal pathogen.

Further studies demonstrated that sugars stimulated isoflavonoid production, increased β-glucosidase activity, and enhanced genistein accumulation in infected tissues. Infection also affected actin and tubulin organization, varying with sucrose levels. Additionally, high sugar levels reduced fungal sporulation, ergosterol accumulation, and mycotoxin moniliformin production, while increasing salicylic acid and hydrogen peroxide, indicating an enhanced defense response. These findings highlight that sugars disrupt the pathogen's metabolic

mechanisms and stimulate efficient plant defense pathways, essential for improving crop resistance (Formela et al., 2014; Formela-Luboińska et al., 2020)

Recent advances in screening methods have enhanced breeding for disease resistance in grain legumes. Molecular markers linked to resistance genes have enabled marker-assisted selection (MAS), leading to varieties resistant to multiple pathogens. Improved understanding of fungal genetics, diagnostics, and host-pathogen interactions has further driven these improvements. Accurate screening has facilitated genetic mapping and the introgression of resistance genes into commercial cultivars. Ongoing research in candidate resistance genes and comparative genomics is expected to accelerate marker-assisted breeding of disease-resistant legumes (Infantino et al., 2006).

While the identification of resistance genes (R-genes) and loci is important, the analysis of susceptible accessions, such as L49, can also provide valuable insights. Susceptible accessions may help identifying susceptibility-promoting genes (S-genes) that contribute to their vulnerability to Fol infection. Raza et al. (2000) acknowledges the importance of having a susceptibility check in screening studies. By comparing and contrasting the genetic factors in resistant accessions like L26, resistance related genes can be identified. Further research regarding the exact resistance mechanisms on molecular level is required.

VI. FUTURE DIRECTIONS AND PERSPECTIVES

Studies on plant infection with *F. oxysporum* highlight the importance of using multiple time points in RNA-seq experiments to identify DEGs (differently expressed genes) and capture dynamic changes in gene expression. Thatcher et al. (2016) conducted transcriptome analysis of *F. oxysporum* f.sp. *medicaginis* on susceptible and resistant varieties of *Medicago truncatula* using RNA-seq at 2- and 7-days post-inoculation, emphasizing the need to capture early-stage dynamics during host colonization. Similarly, Boba et al. (2021) investigated susceptible and resistant flax seedlings post *F. oxysporum* f.sp. *lini* infection using RNA-seq at 24- and 48-hours post-inoculation to elucidate mechanisms activated during the initial stages of infection.

L. Chen et al. (2019) revealed that common bean plants exhibit a complex defense response to *F. oxysporum* f. sp. *phaseoli* (FOP) infection, involving structural, signaling, and metabolic adaptations. RNA-seq and transcriptomic analysis showed upregulation of cell wall-related genes, pathogen recognition receptors, defense signaling pathways, and pathogenesis-related genes. The flavonoid biosynthesis pathway was significantly enriched, supporting the importance of flavonoids in plant defense, as also noted in studies of lupins by Morkunas et al. (2011). The upregulation of structural defense genes and ethylene-responsive transcription factors like *ERF-RAP2-7* highlights their roles in resistance. These findings underscore the multifaceted nature of common bean defense against FOP infection.

By targeting gene expression at multiple time points, we gain an understanding of the temporal regulation of fundamental biological processes, such as pathogen infections and plant defense mechanisms, as well as the identification of key genes or pathways that are involved in the colonization of the pathogen and plant defense mechanism (Galindo-González & Deyholos, 2016; Yang et al., 2022).

The findings from the planned RNA-seq experiment will not only contribute to a deeper understanding of the Fol-lupin pathosystem but may also have broader implications for the development of effective strategies to manage Fusarium wilt in other important legume crops. However, other diseases affecting lupins, such as anthracnose, have been more extensively investigated, providing valuable insights that can guide future research directions.

Fischer et al. (2015) identified a novel and effective anthracnose resistance locus in blue lupin, designated as *LanrBo*, which was mapped to linkage group NLL-11. Molecular markers linked to the *LanrBo* locus were developed for use in lupin breeding programs. Similarly, Książkiewicz et al. (2021) found that two major unlinked alleles, Phr1 and PhtjR, confer resistance to the fungus *Diaporthe toxica*, which causes lupinosis disease in livestock grazing on lupin stubble. Resistant lines showed rapid activation of defense response genes, including those involved in reactive oxygen species and oxylipin biosynthesis, compared to susceptible lines.

Baroncelli et al. (2021) published the complete genome sequence of the *Colletotrichum lupini* strain RB221, providing a valuable resource for understanding the biology and pathogenicity of the anthracnose pathogen. Furthermore, Książkiewicz et al. (2022) used high-throughput

sequencing to profile DEGs in blue lupin against anthracnose. The study revealed that the *Lanr1* gene triggers an immediate defense response, upregulating genes involved in defense, oxidation-reduction, and photosynthesis. In contrast, the *AnMan* gene provides a delayed, less effective resistance. These findings highlight the importance of transcriptomic profiling in understanding the molecular mechanisms underlying disease resistance in lupins.

In addition to the insights from anthracnose research, the latest advancements in lupin genomics have also laid the groundwork for further exploration of disease resistance. Garg et al. (2022) developed a high-quality, chromosome-length reference genome for *L. angustifolius* and a comprehensive pan-genome assembly comprising 55 diverse lupin lines. This study revealed insights into the genomic diversity, core and variable genes, and key traits such as alkaloid biosynthesis and disease resistance in narrow-leafed lupin.

While disease resistance is an important consideration for lupin breeding, the primary focus has often been on reducing the alkaloid content to develop "sweet" lupin varieties. The *iucundus* locus in *L. angustifolius* and the *pauper* locus in *L. albus* are the major genetic regions that confer low alkaloid "sweet" phenotypes when present in the recessive homozygous state. Molecular markers linked to these loci have been developed and are used in marker-assisted selection to efficiently introgress the low-alkaloid traits into new breeding lines and cultivars (Rychel & Książkiewicz, 2019).

However, the presence of quinolizidine alkaloids in lupins is highly related to their resistance against certain diseases and pests. The previously mentioned transcription factor *RAP2-7* has been identified as a key regulator of QAs biosynthesis in blue lupin with *RAP2-7* and other QA biosynthesis genes upregulated in the aerial organs of bitter cultivars. While QA synthesis may occur independently in different organs, further research is needed to understand how *RAP2-7* regulation is altered in sweet lupins. Interestingly, seed alkaloid content was unaffected by anthracnose infection, but other lysine-derived metabolites like polyamines may play a role in defense against this fungal disease. Investigating lupin polyamines could provide insights into the relationship between alkaloids, disease resistance, and the complex regulation of QA biosynthesis by *RAP2-7* (Czepiel et al., 2021).

In this regard, Vishnyakova et al., (2020) proposed creating 'bitter/sweet' lupine varieties that combine high alkaloid content in the vegetative organs for insect resistance and low alkaloid levels in the seeds for food and feed purposes, highlighting the potential of lupine alkaloids as natural pesticides and their selective regulation within the plant. The latest finding of Namdar et al. (2024) reviewed and re-analyzed the suggested quinolizidine alkaloid biosynthesis pathway, including the relationship between the amino acid precursor L-lysine and the different quinolizidine alkaloids occurring in seeds of lupin species. Revealing alkaloid compositions and highlighting some aspects of their formation pathway are important steps in evaluating the use of wild lupins as a novel legume crop. The findings suggest that the presence and distribution of specific alkaloids may contribute to the plant's disease resistance mechanisms.

VII. CONCLUSIONS

This study aimed to investigate the resistance mechanisms of blue lupins (*Lupinus angustifolius*) against Fusarium wilt (*Fusarium oxysporum* f. sp. *lupini*) and to identify potential resistant accessions for breeding programs. Through a series of two screenings and molecular analyses, we have identified significant differences in the resistance levels among 20 uncharacterized blue lupin accessions. These screenings were supported by quantitative real-time PCR (qPCR) analyses to quantify Fusarium DNA levels in infected seedlings. A ratio to assess susceptibility levels was calculated for each accession as dividing lupin DNA Cq to Fusarium DNA Cq, with lower ratios indicating higher resistance and higher ratios indicating greater susceptibility.

The statistical analysis confirmed the reliability and consistency of the findings, identifying accessions L49 as highly susceptible and L26 as potentially resistant to Fusarium wilt. These results provide a solid basis for further research to validate the resistance of L26 and susceptibility of L49, as well as investigate the genetic mechanisms underlying disease resistance, which will be crucial for developing Fusarium wilt-resistant lupin varieties through breeding programs. Despite encountering technical issues with RNA sample quality, the study's preliminary RNA-seq data aimed to provide insights into the gene expression changes associated with resistance and susceptibility at early infection stages. The RNA-seq experiments, although needing repetition due to RNA quality issues, are crucial for future comparative genomics analyses to further elucidate the molecular pathways involved in Fusarium resistance.

In conclusion, this study provides valuable insights into the susceptibility of different lupin accessions to Fol using a qPCR-based approach. The successful identification of potentially resistant and susceptible accessions, such as L26 and L49, is an important step towards developing Fol-resistant lupin varieties through breeding programs. This work has laid the groundwork for further research aimed at understanding and enhancing resistance to Fusarium wilt, a critical step in advancing lupin breeding programs and securing crop yields against this pathogenic threat. The planned transcriptome analysis will shed light on the molecular basis of the defense mechanisms, guiding future efforts to improve lupin disease resistance. The integration of germplasm screening with molecular analyses holds great promise for developing diagnostic tools to assess plant material and determine susceptibility to Fusarium wilt and other fungal diseases.

VIII. APPENDIX

1. Ratios and mean ratios from first and second screening (S1 and S2)

Table 15. *Ratios and mean ratios from S1 and S2*

Accession	S1 ratios	Mean ratio	S2 ratios		Mean ratio
L3	0.8335238 0.8918176 0.81764708	0.84766283	0.910714001 0.866457798 0.814500429	0.875508199 0.879092818 0.928283661	0.87909282
L4	0.84905653 0.84062552 0.85413205	0.84793803	0.841581992 0.857292419 0.871960949	0.782033961 0.860298477 0.800954745	0.83568709
L18-1	0.77815592 0.7962904 0.74298499	0.77247711	0.778489099 0.861228303 0.882904684	0.824956234 0.811186423 0.796449374	0.8258690
L19	0.84336429 0.84882306 0.88025946	0.85748227	0.897143576 0.941441757 0.856935052	0.92737216 0.947002889 0.818459448	0.89805915
L23	0.90864186 0.88801383 0.89832784	0.89832784	0.801464671 0.783503625 0.790580567	0.810891532 0.874481571 0.864257888	0.82086331
L26	0.90675009 0.78402496 0.83588709	0.84222071	0.683474794 0.743645307 0.770890544	0.776095485 0.785339722 0.772231224	0.75527951
L28	0.86863369 0.87795699 0.82820257	0.85826442	0.869231576 0.868889399 0.865062936	0.858446015 0.841143193 0.888091222	0.86514406
L30	0.89875758 0.865297 0.88457816	0.88287758	0.919214446 0.900421238 0.854963879	0.821809161 0.820422956 0.732550689	0.84156373
L37-1	0.83276765 0.79028011 0.83562773	0.8195585	0.753084574 0.802022346 0.801434302	0.779599719 0.775634769 0.787949865	0.7832876
L46	0.88443921 0.77650397 0.89439843	0.85178054	0.826519017 0.855895529 0.84393352	0.967285575 0.871353096 0.904815669	0.8783004
L47	0.83420604 0.83485024 0.82501709	0.83135779	0.829684193 0.853349358 0.805917866	0.830036807 0.812762587 0.703756385	0.805918
L49	0.98048312 0.85996193 0.8307199	0.89038832	0.870244071 0.922988155 0.9436246	0.935264428 0.916503697 0.960435329	0.92484338
L54	0.85787576 0.93880345 0.80579382	0.86749101	0.852888022 0.867072273 0.898802846	0.862427142 0.864911151 0.828461419	0.86242714
L55-1	0.74239885 0.80504823 0.80848969	0.78531226	0.848156305 0.83364353 0.788811622	0.892861748 0.813445102 0.826277346	0.83386594
L57	0.88868911 0.89388179 0.86870497	0.88375863	0.958635553 0.893766233 0.950339514	0.825221223 0.906449684 0.911474037	0.90764771

L58	0.83165992 0.7994878 0.69102793	0.77405855	0.837492526 0.800692407 0.806176011	0.804448112 0.741807623 0.812566905	0.8005306
L64	0.78158887 0.75717906 0.7768945	0.77188748	0.810602662 0.902406798 0.778642133	0.832217835 0.816996606 0.814324526	0.82586509
L66	0.81880592 0.83369773 0.85127219	0.83459195	0.940103354 0.84402712 0.914728871	0.905979173 0.928314545 0.902721974	0.90597917
L80	0.80138115 0.86015868 0.86947157	0.84367047	0.976655995 0.887996535 0.908855557	0.928456993 0.953703018 0.843623148	0.91654854
L81	0.78725038 0.79784598 0.8271475	0.80408129	0.781918143 0.824572011 0.810186488	0.778578206 0.819183225 0.877032794	0.81524514

2. Standard curves

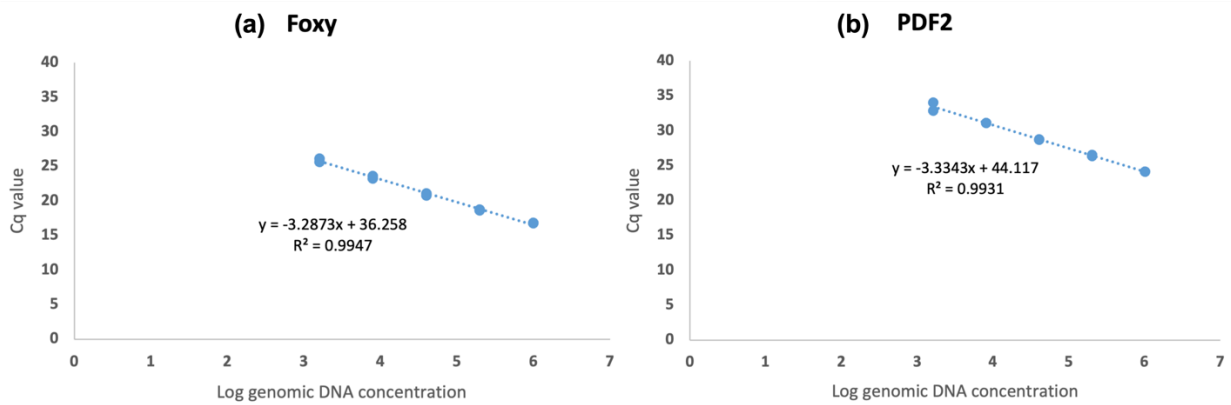

Figure 23. *Standard curves for determination of primer efficiency a: Foxy primer for Fol (primer efficiency 101,5%). b: PDF2 primer for L. angustifolius (primer efficiency 99,5%)*

IX. BIBLIOGRAPHY

Abdullah, A. S., Turo, C., Moffat, C. S., Lopez-Ruiz, F. J., Gibberd, M. R., Hamblin, J., & Zerihun, A. (2018). Real-Time PCR for Diagnosing and Quantifying Co-infection by Two Globally Distributed Fungal Pathogens of Wheat. *Frontiers in Plant Science*, *9*, 1086. https://doi.org/10.3389/fpls.2018.01086

Abraham, E., Ganopoulos, I., Madesis, P., Mavromatis, A., Mylona, P., Nianiou-Obeidat, I., Parissi, Z., Polidoros, A., Tani, E., & Vlachostergios, D. (2019). The Use of Lupin as a Source of Protein in Animal Feeding: Genomic Tools and Breeding Approaches. *International Journal of Molecular Sciences*, *20*(4), 851. https://doi.org/10.3390/ijms20040851

Adams, G. (2020). A beginner's guide to RT-PCR, qPCR and RT-qPCR. *The Biochemist*, *42*(3), 48–53. https://doi.org/10.1042/BIO20200034

Armstrong, G. M., & Armstrong, J. K. (1964). Lupinus species-common hosts for wilt fusarium from alfalfa, bean, Cassia, cowpea, lupine, and US cotton.

Askun, T. (2018). Introductory Chapter: Fusarium: Pathogenicity, Infections, Diseases, Mycotoxins and Management. In T. Askun (Ed.), *Fusarium—Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers*. InTech. https://doi.org/10.5772/intechopen.76507

Australian Government. (2013). *The Biology of Lupinus L. (lupin or lupine)*. Office of the Gene Technology Regulator. https://ogtr.gov.au/resources/publications/biology-lupinus-l-lupin-or-lupine

Babadoost, M. (2018). Fusarium: Historical and Continued Importance. In T. Askun (Ed.), *Fusarium—Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers*. InTech. https://doi.org/10.5772/intechopen.74147

Bani, M., Pérez-De-Luque, A., Rubiales, D., & Rispail, N. (2018). Physical and Chemical Barriers in Root Tissues Contribute to Quantitative Resistance to Fusarium oxysporum f. Sp. Pisi in Pea. *Frontiers in Plant Science*, *9*, 199. https://doi.org/10.3389/fpls.2018.00199

Bani, M., Rubiales, D., & Rispail, N. (2012). A detailed evaluation method to identify sources of quantitative resistance to *Fusarium oxysporum* f. Sp. *Pisi* race 2 within a *Pisum* spp. Germplasm collection. *Plant Pathology*, *61*(3), 532–542. https://doi.org/10.1111/j.1365-3059.2011.02537.x

Baroncelli, R., Pensec, F., Da Lio, D., Boufleur, T., Vicente, I., Sarrocco, S., Picot, A., Baraldi, E., Sukno, S., Thon, M., & Le Floch, G. (2021). Complete Genome Sequence of the Plant-Pathogenic Fungus *Colletotrichum lupini*. *Molecular Plant-Microbe Interactions®*, *34*(12), 1461–1464. https://doi.org/10.1094/MPMI-07-21-0173-A

Bebeli, P. J., Lazaridi, E., Chatzigeorgiou, T., Suso, M.-J., Hein, W., Alexopoulos, A. A., Canha, G., Van Haren, R. J. F., Jóhannsson, M. H., Mateos, C., Neves-Martins, J., Prins, U., Setas, F., Simioniuc, D. P., Talhinhas, P., & Van Den Berg, M. (2020). State and Progress of Andean Lupin Cultivation in Europe: A Review. *Agronomy*, *10*(7), 1038. https://doi.org/10.3390/agronomy10071038

Boba, A., Kostyn, K., Kozak, B., Zalewski, I., Szopa, J., & Kulma, A. (2021). Transcriptomic profiling of susceptible and resistant flax seedlings after Fusarium oxysporum lini infection. *PLOS ONE*, *16*(1), e0246052. https://doi.org/10.1371/journal.pone.0246052

Bolland, M. D. A., & Brennan, R. F. (2008). Comparing the phosphorus requirements of wheat, lupin, and canola. *Australian Journal of Agricultural Research*, *59*(11), 983–998. https://doi.org/10.1071/AR07430

Borreani, G., Chion, A. R., Colombini, S., Odoardi, M., Paoletti, R., & Tabacco, E. (2009). Fermentative profiles of field pea (*Pisum sativum*), faba bean (*Vicia faba*) and white lupin (*Lupinus albus*) silages as affected by wilting and inoculation. *Animal Feed Science and Technology*, *151*(3), 316–323. https://doi.org/10.1016/j.anifeedsci.2009.01.020

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

CABI. (2022). *Fusarium oxysporum f.sp. Lupini (wilt of lupin)* (p. 24659) [dataset]. https://doi.org/10.1079/cabicompendium.24659

Cenis, J. L. (1992). Rapid extraction of fungal DNA for PCR amplification. Nucleic Acids Research, 20(9), 2380. https://doi.org/10.1093/nar/20.9.2380

Chauhan YS, Nene YL, Johansen C, Haware MP, Saxena NP, Singh S, Sharma SB, Sahrawat KL, Burford JR, Rupela OP, Kumar Rao JVDK, Sithanantham S.(1988). Effects of Soil Solarization on Pigeonpea and Chickpea. Information Bulletin No. 11. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Chen, L., Wu, Q., He, W., He, T., Wu, Q., & Miao, Y. (2019). Combined De Novo Transcriptome and Metabolome Analysis of Common Bean Response to Fusarium oxysporum f. Sp. Phaseoli Infection. *International Journal of Molecular Sciences*, *20*(24), 6278. https://doi.org/10.3390/ijms20246278

Chen, Y. C., Wong, C. L., Muzzi, F., Vlaardingerbroek, I., Kidd, B. N., & Schenk, P. M. (2014). Root defense analysis against Fusarium oxysporum reveals new regulators to confer resistance. *Scientific Reports*, *4*(1), 5584. https://doi.org/10.1038/srep05584

Choi, H.-W., Kim, J.-M., Hong, S.-K., Kim, W.-G., Chun, S.-C., & Yu, S.-H. (2009). Mating Types and Optimum Culture Conditions for Sexual StateFormation of Fusarium fujikuroi Isolates. *Mycobiology*, *37*(4), 247–250. https://doi.org/10.4489/MYCO.2009.37.4.247

Chu, S., Wang, J., Cheng, H., Yang, Q., & Yu, D. (2014). Evolutionary study of the isoflavonoid pathway based on multiple copies analysis in soybean. BMC Genetics, 15(1), 76. https://doi.org/10.1186/1471-2156-15-76

Czepiel, K., Krajewski, P., Wilczura, P., Bielecka, P., Święcicki, W., & Kroc, M. (2021). Expression Profiles of Alkaloid-Related Genes across the Organs of Narrow-Leafed Lupin (Lupinus angustifolius L.) and in Response to Anthracnose Infection. International Journal of Molecular Sciences, 22(5), 2676. https://doi.org/10.3390/ijms22052676

Dewey, J., Hicks, J., & Schuchardt, A. (2022). Improving Students' Understanding of Biological Variation in Experimental Design and Analysis through a Short Model-Based Curricular Intervention. *CBE—Life Sciences Education*, *21*(1), ar11. https://doi.org/10.1187/cbe.21-03-0062

Diaz, J. M. R. (2015). *Use of amaranth, quinoa, kañiwa and lupine for the development of gluten-free extruded snacks*. University of Helsinki.

DPIRD. (2023, February 11). *Understanding and managing sclerotinia stem rot in lupins* [Text]. https://agric.wa.gov.au/n/9319

Edel-Hermann, V., & Lecomte, C. (2019). Current Status of *Fusarium oxysporum Formae Speciales* and Races. *Phytopathology®*, *109*(4), 512–530. https://doi.org/10.1094/PHYTO-08-18-0320-RVW

Eickmeyer, F. (2023, January). *Lupinenzüchtung für Food und Technik – Beitrag der Bitterlupinen*. GFL-Jahrestagung 2023. https://lupinenverein.de/wp-content/uploads/2022/10/8_Eickmeyer_ESKUSA_GFL-JT2023.pdf

Ekwomadu, T. I., & Mwanza, M. (2023). Fusarium Fungi Pathogens, Identification, Adverse Effects, Disease Management, and Global Food Security: A Review of the Latest Research. *Agriculture*, *13*(9), 1810. https://doi.org/10.3390/agriculture13091810

El-Rahman, S. S., Mazen, M. M., Mohamed, H. I., & Mahmoud, N. M. (2012). Induction of defence related enzymes and phenolic compounds in lupin (Lupinus albus L.) and their effects on host resistance against Fusarium wilt. *European Journal of Plant Pathology*, *134*(1), 105–116. https://doi.org/10.1007/s10658-012-0028-z

European Commission. (2023, July 27). *Boosting innovation in breeding for the next generation of legume crops for Europe | Legume Generation Project | Fact Sheet | HORIZON*. CORDIS | European Commission. https://cordis.europa.eu/project/id/101081329

Food and Agriculture Organization of the United Nations (FAO). (2017). ECOCROP | GAEZ v4 Data Portal. FAO, Rome, Italy. https://gaez.fao.org/pages/ecocrop

FAOSTAT. (2023). *Crops and livestock products* [dataset]. https://www.fao.org/faostat/en/#data/QCL

Fischer, K., Dieterich, R., Nelson, M. N., Kamphuis, L. G., Singh, K. B., Rotter, B., Krezdorn, N., Winter, P., Wehling, P., & Ruge-Wehling, B. (2015). Characterization and mapping of LanrBo: A locus conferring anthracnose resistance in narrow-leafed lupin (Lupinus angustifolius L.). *Theoretical and Applied Genetics*, *128*(10), 2121–2130. https://doi.org/10.1007/s00122-015-2572-3

Formela, M., Samardakiewicz, S., Marczak, Ł., Nowak, W., Narożna, D., Bednarski, W., Kasprowicz-Maluśki, A., & Morkunas, I. (2014). Effects of Endogenous Signals and Fusarium oxysporum on the Mechanism Regulating Genistein Synthesis and Accumulation in Yellow Lupine and Their Impact on Plant Cell Cytoskeleton. *Molecules*, *19*(9), 13392–13421. https://doi.org/10.3390/molecules190913392

Formela-Luboińska, M., Remlein-Starosta, D., Waśkiewicz, A., Karolewski, Z., Bocianowski, J., Stępień, Ł., Labudda, M., Jeandet, P., & Morkunas, I. (2020). The Role of Saccharides in the Mechanisms of Pathogenicity of Fusarium oxysporum f. Sp. Lupini in Yellow Lupine (Lupinus luteus L.). *International Journal of Molecular Sciences*, *21*(19), 7258. https://doi.org/10.3390/ijms21197258

Frick, K. M., Kamphuis, L. G., Siddique, K. H. M., Singh, K. B., & Foley, R. C. (2017). Quinolizidine Alkaloid Biosynthesis in Lupins and Prospects for Grain Quality Improvement. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00087

Fumagalli, P., Comolli, R., Ferrè, C., Ghiani, A., Gentili, R., & Citterio, S. (2014). The rotation of white lupin (Lupinus albus L.) with metal-accumulating plant crops: A strategy to increase the benefits of soil phytoremediation. *Journal of Environmental Management*, *145*, 35–42. https://doi.org/10.1016/j.jenvman.2014.06.001

Galindo-González, L., & Deyholos, M. K. (2016). RNA-seq Transcriptome Response of Flax (Linum usitatissimum L.) to the Pathogenic Fungus Fusarium oxysporum f. Sp. Lini. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.01766

Gao, X., Jackson, T. A., Lambert, K. N., Li, S., Hartman, G. L., & Niblack, T. L. (2004). Detection and Quantification of *Fusarium solani* f. Sp. *Glycines* in Soybean Roots with Real-Time Quantitative Polymerase Chain Reaction. *Plant Disease*, *88*(12), 1372–1380. https://doi.org/10.1094/PDIS.2004.88.12.1372

Garg, G., Kamphuis, L. G., Bayer, P. E., Kaur, P., Dudchenko, O., Taylor, C. M., Frick, K. M., Foley, R. C., Gao, L., Aiden, E. L., Edwards, D., & Singh, K. B. (2022). A pan-genome and chromosome-length reference genome of narrow-leafed lupin (*Lupinus angustifolius*

) reveals genomic diversity and insights into key industry and biological traits. *The Plant Journal*, *111*(5), 1252–1266. https://doi.org/10.1111/tpj.15885

Georgieva, N. A., Kosev, V. I., Genov, N. G., & Butnariu, M. (2018). Morphological and Biological Characteristics of White Lupine Cultivars (Lupinus albus L.). *Romanian Agricultural Research*, *35*, 109–119. https://doi.org/10.59665/rar3515

Giménez-Ibánez, S. (2020). Designing disease-resistant crops: From basic knowledge to biotechnology. *Mètode Revista de Difusió de La Investigació*, *11*. https://doi.org/10.7203/metode.11.15496

Gresta, F., Wink, M., Prins, U., Abberton, M., Capraro, J., Scarafoni, A., & Hill, G. (2017). Lupins in European cropping systems. In D. Murphy-Bokern, F. L. Stoddard, & C. A. Watson (Eds.), *Legumes in cropping systems* (1st ed., pp. 88–108). CABI. https://doi.org/10.1079/9781780644981.0088

Haware MP (1998) Diseases of chickpea. In: Allen DJ and Lenné JM (eds.) The Pathology of Food and Pasture Legumes (pp. 473-516) CAB International and ICRISAT, UK.

Heuzé, V., Thiollet, H., Tran, G., Nozière, P., & Lebas, F. (2019). *Blue lupin (Lupinus angustifolius) forage. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO*. https://www.feedipedia.org/node/24503

Hogg, A. C., Johnston, R. H., & Dyer, A. T. (2007). Applying Real-Time Quantitative PCR to Fusarium Crown Rot of Wheat. *Plant Disease*, *91*(8), 1021–1028. https://doi.org/10.1094/PDIS-91-8-1021

Holtz, M. D., Chang, K. F., Hwang, S. F., Gossen, B. D., & Strelkov, S. E. (2013). Characterization of *Fusarium* spp. Associated with lupin in central Alberta, Canada. *Canadian Journal of Plant Pathology*, *35*(1), 56–67. https://doi.org/10.1080/07060661.2012.729538

Hwang, S. F., Chang, K. F., Strelkov, S. E., Gossen, B. D., & Howard, R. J. (2014). The impact of *Fusarium avenaceum* on lupin production on the Canadian prairies. *Canadian Journal of Plant Pathology*, *36*(3), 291–299. https://doi.org/10.1080/07060661.2014.925507

Infantino, A., Kharrat, M., Riccioni, L., Coyne, C. J., McPhee, K. E., & Grünwald, N. J. (2006). Screening techniques and sources of resistance to root diseases in cool season food legumes. *Euphytica*, *147*(1–2), 201–221. https://doi.org/10.1007/s10681-006-6963-z

Institute of Agricultural and Food Economics National Research Institute (IAFE-NRI). (2021). Protein crop market in Poland. Warsaw, Poland. Retrived from: https://www.agroberichtenbuitenland.nl/landeninformatie/polen/achtergrond/protein-crop-market-in-poland

Jangir, P., Mehra, N., Sharma, K., Singh, N., Rani, M., & Kapoor, R. (2021). Secreted in Xylem Genes: Drivers of Host Adaptation in Fusarium oxysporum. *Frontiers in Plant Science*, *12*, 628611. https://doi.org/10.3389/fpls.2021.628611

Jansen, P. C. M., 2006. Lupinus albus L. Record from PROTA4U, Brink, M. & Belay, G. (Editors), PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands

Jørnsgård, B., Kuptsov, V., Jensen, B., Bødker, L., Knudsen, J. C., & Nielsen, J. (2007). *Resistance against Fusarium spp. In narrowed leafed lupin (Lupinus angustifolius L.)*. https://orgprints.org/id/eprint/10996/

Kingwell, R. (2003). Economic considerations for Lupin Protein Concentrate production. In Proceedings of the 1st Workshop for Seeding a Future for Grains in Aquaculture Feeds, Fremantle, Australia; pp. 16–20.

Knight, N. L., Sutherland, M. W., Martin, A., & Herde, D. J. (2012). Assessment of Infection by *Fusarium pseudograminearum* in Wheat Seedling Tissues Using Quantitative PCR and a Visual Discoloration Scale. *Plant Disease*, *96*(11), 1661–1669. https://doi.org/10.1094/PDIS-12-11-1050-RE

Koblitz, J., Halama, P., Spring, S., Thiel, V., Baschien, C., Hahnke, R. L., Pester, M., Overmann, J., & Reimer, L. C. (2023). Media *Dive*: The expert-curated cultivation media database. *Nucleic Acids Research*, *51*(D1), D1531–D1538. https://doi.org/10.1093/nar/gkac803

Książkiewicz, M., Rychel-Bielska, S., Plewiński, P., Bielski, W., Nuc, M., Kozak, B., Krajewski, P., & Jędryczka, M. (2022). A successful defense of the narrow-leafed lupin against anthracnose involves quick and orchestrated reprogramming of oxidation–reduction, photosynthesis and pathogenesis-related genes. *Scientific Reports*, *12*(1), 8164. https://doi.org/10.1038/s41598-022-12257-7

Książkiewicz, M., Rychel-Bielska, S., Plewiński, P., Nuc, M., Irzykowski, W., Jędryczka, M., & Krajewski, P. (2021). The Resistance of Narrow-Leafed Lupin to Diaporthe toxica Is Based on the Rapid Activation of Defense Response Genes. *International Journal of Molecular Sciences*, *22*(2), 574. https://doi.org/10.3390/ijms22020574

Kupstou, N., Christiansen, J. L., Raza, S., & Joernsgaard, B. (2002, June). *Greenhouse screening for fusarium wilt resistance in lupine*. 10th International Lupin Conference, Iceland.

Kuptsov, V. (2000). *Lupin: An ancient crop for the new millenium; proceedings of the 9th International Lupin Conference* (E. van Santen & M. Wink, Eds.). International Lupin Association.

Kurlovich, B., Korneichuk, N. S., & Kiselev, I. I. (1995). Breeding Fusarium resistant lupin forms for agricultural environment of Russia based on ecologico-geographical approach. *Journal of Applied Genetics*, *36*(3). http://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-5f499b62-b2b6-4cbe-9574-7535e9d173ef

Kurlovich, B. S., Stankevich, A. K., & Stepanova, S. I. (2002). The history of lupin domestication. Lupins (Geography, classification, genetic resources and breeding). OY International North Express. St. Petersburg, Russia-Pellosniemi, Finland, 147-164.

Lamberts, H., 1955. Broadening the bases for the breeding of yellow sweet lupine. Euphytica 4: 97–106.

Lawrance, L. (2007). Lupins: Australia's Role in World Markets. *Australian Commodities: Forecasts and Issues*, *14*(2), 353–356. https://doi.org/10.3316/informit.929412013106520

Lindbeck, K. (2009). *Threat Specific Contingency Plan: Fusarium wilt (of chickpea, lentil & lupin)*. Plant Health Australia.

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frías, J., Martínez-Villaluenga, C., Sussmann, D., Duranti, M., Seger, A., Zander, P. M., & Pueyo, J. J. (2015). The future of lupin as a protein crop in Europe. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00705

Malvick, D. K., & Impullitti, A. E. (2007). Detection and Quantification of *Phialophora gregata* in Soybean and Soil Samples with a Quantitative, Real-Time PCR Assay. *Plant Disease*, *91*(6), 736–742. https://doi.org/10.1094/PDIS-91-6-0736

Mezzomo, R., Rolim, J. M., Poletto, T., Oliveira, M. B. D., Lazarotto, M., & Muniz, M. F. B. (2018). MYCELIAL GROWTH AND SPORULATION OF Fusarium spp. PATHOGENIC TO Ilex paraguariensis IN DIFFERENT CULTURE MEDIA AND UNDER EXPOSURE TO

DIFFERENT LIGHT LEVELS. *Scientia Agraria*, *19*(1), 14. https://doi.org/10.5380/rsa.v19i1.55844

Milgate, A., Baxter, B., Simpfendorfer, S., Herdina, Giblot-Ducray, D., Yang, N., Orchard, B., & Ovenden, B. (2023). Improved quantification of Fusarium pseudograminearum (Fusarium crown rot) using qPCR measurement of infection in multi-species winter cereal experiments. *Frontiers in Plant Science*, *14*, 1225283. https://doi.org/10.3389/fpls.2023.1225283

Mohamed, H. I., Abd El-Rahman, S., & Mazen, M. (2012). Influence of some biotic and abiotic inducers on Fusarium wilt disease incidence of lupin (Lupinus albus) on disease resistance and protein pattern. *Plant Pathology & Quarantine*, *2*(2), 179–188. https://doi.org/10.5943/ppq/2/2/11

Mondal, R., Kumar, A., & Gnanesh, B. N. (2023). Crop germplasm: Current challenges, physiological-molecular perspective, and advance strategies towards development of climate-resilient crops. *Heliyon*, *9*(1), e12973. https://doi.org/10.1016/j.heliyon.2023.e12973

Morkunas, I., Bednarski, W., & Kozłowska, M. (2004). Response of embryo axes of germinating seeds of yellow lupine to Fusarium oxysporum. *Plant Physiology and Biochemistry*, *42*(6), 493–499. https://doi.org/10.1016/j.plaphy.2004.05.007

Morkunas, I., Formela, M., Floryszak-Wieczorek, J., Marczak, Ł., Narożna, D., Nowak, W., & Bednarski, W. (2013). Cross-talk interactions of exogenous nitric oxide and sucrose modulates phenylpropanoid metabolism in yellow lupine embryo axes infected with Fusarium oxysporum. *Plant Science*, *211*, 102–121. https://doi.org/10.1016/j.plantsci.2013.07.007

Morkunas, I., & Gmerek, J. (2007). The possible involvement of peroxidase in defense of yellow lupine embryo axes against Fusarium oxysporum. *Journal of Plant Physiology*, *164*(2), 185–194. https://doi.org/10.1016/j.jplph.2005.11.005

Morkunas, I., Marczak, Ł., Stachowiak, J., & Stobiecki, M. (2005). Sucrose-induced lupine defense against Fusarium oxysporum. *Plant Physiology and Biochemistry*, *43*(4), 363–373. https://doi.org/10.1016/j.plaphy.2005.02.011

Morkunas, I., Narożna, D., Nowak, W., Samardakiewicz, S., & Remlein-Starosta, D. (2011). Cross-talk interactions of sucrose and Fusarium oxysporum in the phenylpropanoid pathway and the accumulation and localization of flavonoids in embryo axes of yellow lupine. *Journal of Plant Physiology*, *168*(5), 424–433. https://doi.org/10.1016/j.jplph.2010.08.017

Namdar, D., Mulder, P. P. J., Ben-Simchon, E., Hacham, Y., Basheer, L., Cohen, O., Sternberg, M., & Shelef, O. (2024). New Analytical Approach to Quinolizidine Alkaloids and Their Assumed Biosynthesis Pathways in Lupin Seeds. *Toxins*, *16*(3), 163. https://doi.org/10.3390/toxins16030163

New England Biolabs. (n.d.). *Luna® Universal qPCR Master Mix | NEB*. Retrieved May 20, 2024, from https://www.neb.com/en/products/m3003-luna-universal-qpcr-master-mix#Citations---Technical-Literature

Notz, I., & Reckling, M. (2022, November 4). Why farmers grow lupin: Insights from a survey of German farmers. *Legumehub.Eu*. https://www.legumehub.eu/is_article/why-farmers-grow-lupin/

Osman, M., Stigloher, C., Mueller, M. J., & Waller, F. (2020). An improved growth medium for enhanced inoculum production of the plant growth-promoting fungus Serendipita indica. *Plant Methods*, *16*(1), 39. https://doi.org/10.1186/s13007-020-00584-7

Ozdemir, F., Koc, N. K., Paulitz, T., Nicol, J. M., Schroeder, K. L., & Poole, G. (2020). Determination of fusarium crown rot resistance in wheat to Fusarium culmorum and Fusarium pseudogramineaum using real time PCR. *Crop Protection*, *135*, 105204. https://doi.org/10.1016/j.cropro.2020.105204

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), 45e–445. https://doi.org/10.1093/nar/29.9.e45

Pulse Crop Database. (2024). *Lupinus angustifolius* [dataset]. https://www.pulsedb.org/organism/983370

Rataj-Guranowska, M., Wiatroszak, I., & Hornok, L. (1984). Serological Comparison of Two Races of *Fusarium oxysporum* f. Sp. *Lupini*. *Journal of Phytopathology*, *110*(3), 221–225. https://doi.org/10.1111/j.1439-0434.1984.tb00752.x

Raza, S., Christiansen, J. L., Jørnsgå;rd, B., & Ortiz, R. (2000). Partial resistance to a Fusarium root disease in Egyptian white lupin landraces. *Euphytica*, *112*(3), 233–237. https://doi.org/10.1023/A:1003904805737

Rhodes, L., & Maxted, N. (2014). *Lupinus angustifolius: The IUCN Red List of Threatened Species 2016: e.T174707A19404942* [dataset]. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T174707A19404942.en

Rispail, N., & Rubiales, D. (2015). Rapid and Efficient Estimation of Pea Resistance to the Soil-Borne Pathogen Fusarium oxysporum by Infrared Imaging. *Sensors*, *15*(2), 3988–4000. https://doi.org/10.3390/s150203988

Rocha, L. F., Srour, A. Y., Pimentel, M., Subedi, A., Bond, J. P., Fakhoury, A., & Ammar, H. A. (2023). A panel of qPCR assays to detect and quantify soybean soil-borne pathogens. *Letters in Applied Microbiology*, *76*(1), ovac023. https://doi.org/10.1093/lambio/ovac023

Rojas-Sandoval, J. (2022). *Lupinus angustifolius (narrow-leaf lupin)* (p. 31706) [dataset]. https://doi.org/10.1079/cabicompendium.31706

Roman, L., Tsochatzis, E., Tarin, K., Röndahl, E. M., Ottosen, C.-O., & Corredig, M. (2023). Compositional Attributes of Blue Lupin (Lupinus angustifolius) Seeds for Selection of High-Protein Cultivars. *Journal of Agricultural and Food Chemistry*, *71*(45), 17308–17320. https://doi.org/10.1021/acs.jafc.3c04804

Rychel, S., & Książkiewicz, M. (2019). Development of gene-based molecular markers tagging low alkaloid pauper locus in white lupin (Lupinus albus L.). *Journal of Applied Genetics*, *60*(3–4), 269–281. https://doi.org/10.1007/s13353-019-00508-9

Salleh, B., & Owen, H. (1984). Xylem Fluids of White Lupin (*Lupinus albus*) Cultivars Susceptible and Resistant to *Fusarium oxysporum* f. Sp. Lupini Race 3. *Journal of Phytopathology*, *111*(3–4), 189–198. https://doi.org/10.1111/j.1439-0434.1984.tb00760.x

Schena, L., Li Destri Nicosia, M. G., Sanzani, S. M., Faedda, R., Ippolito, A., & Cacciola, S. O. (2013). Development of quantitative PCR detection methods for phytopathogenic fungi and oomycetes. *Journal of Plant Pathology*, *95*(1). https://doi.org/10.4454/JPP.V95I1.016

Schrenk, D., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Leblanc, J., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Alexander, J., Cottrill, B., Dusemund, B., … Bignami, M. (2019). Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products. *EFSA Journal*, *17*(11). https://doi.org/10.2903/j.efsa.2019.5860

Schumacher, H., Paulsen, H. M., Gau, A. E., Link, W., Jürgens, H. U., Sass, O., & Dieterich, R. (2011). Seed protein amino acid composition of important local grain legumes Lupinus

angustifolius L., Lupinus luteus L., Pisum sativum L. and Vicia faba L. *Plant Breeding*, *130*(2), 156–164. https://doi.org/10.1111/j.1439-0523.2010.01832.x

Sedláková, K., Straková, E., Suchý, P., Krejcarová, J., & Herzig, I. (2016). Lupin as a perspective protein plant for animal and human nutrition – a review. *Acta Veterinaria Brno*, *85*(2), 165–175. https://doi.org/10.2754/avb201685020165

Shaban, W. I., & Zian, H. (2011). *Control of Lupine Fusarium Wilt by Biofumigation with Mustard and Canola Seed Meal*. *6*(2).

Singh, G., Chen, W., Rubiales, D., Moore, K., Sharma, Y. R., & Gan, Y. (2007). Diseases and their management. In S. S. Yadav, R. J. Redden, W. Chen, & B. Sharma (Eds.), *Chickpea breeding and management* (1st ed., pp. 497–519). CABI. https://doi.org/10.1079/9781845932138.024

Stark, R., Grzelak, M., & Hadfield, J. (2019). RNA sequencing: The teenage years. *Nature Reviews Genetics*, *20*(11), 631–656. https://doi.org/10.1038/s41576-019-0150-2

Steward, K. (2024, February 23). *qPCR Analysis, How a qPCR Machine Works and qPCR Protocol*. Analysis & Separations from Technology Networks. http://www.technologynetworks.com/analysis/articles/qpcr-analysis-how-a-qpcr-machine-works-and-qpcr-protocol-356835

Talhinhas, P., Baroncelli, R., & Le Floch, G. (2016). Anthracnose of lupins caused by Colletotrichum lupini: A recent disease and a successful worldwide pathogen. *Journal of Plant Pathology*, *98*(1). https://doi.org/10.4454/JPP.V98I1.040

Taylor, C. M., Jost, R., Erskine, W., & Nelson, M. N. (2016). Identifying Stable Reference Genes for qRT-PCR Normalisation in Gene Expression Studies of Narrow-Leafed Lupin (Lupinus angustifolius L.). *PLOS ONE*, *11*(2), e0148300. https://doi.org/10.1371/journal.pone.0148300

Thatcher, L. F., Williams, A. H., Garg, G., Buck, S.-A. G., & Singh, K. B. (2016). Transcriptome analysis of the fungal pathogen Fusarium oxysporum f. Sp. Medicaginis during colonisation of resistant and susceptible Medicago truncatula hosts identifies differential pathogenicity profiles and novel candidate effectors. *BMC Genomics*, *17*(1), 860. https://doi.org/10.1186/s12864-016-3192-2

Van Andel, O. M. (1956). The importance of amino acids for the development of Fusarium oxysporum (F. lupini) sn.et h. In the xylem of lupins. *Acta Botanica Neerlandica*, *5*(3), 280–286. https://doi.org/10.1111/j.1438-8677.1956.tb00562.x

Vishnyakova, M. A., Kushnareva, A. V., Shelenga, T. V., & Egorova, G. P. (2020). Alkaloids of narrow-leaved lupine as a factor determining alternative ways of the crop's utilization and breeding. *Vavilov Journal of Genetics and Breeding*, *24*(6), 625–635. https://doi.org/10.18699/VJ20.656

Wahid, O. A. A. (2006). Improving control of Fusarium wilt of leguminous plants by combined application of biocontrol agents. *Phytopathologia Mediterranea*, *45*(3).

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: A revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*(1), 57–63. https://doi.org/10.1038/nrg2484

Weßling, R., & Panstruga, R. (2012). Rapid quantification of plant-powdery mildew interactions by qPCR and conidiospore counts. *Plant Methods*, *8*(1), 35. https://doi.org/10.1186/1746-4811-8-35

White, P., French, B., McLarty, A., & Grains Research and Development Corporation. (2008, August). *Producing lupins*. *Bulletin 4720*. https://library.dpird.wa.gov.au/bulletins/10

Yang, T., Liu, J., Li, X., Amanullah, S., Lu, X., Zhang, M., Zhang, Y., Luan, F., Liu, H., & Wang, X. (2022). Transcriptomic Analysis of Fusarium oxysporum Stress-Induced Pathosystem

and Screening of Fom-2 Interaction Factors in Contrasted Melon Plants. *Frontiers in Plant Science*, *13*, 961586. https://doi.org/10.3389/fpls.2022.961586

Zeise, K., Eickmeyer, F., Schmidt, F., Rose, J., Noll, G., Prüfer, D., Baum, C., Vitow, N., Schwinkendorf, L.-M., Ladach, T.-H., Christiansen, F., Kollar, F., & Leinweber, P. (2018). *Zuchtmaterialerstellung durch Erschließung und Selektion bisher nicht erfasster Anbau— Und Verwertungsmerkmale in bisher nicht genutzten genetischen Ressourcen der Schmalblättrigen Lupine (Lupinus angustifolius L.)*.

Zian, A. H., El-Demardash, I. S., El-Mouhamady, A. A., & El-Barougy, E. (2013). *Studies the Resistance of Lupine for Fusarium oxysporum F. sp Lupini) Through Molecular Genetic Technique*.