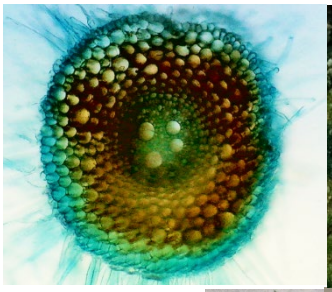
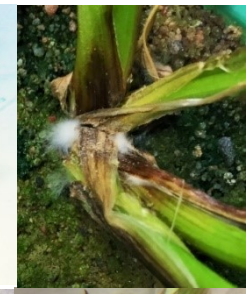
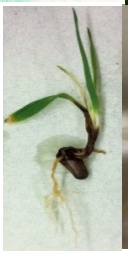
Assessing the effect of *Fusarium graminearum* inoculation on the root structure of Swedish winter wheat cultivars (*Triticum aestivum*)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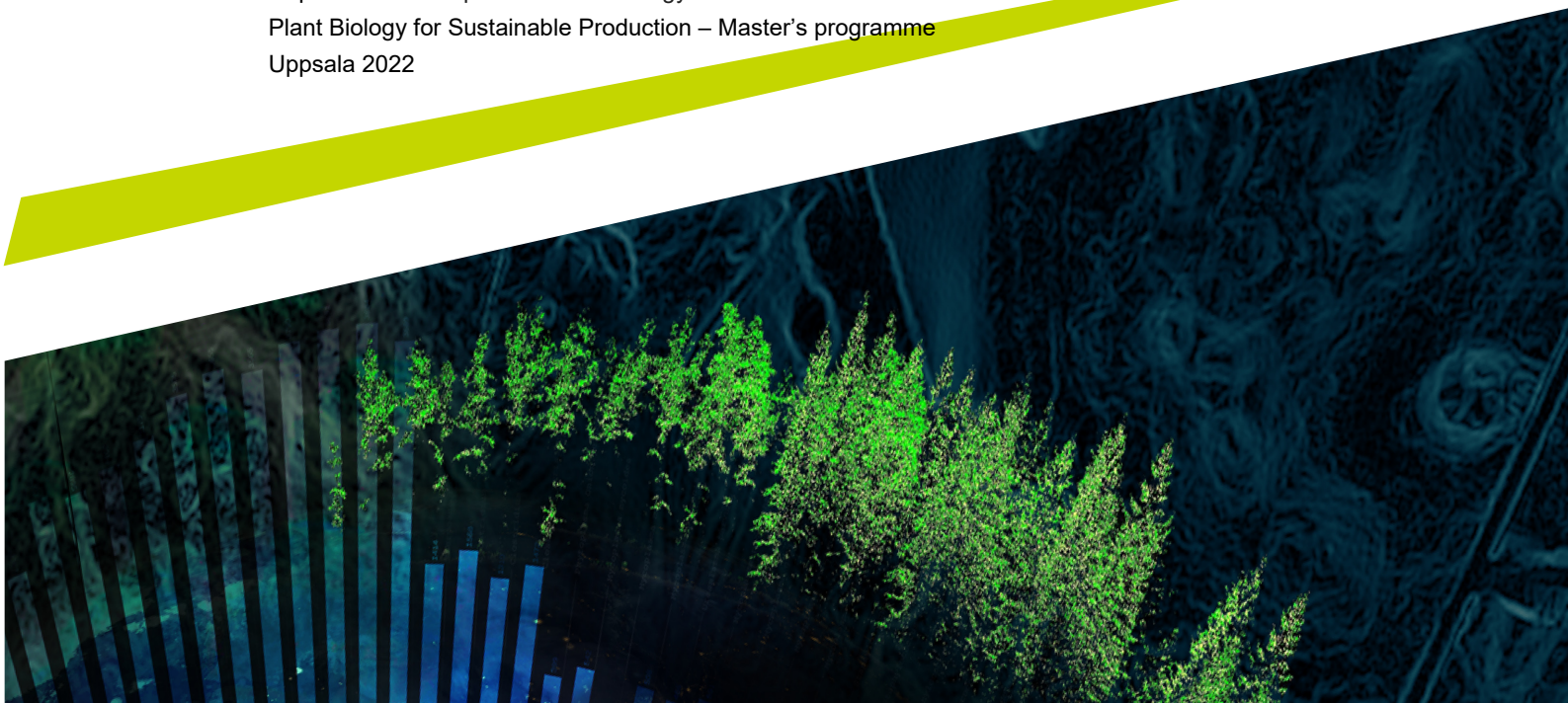

Assessing the effect of *Fusarium graminearum* inoculation on the root structure of Swedish winter wheat cultivars (*Triticum aestivum*

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Abstract

Several diseases attack the wheat crop and reduce the yield, such as root disease, which causes a tremendous loss in wheat production. Root rot has a variety of causal agents, such as nematodes, viruses, and bacteria, but the most common pathogens causing wheat root rot are oomycetes and fungi. The subject of this study is one of the fungus species from the *Fusarium* genus, *Fusarium graminearum*. This thesis aimed to evaluate the susceptibility of several wheat genotypes to root rot caused by *F. graminearum* under two concentrations of nutrients.

This study assessed five winter wheat cultivars - Julius, Brons, Reform, Informer, and Ceylon - in their development with *Fusarium* root rot. The study consisted of 80 pots of un-inoculated seedlings, 80 pots inoculated with a conidium suspension (62500 conidia/ml), and 40 pots inoculated with a hypha agar plug. In addition, an application of a single nutrient concentration was added to half of the pot numbers of inoculated and uninoculated samples. Then, a double nutrient concentration was added to another half of the pot numbers of inoculated and uninoculated samples. High seedling death was noted with the agar plug treatment, so seedling survival was measured to compare between genotypes—the conidia suspension allowed us to observe the interaction between the genotypes and different nutrient levels. The seedling can grow and use nutrients to support the immune system in the initial stages of development, while the conidial solution needs time to germinate and grow. Three variables were examined to compare these factors, i.e., visual shoot traits, visual root architectural traits, and microscopic root structural traits.

Differences in the inoculation method could be due to the difference in hypha development and, thus, the time before the *F. graminearum* hypha can launch its aggressive offensive against the seedling. With the mycelium growing, the agar plug method represents the living soil-borne pathogen growing on crop debris, with its grown mycelium ready to attach to the young seedlings at germination. The conidia suspension means transferring conidia to new soil (e.g., dispersed by wind or rain). These conidia will need time to germinate before infecting the more advanced seedlings. Then these seedlings will have had more time to use the nutrients and build defences against the pathogen.

The shoot traits include fresh and dry shoot biomass and leaf numbers. The plants were provided with two levels of nutrient doses to assist them in tolerating the pathogen. The results showed significantly more leaves developed in Ceylon than other cultivars under the conidia suspension inoculation method. On the other hand, many leaves were developed in Brons under agar plug mycelium inoculation.

The essential cellular structure traits, such as xylem number and diameter, are believed to create a kind of elasticity for transporting water and nutrients to other plant tissues. The xylem case was preserved in Julius and Reform cultivars despite the *F. graminearum* inoculations. In addition, Reform's live cortex cells percentage was higher than the other genotypes, suggesting higher nutrient storage ready to help the plant immune system.

The germination survival ratio suggests Brons and Julius are less susceptible to *F. graminearum* at the germination stage.

The three groups of studied traits play a role in understanding the genotypes' susceptibility to *F. graminearum*, helping to recognize tolerant genotypes.

The most distinguished cultivar in this experiment was Reform, showing a high germination survival ratio with the agar plug method compared to other cultivars. This high germination suggests that Reform can be a candidate to establish a molecular insight to study the resistance mechanism. The traits associated with the high germination ratio of Reform are high root and shoot fresh biomass weight. The quantification results of the traits, for instance, dry root and shoot biomass, xylem number, and the most recognized trait, the roots living cortex cells percentage, will support selecting the candidate cultivar and then implement this information in developing a breeding program for sustainable resistance.

Keywords: root rot, conidium suspension, hypha agar plug, root architecture, root cellar structure traits, shoot traits, genotypes.

Popular scientific abstract

Wheat is one of the essential foods for humanity, and with the high population, which continues to increase, there is a steadily increasing need for wheat. Furthermore, global weather changes will affect wheat production by changing the temperature and humidity of agricultural soil, changing the soil microbial community, and increasing the growth rate of disease-causing fungi (pathogens).

This increased growth of pathogens and, thus, increased level of disease in wheat is one of the challenges facing wheat production, affecting wheat's quantity and quality production. One disease with economic significance is fusarium root rot, which causes damages such as yield losses of 25% in Nordic European countries. This fungus also produces a toxin that can contaminate healthy grain. If this infected toxin grain is processed into flour, it will affect human health. Furthermore, if toxin-contaminated crop residue is provided as fodder to livestock, it will also affect the animal's health.

In the initial stages of infection, symptoms are hard to detect as they occur beneath the soil and cannot be seen. After a few days from the sowing date, seedlings do not produce any shoot above the soil and leave a space; another seedling produces weak shoot that wilt, dry and dies; other seedlings stay longer time alive. However, the fungi start to show a colony at the stem base after they wilt and die; some seedlings grow up to spike time and are infected with head blight. So, on the field scale, the infected plant will appear as spots of dry plants or empty or from white to light pink spikes.

Five wheat cultivars were grown in pots with two different nutrient regimes and inoculated with Fusarium spores. The roots were inoculated using two different methods, either in suspension or on agar plugs. The assessment measured shoot traits, root architecture, and cellular structure.

The first traits measured were the shoot traits, such as leaf number and the shoot's fresh and dry weight. So, the cultivar is less susceptible to the fungus under the nutrient's applications when one cultivar has a higher number than the other cultivars. The second set of traits was the root architecture traits, looking at two main types of roots. The first type is nodal roots with a shallow location and appears in the advanced development of the seedling. Our results have shown that when a cultivar has a high number of these roots, it increases the possibility of surviving the fusarium root rot, making the cultivar less susceptible to the pathogen. The second type of root we assessed was the seminal root that first grew deep in the soil. Our results show that they are the first front against the fungus, so it is less susceptible to the pathogen when the cultivar has high numbers of this type of root.

The third group of traits we assessed is the root cellular structure traits, which present the anatomical formation of stem cell tissues. The live cortex cell percentage was studied, which expresses the root cortex cells' life period. It can be estimated first from the root live cortex cells percentage that their capability of carbon food conservation if those cells are alive. The second estimation of the cell wall thickness formed a defensive front against the fungus's offensive ambush penetration in the root. In the experiment, the thickness

of the wall was estimated by realizing that the cultivar root has a high percentage of cortical living cells; the cells look small and denser when the cell percentage of the live cells is high. The assessment found that one cultivar, Reform, had the highest score of all cultivars evaluated, making Reform a candidate for breeding. Finally, the xylem number trait presents the vascular tissues bundle in the centre of the root responsible for transferring minerals and water to the leaves. When the fungus reaches the xylem, it has passed the first defensive line of cortex tissue towards the xylems to block them. The fungus uses water nutrients to propagate. There was no significance between the xylem number and disease interaction. However, only in nutrient applications does one cultivar have a higher number under higher nutrient applications. In lower applications, one cultivar has a higher number than the other. It can be estimated that when the cultivar has a higher number of xylem bundles, the chance of avoiding the fungus blocking them will increase, so the water and mineral will run through uninfected xylem bundles. As a result, the plant will survive and has more time to live.

Of the three groups of traits, most were significant and some insignificant. The aim was to find a scale to quantify the assessment process between the studied cultivars. I hope this study's results will reveal a pathway for building a plant with less exposed roots to disease and developing a sustainable resistance against the pathogen. In addition, global climate change introduces diseases to emerging areas or aggravates controlled diseases.

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Abbreviations

FRR	Fusarium Root Rot
FHB	Fusarium head blight
DON	Deoxynivalenol
Ggt	*Gaeumannomyces graminis var. tritici*
PCD	Programmed cell death
RCS	Root Cortical Senescence
ROS	Reactive oxygen species
MAMPs	Microbe-associated molecular patterns
DAMPs	Damage-associated molecular patterns
DNA	Deoxyribonucleic Acid
VPE	Volatile-producing endophytes
PDA	Potato Dextrose Agar
SNA	Synthetic Nutrient-Poor Agar
UV-A light	Ultraviolet A
ANOVA	Analysis of variance

1. -Introduction

1.1. The history and importance of the wheat

Food is essential for life, so we ask ourselves how our early ancestors domesticated wheat between 300,000 and 200,000 years (Richter et al., 2012). First, the early humans depended on hunting for meat and gathering plants for their food. Then, around 12,000 years ago, the early domestication of the earliest crops in the Neolithic era were wheat, barley, and lentils. This domestication occurred in the lower fertile region of Mesopotamia, the zone between two rivers, Tigris and the Euphrates. Next, humans learned how to grind the grains into flour and mix it with water to make bread, and this was the beginning of a long story about the crop called wheat (Stalker, 2000)

Wheat is an annual plant that belongs to the family *Poaceae* and the tribe *Triticeae* (Mazid, 2003). There are diverse types of wheat; soft wheat (*Triticum aestival*) is used for bread, cakes, and biscuit industries, and hard wheat, *Triticum durum*, is used in the pasta industry (Mazid, 2003). In Nordic countries, there are two types of wheat. Winter wheat is popular in Sweden, and spring wheat is used in the food industry (Ländell, 2021).

COCERAL European grain sector body expressed that Sweden's grain yield for 2018 was 7800 kg/hectare of winter wheat and 4400 kg/hectare of spring wheat, respectively (Ländell, 2018) (The Swedish Board of Agriculture "Jordbruksverket", 2021). Large consumers of wheat in the Swedish trade are Lilla Harrie Valskvarn mill and Västra Frölunda mill, which consume 120,000 tons of wheat grain per year to mill into bakery flour, primarily solid to Pågen bakery (Lyddon, 2018).

1.2. *Fusarium* diseases in wheat

Cereal production and wheat agriculture suffer from pathogenic *Fusarium* species (Vogelsang et al., 2019). As a result, there are losses and harmful influences. The losses range from 20 to 40% of grain yield, inadequately reflecting the actual costs of crop losses to the financial industry and farmers. The harmful effect is that seeds are contaminated with fungal toxins, which affect consumers, public health, societies, and environments (Savary et al., 2012).

Common field weeds and crop residues act as the pathogen's green bridge. Therefore, the crop residues must be removed to eliminate foci of infection, use fungicides targeted against the remains of fungi on the soil surface, and design agricultural rotation (Lamichhane et al., 2017).

Certain environmental conditions, such as contamination with industrial waste, can increase the ability of soil-borne pathogens, such as *Fusarium*, to infect plants. Chromium can be found in industrial waste and affect plant growth and development. Several *Fusarium* species, such as *Fusarium oxysporum*, can tolerate soils with elevated levels of chromium, which is much more threatening than other *Fusarium spp* in assailing the plant root in the polluted soil with industrial waste, especially chromium waste (Shoaib et al., 2019).

Agricultural rotation was *Fusarium. graminearum* infection results in the diseases *Fusarium Root Rot (FRR) and Fusarium Head Blight (FHB)* and is usually more predominant when wheat follows maize than grain follows other crops*. F. graminearum* is more aggressive than other toxigenic species*,* including *F. proliferatum, F. moniliforme, and F. culmorum/F. poae, and F. avenaceum* In Xu et al. (2007*),* it is declared that *F. graminearum* is the most aggressive of the *4 Fusarium spp*, such as *F. graminearum , F.avenaceum, F.culmorum, F.poae* test, resulting in more significant quantities of biomass produced by pathogen *"F. graminearum"* when competing."

The standard *Fusarium* population in European soils are *F. poae, F. avenaceum, F. equiseti, F. langsethiae, and F. sporotrichioides* (Kuzdralinski et al., 2017), especially *F. culmorum F. graminearum*, *F. pseudograminearum*, have the most effect on lowering yield production (Dyer et al., 2009).

Damages caused by *F. graminearum* can reduce the seed's quality by contaminating it with the pathogen's spores and yield loss associated with climate factors such as rainfall ratio and temperature. As (Evtić et al.,2021) scored in 2014 and 2015, respectively, the plants' infection was 31% 10%, yield loss of 15%, 5%, the average temperature of 16,3 °C, 18 °C and average rainfall 202.1 mm, 191.7 mm. Yield loss can occur when the pathogen causes FHB, characterised by blight in the spikes, and this disease caused $2.7 billion in losses between 1998 to 2002 in central and north America (Nganje et al. 2002). In a developing country like Syria, *F. graminearum* and other *Fusarium species* causing high yield loss can be very damaging as bread is the primary food source (Alkadri et al.,2013). Several *Fusarium species* cause root rot (Minati, 2020), but root rot has several causal agents, and not all root rot is caused by *Fusarium* (Bodah, 2017). The asexual sporulation phase of the pathogen is characterised by orange/pink sporodochia. It is emerging on the spikelet in moist climates (Scherm et al., 2013).

One of the most critical consequences of FRR and FHB, together with direct yield losses, is the contamination of the kernels with mycotoxins (Balmas et al., 2015). The seeds contaminated with mycotoxin will not be utilized for food and feed (Boenish et al., 2011).

During infection of the vascular disease *Fusarium* root rot, the pathogen can throw out hypha that can infect the bundle sheath before the defensive lignification of the parenchyma cells occurs. This period then allows the pathogen to diffuse towards the leaves, followed by spikes causing the head blight, producing a mycotoxin (Wang et al., 2015). Mycotoxin contamination of the crop residue is also a dilemma when the straw is used for animal feed (Beccari et al., 2018).

1.2.1. The life cycle of the disease

The best shelter for *F. graminearum* during the winter is in the inoculated wheat residues in the field. For example, wheat straw and debris from previous crops. The contamination was high in agricultural rotation crops such as maize and oilseed rape. Still, in other crops, such as mustard and alfalfa, the inoculation was low because these crops produce anti-mycotic agents (Leplat et al., 2016).

Suppose we keep crops in fields under suitable temperatures and humidity. In that case, *F. graminearum* will find an excellent ecology to propagate, spread, and last in the soil for a long time, causing yield loss problems (Leplat et al., 2013). The fungus produces asexual spores on contaminated residues (macroconidia) by rain-splash or wind dispersal (Basavaraj et al., 2020).

Another way of positioning the spores produced by *Fusarium* on roots/crowns is the spread to the heads by rain splashing (Zinkernagel et al.,1997).

F. graminearum is the name of the anamorph phase of the fungi; the sexual phase of the fungus is called *Gibberella zeae*. It thrives on the inoculated crop residues in warm and wet conditions, releasing sexual spores (ascospores) into the air. When those spores land on the plant surface, they look at the shape of the bluish-ebony granules (Trail, 2009). The turbulent wind flow lifts the ascospores to a far distance. When the ascospores touch down on wheat spikes, they start the infection and produce conidia, and then contagion transpires to the soil and the roots. (Schmale & Bergstrom, 2003). The site of primary contamination occurs during the flowering period of wheat. The florets will be eliminated, and grains will not fill if the anthers are contaminated just after their appearance.

Infected kernels develop from contaminated florets. Growing grains, which are tolerant to the pathogen, are contaminated with several mycotoxins produced by the fungus, like deoxynivalenol (DON), which causes health problems in animals (Lessard et al., 2015). Kernels contaminated with DON can be used to grow the next season's crops, but they should be treated with a chemical to prevent the germination of the fungus spores on the seeds' surface. Otherwise, they may result in blighted seedlings (Obanor et al., 2013). The infection percentage of contaminated kernels and soil conditions like pH, moisture, and other abiotic soil stress influence the germination and development of the seedlings; it counts on the effectiveness of inoculation transferring into the field (Drakopoulos et al., 2019).

The infection spreads rapidly and achieves its optimum in the "milky-waxy" maturing stage, affecting the developing kernels, and reducing yield. However, rains, prolonged instances of crop moisture, mist in maturing, and long harvesting time increase the contamination of the seeds (Mykhalska et al., 2019).

1.2.2. Symptoms of Fusarium Root Rot

A root infected with *F. graminearum* shows symptoms that mimic abiotic stress effects, such as wilting and drought of the shoot, as found by (Zhang et al., 2005; Deshmukh et al., 2006; Deshmukh & Kogel, 2007; Liu et al., 2015; Knight & Sutherland, 2016). The necrosis of lower stem tissues is a delayed symptom that occurs because of fusarium root rot due to an early infected root, which is challenging to recognise at the beginning of the root infection (Brennan et al., 2005). The early symptoms of the root seedlings are deep brown spots on the roots (Akinnuoye-Adelabu et al., 2019).

The level of root infection will explain how much of the thickness of necrotic infection. On thin root cells, walls will be more significantly infected than the thick ones, according to the cell wall gene, which controls the chitin deposition operation; this trait differs from one cultivar to another. The FRR mycelium breaks through the surface of the roots using hyphen swellings and moves into cortex cells (Beccari et al., 2011).

The wilting and drought symptoms will become apparent if the conidia hypha breaks through the cell's walls. The pathogen mycelium will block the seedling's vascular bundles and prevent them from conveying carbohydrates, nutrients, and water into its organs (Wang et al., 2015).

The alkaloid soil at pH 7 enables the pathogen mycelium mycotoxin to degrade the enzymes of the root's cell walls (Perincherry et al., 2019). As a result, the infection leads to reduced grain germination or, in the advanced stage of infection, will appear on the seedling stalk and roots, which causes the seedling to wilt (Ferrigo et al., 2016).

A specific "Pseudomonas " Plant growth promote rhizobacteria, which has symbiosis with the plant's root. As a result, the bacteria will help the plant's fitness by creating unsuitable environments for the pathogen to aggravate and propagate. (Barrett et al., 2009).

The hypha blocks the xylem centre and stops water and nutrients from running to the areal part, which causes dry leaves. Thus, the senescent cortex cells will be empty of any storage substances. However, the vascular bundles, including phloem, xylem numbers, and diameters, will still flexibly convey the water, minerals, and storage substances to reduce pathogen affection (Keenan et al., 2015).

When the soil gets polluted with the pathogen, which causes a primary degrading of plant biomass by enormous pathogen inoculation and propagation for releasing spores to spread the contamination on adjacent plants; in addition, flourishing root colonization leads to excessive necrosis of the roots and the stem base, hence the death of the plant (Winter et al., 2018).

The pathogen contamination affects the shoot length, which will take dwarven, root length and biomass. The symptoms can be visualized in the root cross-section under the microscope in colours from murky black to burnt brown possessed by the take-all hypha (Freeman & Ward, 2004).

1.2.3. A different pathogen causes root rot

Another pathogen that causes root disease is, Take All, caused by *Gaeumannomyces graminis var. tritici (Ggt)*, which causes more severe symptoms than *F. graminearum*. Fast-growing necrotrophic hyphae cause cell death through the epidermis, cortex, endodermis, vascular tissues, phloem, and xylem (Barret et al., 2009). When will the pathogen gene interact with the symbiotic root plant growth-promoting rhizobacteria gene "*Pseudomonas* spp" to prevent the pathogen RNA replication by bacterial RNA (Barret et al., 2009). The effect was shown on the shoot and root length and biomass. The symptoms can be visualized in the root cross-section under the microscope in colour from ebony to deep brown occupied by the hypha (Freeman & Ward, 2004). The hypha blocks the xylem centre and stops incomplete sap from running to the chloroplast in the leaves, which causes dry leaves. The cortex cells will be vacuous from any storage components, but the xylem number and diameter will adjust the plasticity to reduce the pathogen effect (Keenan et al., 2015). The pathogen hypha will minimize or cease the metabolism process in the host cells to amplify the pathogen life cycle, increment the soil rhizosphere's population, and contaminate emerging plant roots (Kwak et al., 2013). With root biomass, pseudomonas bacteria and soil nutrients deprive the pathogen of completing the life circle, infection and minimizing the pathogen inoculation. On the other hand, it promotes plant root tolerance (Kwak et al., 2013).

The second type of root disease is the common root rot caused by the causal agent, *Bipolaris sorokiniana*, which is determined to minimize the yield from 6% to 24% (Al-Sadi, 2021). The yield loss can be controlled by a specific type of symbiotic root bacteria that could engender antimicrobial substances that could help degreed the pathogen. However, on the other hand, the host root can increment auxin hormone engendering. These chitinases benefit the root in lowering susceptibility (Al-Sadi, 2021).

The third type of root disease is *Rhizoctonia solani*, and *R. oryzae* are causal agents of Rhizoctonia root rot, symptoms of rusty-brown spots on the roots path that are facile to uproot (Brown et al., 2021). Therefore, the solution is to obtain the less vulnerable plant root to the pathogen by establishing a quantification study of the root traits such as root length, fresh weight, and shoot biomass, which in

their turn, they will identify the suitable plant candidate for the breeding program (Okubara et al., 2009). Another method uses *Pseudomonas* bacteria suitable strains to produce antimicrobial bodies, which will help degrade the pathogen (Mavrodi et al., 2012).

The fourth type of root disease is an outbreak of root rot by several causal agents such as *Pythium spinosum, P. vanterpoolii*, and *P. eccentric*. The infection appears in late winter to early spring. The symptoms are lamentably necrotic root tumbling tissue was often indurate and lignified without hairy roots (Reeves et al., 2021). Transgenic methods to genes responsible for chitinase and exochitinase output are helping the plant roots cells tolerate the pathogen. Still, it only can be operated on the diploid genome, not on hexaploidy plants (Cook, 2001).

1.3. Defence mechanisms

The appearance structure of the root rot was like those formed by *F. graminearum* on wheat florets, supposed to be bulbous inoculation hyphae; this means that FRR leads to FHB (Wang et al., 2015). The vulnerable root cell tissues - sclerenchyma, phloem, and xylem - are blocked by the mycelium of *F. graminearum* (Rebib et al., 2014).

Upon breaking through the vascular bundles, the hyphae destroy root core parenchyma in the bundle sheath, triggering Programmed Cell Death (PCD) in resistant genotypes (Wang et al., 2015). This PCD stops the fungus hypha from spreading, keeping the remaining root tissues alive. Vulnerable genotypes would exhibit less root biomass and short root length leading to plant death (Zeilinger et al., 2016).

Fusarium graminearum can be distinguished by the mycotoxin production of deoxynivalenol (DON) from other *Fusarium species* (van der Lee et al., 2015). The pathogen hypha penetration starts from the root epidermis to the cortical intercellular, and then the hyphae advancement is an assault on the endodermis towards vascular tissues (Jaroszuk-Ściseł et al., 2008). *F. graminearum* has a saprophytic model, which means two types of classification (biotrophic and necrotrophic). It was realized that high amino acid production in the host, under low pH, in the less susceptible plant to the pathogen. Therefore, the plant would prompt gene signalling to reduce the secondary metabolism of amino acids, which creates an environment suppressing the pathogen development (Boedi et al., 2016).

If the exodermis mycelium of the root epidermis cell walls is thin, then the mycelium will penetrate the cortex's root cells (Beccari et al., 2011). However, the cortical root and shoot cells have a thick wall layer containing suberin, lignin, chitin, cellulose, and activate genes (Subramaniam et al., 2009; Hardham et al., 1980). They also have hormonal signalling, including salicylic acid, jasmonic acid and ethylene (Hong et al., 2017). In addition, it induces reactive oxygen species (ROS) and pushes PCD to suppress pathogen binding (Beccari et al., 2011).

One prime host defence mechanism is the gene-for-gene response that identifies PAMPs or DAMPs and induces defence signalling (basal defence), utilizing Jasmonic acid, ethylene, and salicylic acid (Rathjen et al., 2010). Effectors inhibit PAMP-induced signalling and induce R-protein-mediated defence signalling (habituated defence) (Rathjen et al., 2010; Subramaniam et al.,2009). The security is implemented by producing antimicrobial proteins and thickening the host cell wall by depositing lignin (Rathjen et al., 2010). The responses of the host defences to the pathogen hyphae will decrease the plant's growth since the plant's metabolism will be oriented to produce defences proteins as a

response to the biotic stress and the need to repair the damage caused by the pathogen (Wang et al., 2018).

Once again, the pathogen hypha will minimise or cease the metabolism process to amplify and consummate the pathogen life cycle, increment the soil rhizosphere's population, and contaminate incipient plants' roots—the molecular bulwark (Kwak et al., 2013). Together with root biomass, pseudomonad bacteria deprive the pathogen of soil nutrients and minimize the pathogen inoculation (Kwak et al., 2013).

The plant's molecular components, such as the pathogen receptors in the plant's root cell walls, will play a role in the plant's defence mechanism against the pathogen that causes root rot in the afflicted plant. The receptors will distinguish the microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) from Ggt hyphae, one of the most devastating soil-borne microbe infecting wheat. The Ggt hyphae will excrete proteases, and the pathogen enzymes will degrade the cell wall. Upon the hyphae penetration, the inoculated plant receptors protein will receive the pathogen effectors, induce resistance gene signalling, activate cell programme death, and disinfect the pathogen (Zhang et al., 2020).

1.4. Importance of root architecture for plant health

Nowadays, advanced genomic selection is used in breeding programs. For example, once we perceive and identify the additive marker traits linked with intricate genetic elements affecting FRR susceptibility, it allows the breeder to gain the advantage of gene varieties and execute these details in a breeding program (Voss-Fels et al., 2018).

The effect of soil tiller on nodal root progress is linked with the constructive and interim agents of root trait development, for example, the soil environment and the plant's age. The tilling stage is first optically discerned when the fourth leaf emerges (Rich et al., 2020). At the core bed out of the jointed stem of a plant, the uppermost nodal roots might develop overhead the soil layer, rising as small pegs stick out from the stem (Persson & Baitulin, 1996). Akin more to soil factors, the root system elongates between 1 and 2 m deep. Most of the roots are respiring in the top 30 cm of the soil. "X" format for instances like the old-style electric chandelier, which means as a metaphor, the centre of the "X" is the location of nodal emanating from its four roots which are in its turns after a certain elongation length will develop at the end of the root another four roots "X" at the nodal point for other roots, so every "X" forming four quadrants of a root engenders at each nodal (Klepper et al., 1984) Maybe this shallow structure would explain why the nodal root is less susceptible to the pathogen, as it was found above the crown root (Backhouse et al., 2004).

The genotypes resistant to *F. graminearum* offer flexibility by adjusting the xylem diameter and xylem number throughout the wheat root; furthermore, the quantity of lignin subsists on the vascular tissues (Shah et al., 2016)

Different mineral applications that affected the roots and the disease of *Fusarium* root rot in wheat were evaluated by Gupta et al. (2017). Some specific minerals like zinc have a distinguished act in helping the plant tolerate the pathogen (Khoshgoftarmanesh et al., 2010). The chemical form of the mineral-nitrogen plays a role in decreasing (NH_4) or increasing (NO_3) the pathogen effect in field conditions (Rowaished, 1981)

Mineral nutrition significantly affects yield and plant development (Paez-Garcia et al., 2015). The nutrient applications play a role in the wheat's immunity in fronting *Fusarium graminearum* through different operations. First, it smooths the excretion pathways of several hormones in the immune system and adjusts immune complications (Wang et al.,2018). Secondly, nutrition reduces the root permeability surface of the cell wall against pathogen penetration, in this case, zinc (Luo et al.,2021). Thirdly, nutrients induce the hormone signalling to activate the protein kinases to bind with the defence gene, reactive oxygen species, and the calcium ion (Hao et al.,2022). Finally, for uninoculated plants, the nutrient applications adjust plant evolution (Salim & Raza, 2020).

1.5. Hypotheses

The root structures for the genotypes will be affected when inoculated by *F. graminearum*, examining them in different nutrient concentrations. Therefore, the following hypotheses were evaluated:

i) The percentage of the living cortex cells in an uninoculated root is smaller than in the inoculated root, and the inoculated area improves tolerance against *F. graminearum*.

ii) The number of nodal roots will affect the root size and architecture. Some genotypes will have more nodal roots and tolerate *F. graminearum* inoculation because of their shallow soil location and horizontal branching.

iii) Infestations by the saprophytic pathogen *F. graminearum* will cause disintegration of the root structure with less biomass in the shoot and the root on the host under different nutrient levels.

iv) The plant root supplied with a single concentration application of nutrients will retain more root biomass and large root diameter than the plant root supplied with a double concentration application according to limited soil size in growth pots. Thus, it depends on the ability of roots to nutrient solubilization, increasing the root diameter and biomass weight and helping the root tissues tolerate the pathogen.

v) Inoculating the genotypes with *F. graminearum* could affect root growth, causing fewer seminal roots, decreased root size, and reduced biomass.

1.6. The host immunity

The host immunity, in our case, is what anticipated five winter wheat genotypes against the pathogen root rot on traits parameters such as shoot structure, root architecture, and cellular root structure.

2. Material and Methods

2.1. An experimental plan

The experiment presented in this thesis compared five genotypes of winter wheat and their performance under two different parameters. The first parameter was two inoculation methods with the same pathogen, *F. graminearum*. The second parameter was two concentrations of the same nutrient solution.

While there is a difference in root growth between weeks 6-10 (Weaver et al.,1924; Ellis et al., 1980), the uninoculated plants were kept for four weeks longer to compare the effects of the two nutrient concentrations.

2.2. Preparation of fungal material

The *F. graminearum* isolates (VPE 90, 104, and 105) were grown in Petri dishes at room temperature on two different mediums. First, they were produced on a Potato Dextrose Agar (PDA) medium in Petri dishes for six weeks. They were then grown on Synthetic Nutrient-Poor Agar (SNA) to promote spore production through starvation under UV-A light, using a blacklight, for four weeks.

1-2 ml of sterilized water was applied to each Petri dish's sporulating *F. graminearum* colonies. The fungi were gently disrupted using a fixed stick to release the conidia into the water, removed using a pipette, and pooled into clean tubes. Finally, the harvested isolates were mixed in a new clean tube.

One drop of the suspension was placed microscope glass slide with a grid pattern for counting conidia (Figure 1). The average count from each grid of 24 squares was six conidia, giving an average of 0.25 conidia per cell. This average was multiplied by 250000 to calculate the concentration of 62500 conidia/ml (Fries, 1988).

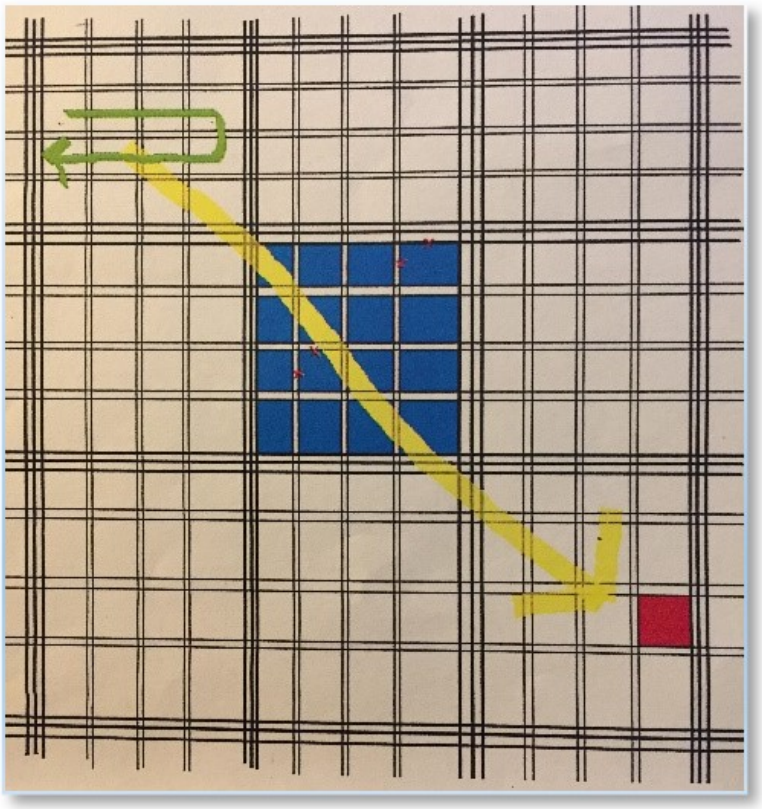


Figure 1. The grid pattern on the microscope slide was used to count the conidia in the Fusarium isolates suspension. Eight squares (defined in red; 0.2x0.2 µm) in three separate grids (defined in blue) were counted - using the cell counting pattern outlined in green and the grid counting pattern outlined in yellow.

Agar plugs for inoculation were prepared by taking a 0.5 cm section of agar with conidia growing on the surface, taking one section for each pot treated with agar plugs. These were then cut in half agar plug for each seed, of which there were two per pot.

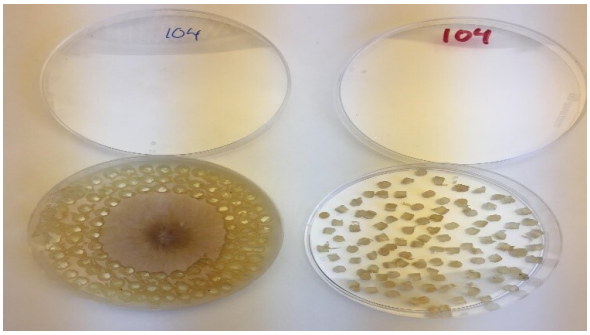


Figure 2. Agar plugs extracted from the plates of conidia grown on SNA are a poor medium of nutrients, leading to a lower growth rate of the fungi. In this artificial ecology, the fungi produce spores, called "sporulation," rather than propagation. Furthermore, agar plugs were cut in halves with a diameter of 0.5 cm.

2.3. Inoculation of wheat roots

The seeds of five winter wheat genotypes chosen, Julius, Brons, Reform, Informer and Ceylon, were provided by Lantmännen, Lantbruk, Sweden.

Two hundred pots (7x7x7 cm, approximately 3.4 dl) were filled with Mineraljord type A soil (Hasselfors Garden, Sweden). The soil was filled to 1 cm from the top of the pots before being gently packed. Two kernels of the same genotype were planted 3 cm apart (Figure 3) - all together, forty pots per genotype.

Figure 3. Kernel distribution within the pots where two kernels were sown 3 cm apart at a depth of 1 cm.

The agar plug method represents the living soil-borne pathogen growing on crop debris, with its grown mycelium ready to attach to the young seedlings. The conidia suspension means transferring conidia to new soil (e.g., dispersed by wind or rain). These conidia will need time to germinate before infecting the seedlings, while simultaneously, at the same time, the seedling has advanced in their maturity. Therefore, these seedlings had more time to use the nutrients and build defences against the pathogen. The reason for choosing the two methods of inoculation is that they have different time agents dedicated to the germination, development and inoculation times of the conidia mycelium, where the agar plug mycelium represents the soil-borne pathogen which is ready to penetrate the young seedling root, so is inoculation time age is short, contrary to the conidia suspension, which represents the transfer pathogen spore by the wind for example which is taking a long time to germanite and to be ready to inoculate the advanced growing seedling root. Hence, it is the inoculation time agent is longer.

The conidia suspension was applied to 16 pots per genotype at 1 ml per seed (2 ml per pot). The 0.25 cm agar plugs were pressed against the grain from above in 40 plots per genotype. After sowing, the seed was then covered with soil, and more soil was added to fill the pot to the top before being gently pressed and watered with one of two nutrient solutions.

The Bio Centre provided the stock nutrient solution staff (Table S1), and it was diluted to two concentrations, 2 ml/l and 4 ml/l of, with water. After sowing, this solution was applied to the pots and then used to water them every second day - half of the replicates with the 2ml/l solutions (S1 table) and the other half with the 4 ml/l solutions. The pots were kept in a growth chamber (Bio Centre, SLU) with a 13/11-hour day/night cycle at 20/15°C, lighting at approximately 350 µmol m^{-2} s^{-1}.

Each of the five genotypes had 40 pots following 16 un-inoculated pots and 24 inoculated with two methods, 16 pots with conidia suspension and 8 treated with agar plugs for each nutrient concentration. Once germination occurred, the first seed germinating in each pot was kept, and the other kernel was removed.

The inoculated replicates of wheat genotypes were grown six weeks before the roots were harvested, whereas the uninoculated ones were grown for ten weeks.

The assessment of germination rate is optically discerned for germinated seedlings and using the lens from 4 to 6 times magnify for killed young seedlings after four weeks from the time of seeding in growth pots.

2.4. Root Harvesting

Excess soil was gently removed from the root system by hand, keeping the root system intact. The remaining soil was removed by soaking the roots in water for 2 hours before washing them with running water until clean. The clean roots were then put on a dry paper towel to remove excess water.

Each root system's nodal and seminal root numbers were counted using a 6x magnifying lens. Next, the root angle was measured by laying the washed roots over a protractor to assess the most significant angle when allowed to rest naturally (Figure 4). Finally, root length was measured using a ruler to measure the primary root.

The fresh roots and shoots were weighed, and the leaf number was counted by hand. A subsection of the root was taken before the shoot, and the remaining root samples were dried in bags in the oven at 85°C for 48 hours before being weighed (Nguyen & Stangoulis, 2019).

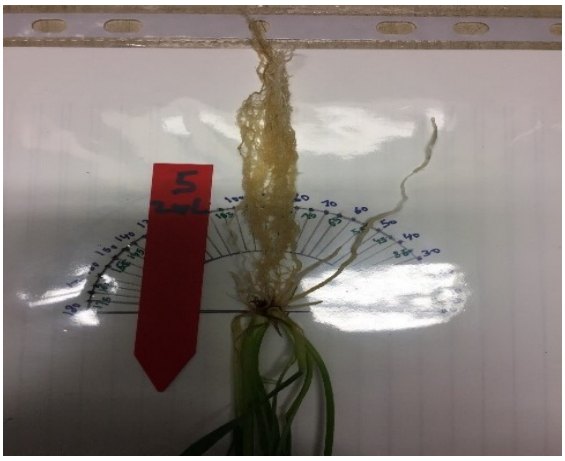


Figure 4. The root angle was measured using a protractor.

Upon harvesting, photos were taken of killed seedlings with complete necrosis of the root; some of the shoots of the young seedlings were buried in soil that could not penetrate the ground (Figure S1). Photos were taken of the seedling infection, brown spots because of tissue death in the stem base and roots with mild necrosis in most of the roots (Figure S2). Symptoms of wilting on the leaf's edges (Figure S3). Also, images were taken off the stem, and the leaves were entirely wilted; (Figures S4 and S5).

2.5. Root Imaging

The subsection of the saved samples was kept in ethanol in 50 ml tubes at 4°C. The root samples were prepared using a razor blade to cut thin cross-sections. Each root sample was coloured using 1-2 drops of Toluidine Blue (0.1% [w/v] in distilled water). After waiting for approximately one minute, the excess dye was removed with water. The coloured root sections were suspended in water on a microscope slide and observed using a bright-field microscope at 10x magnification (KernOptics OBF 122, Kern & Sohn; 0.25 numerical aperture). Photos were taken using a digital microscope camera (Mirazoom MZ808; Oowl Tech) to resolve eight megapixels at 100x magnification (Colombi et al., 2019).

The suitable root image for each sample was then selected to measure the traits. First, the diameter of the image view was measured using a microscope ruler, which was used to set the scale (Figure 5a). Next, the eye counted the xylem and cortex cell layer numbers using a microscope. Finally, the root diameter, cortex width, and xylem diameter were calculated using the line measurement tools in ImageJ (Schneider et al., 2012), as shown in Figure 5b.

The proportion of life to dead cells in the cortex was estimated by measuring the total cortex area and the area of the white cell gaps, as seen in Figure 5b. This was done using the site measurement tools in ImageJ (Schneider et al., 2012).

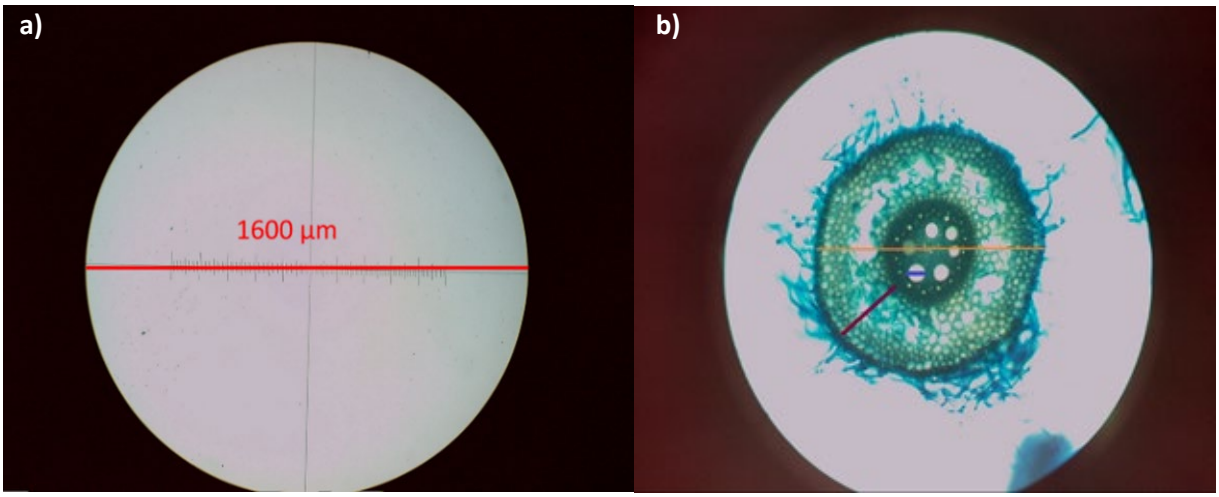


Figure 5. Images are showing a view of the microscope, showing a) the ruler used to set the scale for the images taken (Provided by Dr Tino Colombi), and b) the measurement of the root diameter (orange), Cortex width (purple), and Xylem diameter (blue).

2.6. Statistical Analysis

Statistical analysis for each of the shoot traits (leaf number, shoot fresh biomass, and shoot dry biomass), the root architecture traits (root length, fresh root biomass, root dry biomass, nodal root number, seminal root number, and root angle), and the root cellular structure traits (Root diameter, xylem diameter, xylem number, cortex cell number, cortex width, and live cortex cells) was undertaken. Each trait was measured using a one-way ANOVA, and this was performed using "R" v4.0.4 (RCoreTeam, 2021) with *Fusarium* treatment, nutrient concentration, and genotype as the treatments.

3. Results

3.1. Shoot Traits

3.1.1. Leaf number

There was a significant difference in leaf number between the *Fusarium* treatments, genotype, and nutrient levels, and the interactions of these traits were significant ($p<0.001$). Both inoculation treatments showed significantly fewer leaf numbers in all varieties than when not inoculated. The difference in *Fusarium* treatment showed that the inoculation with agar plugs results in fewer leaves than inoculation with the conidia suspension (Figure 6a). This reduction was seen in all varieties except Brons, which showed no difference between inoculations. Ceylon had significantly more leaves than the other genotypes when inoculated with the suspension. The leaf numbers were significantly higher for the 4ml/l nutrient application than for 2ml/l for all cultivars (Figure 6b). There were no significant differences between the genotypes under the low-nutrient application. However, Ceylon showed a significantly higher number of leaves than the other genotypes with a high nutrient application, producing approximately 30% more leaves than the other genotypes.

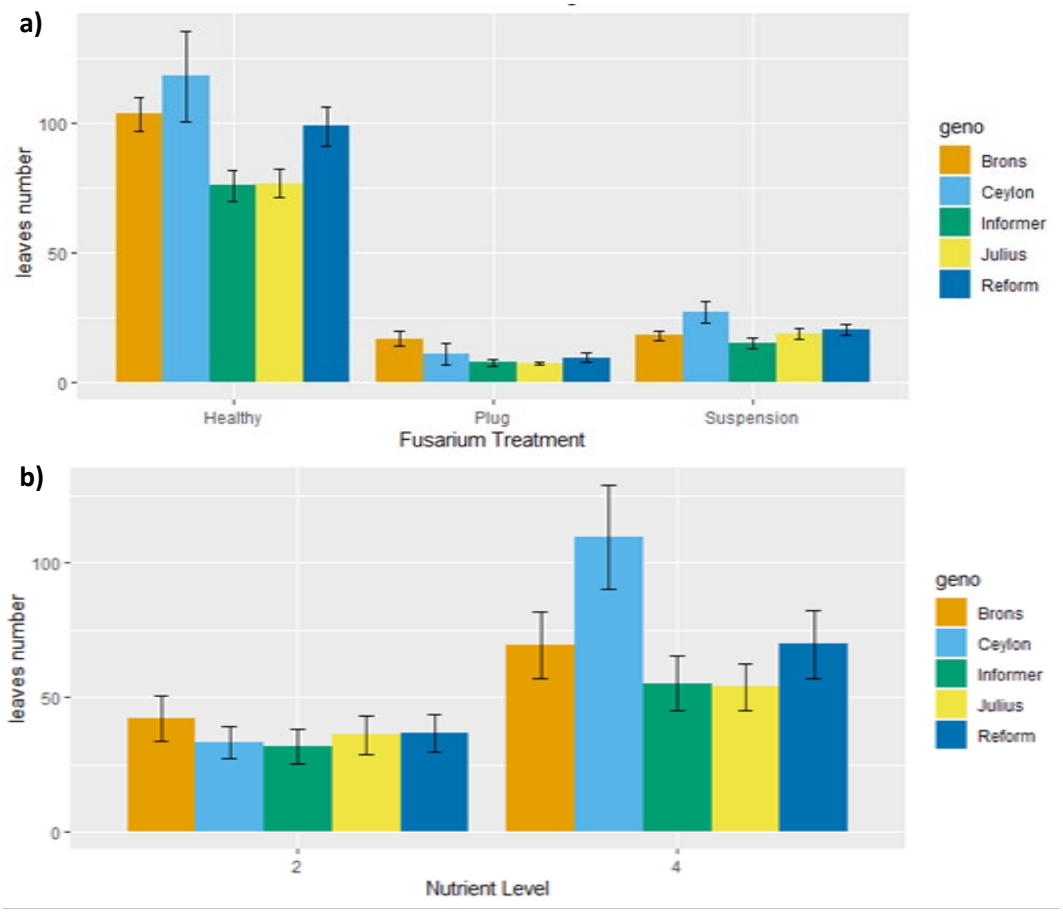
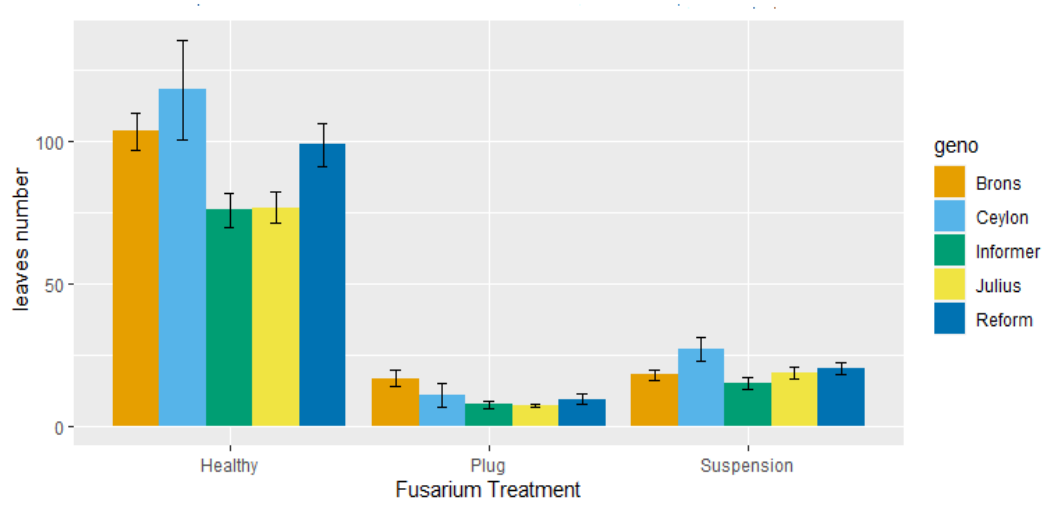


Figure 6. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of leaf numbers per plant. The error bars display the ± standard error.

3.1.2. Fresh Shoot Biomass

There was a significant difference in the fresh shoot biomass for the different *Fusarium* treatments ($p<0.001$), showing a clear distinction between the inoculated and un-inoculated two types of inoculations. Inoculation with an agar plug significantly reduced fresh shoot biomass when inoculated with the suspension (Figure 7a). Additionally, significant differences were seen between the two nutrient concentrations ($p<0.001$), with the higher nutrient concentration resulting in a substantial amount of fresh shoot biomass (Figure 7b). There was also a significant difference in the interaction of the genotype and each treatment ($p<0.001$). Brons retained more quantity of fresh shoot biomass than the other genotypes when infected by the agar plug. The fresh biomass weight for uninoculated root defined by Ceylon and Informer had more biomass than other genotypes in Figure 7(a). However, in Figure 7(b), the other genotypes show no difference at 2m/l concentration but four ml/l nutrient concentration; Ceylon return with larger fresh biomass than the other genotypes.

a)



b)

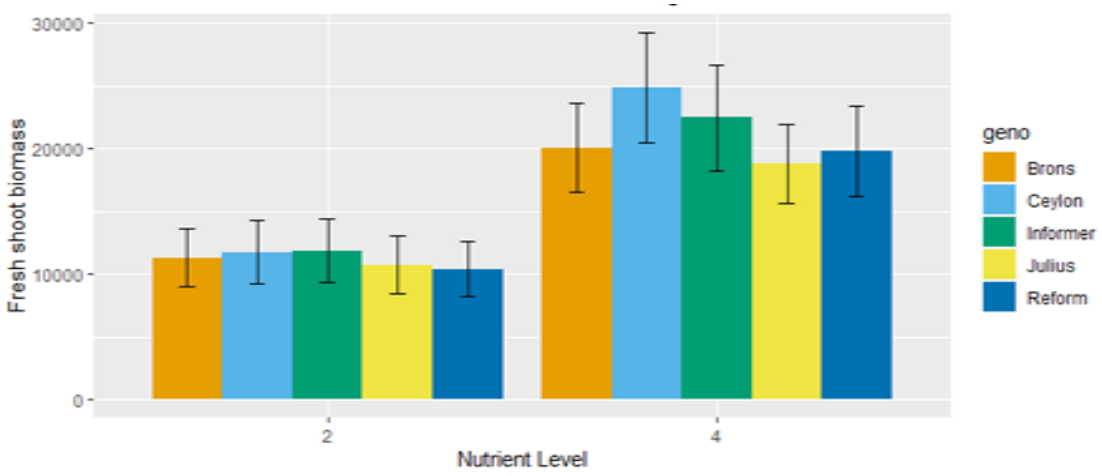


Figure 7. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of fresh shoot biomass weight per mg. The error bars display the ± standard error.

3.1.3. Dry Shoot Biomass

The dry shoot biomass also showed significant differences between the two *Fusarium* treatments and their interaction with the different genotypes ($p<0.001$). The agar plug caused more reduction in dry shoot biomass than the conidial suspension. Significant differences between the nutrient applications were found ($p<0.001$), showing that a 4ml/l application resulted in a higher dry shoot biomass weight than a 2ml/l application. Interactions between the *Fusarium* treatment and the genotypes were significant ($p<0.001$), with Brons producing more dry biomass than the other genotypes, Ceylon, with the agar plug inoculation (Figure 8a). When un-inoculated, there was no difference between genotypes apart from Julius producing less shoot dry weight (Figure 8a). Interaction between nutrient applications and genotypes showed no difference, except Julius, which showed much lower dry shoot biomass (Figure 8b).

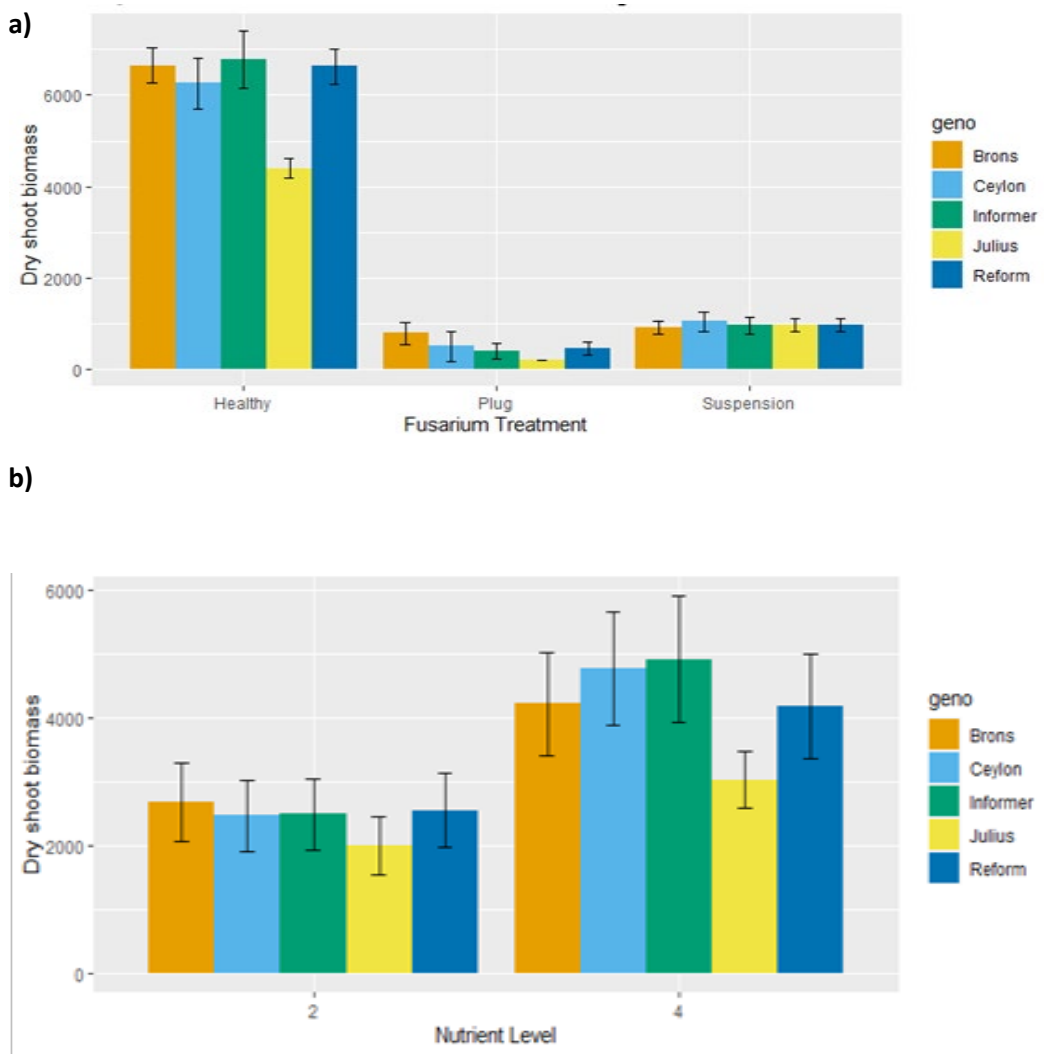


Figure 8. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of dry shoot biomass weight per mg. The error bars display the ± standard error.

3.2. Root Architecture

3.2.1. Root Diameter

The *Fusarium* treatment showed a significant difference (p=0.03) in root diameter when comparing conidial suspension inoculation to un-inoculated plants. The inoculation by agar plug caused extensive root damage. The interaction of genotype and *Fusarium* treatment showed no significance, nor did the interaction of the genotype and the nutrient treatment. There were no significant differences in root diameter between all genotypes when inoculated with the conidial suspension (Figure 9a). Reform had a smaller root diameter for the lower nutrient application than the other genotypes (Figure 9 b), whereas there was no difference for the higher application.

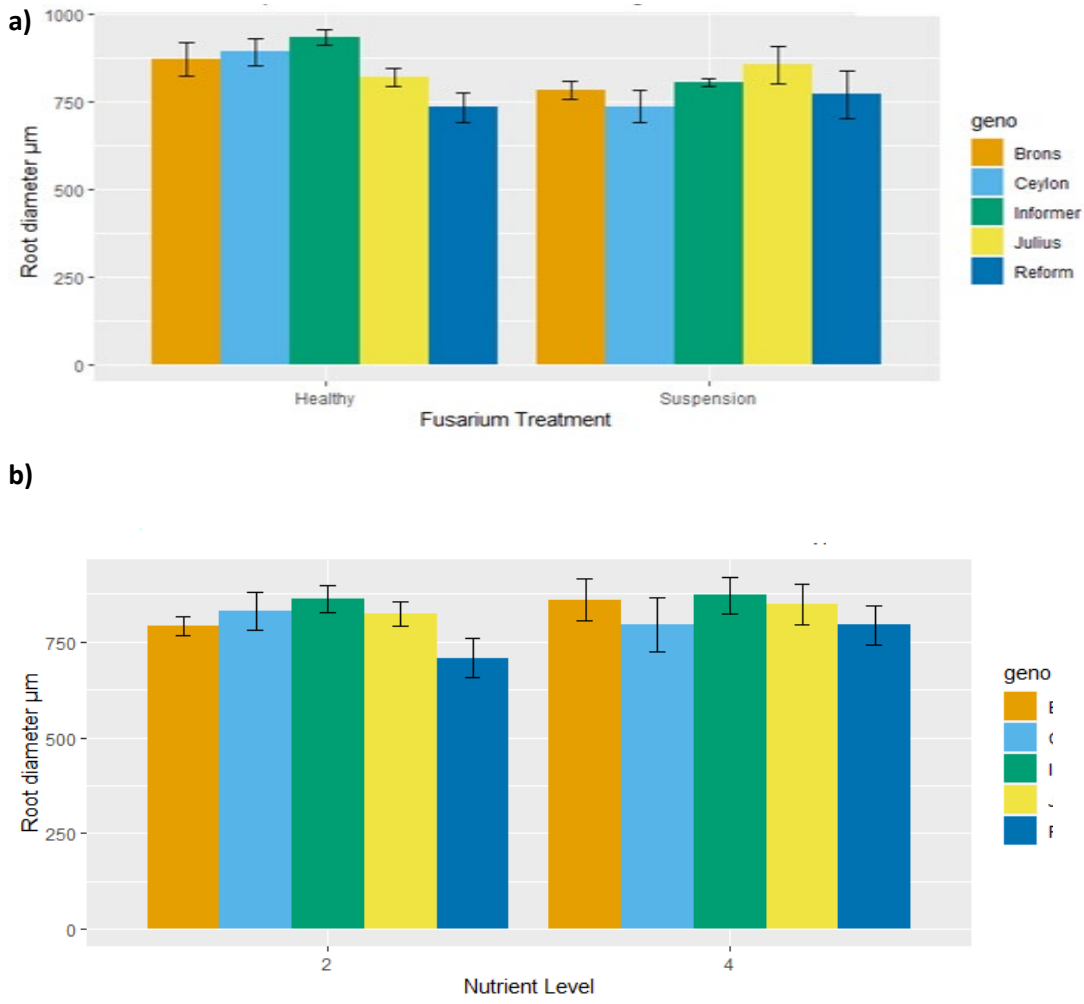


Figure 9. The influence of conidial suspension Fusarium treatment, no data from agar plug mycelium treatment all the plants had extensive root damage (a) and nutrient levels (ml/l) (b) on the development of root diameter per µm. The error bars display the ± standard error.

3.2.2. Root Length

There was a significant difference in root length between the *Fusarium* treatments ($p<0.001$). Both applications decreased the root length compared to the un-inoculated plants (**Figure 10** a). Root length was reduced for both inoculation types, decreasing with the agar plug inoculation. There was no significant difference between the two nutrient levels treatment. The *Fusarium* and the genotype interaction were also significant ($p<0.001$). The uninoculated Ceylon had significantly longer roots than the other genotypes. Reform, Julius, and Brons had a significantly shorter root than Ceylon and Informer under ager plug inoculation. Julius, Brons, and Reform had significantly shorter roots when uninoculated and maintained that length when inoculated by the suspension (Figure 10 b). Informer and Ceylon showed 2% to 4% longer roots than the other genotype under the two nutrition doses.

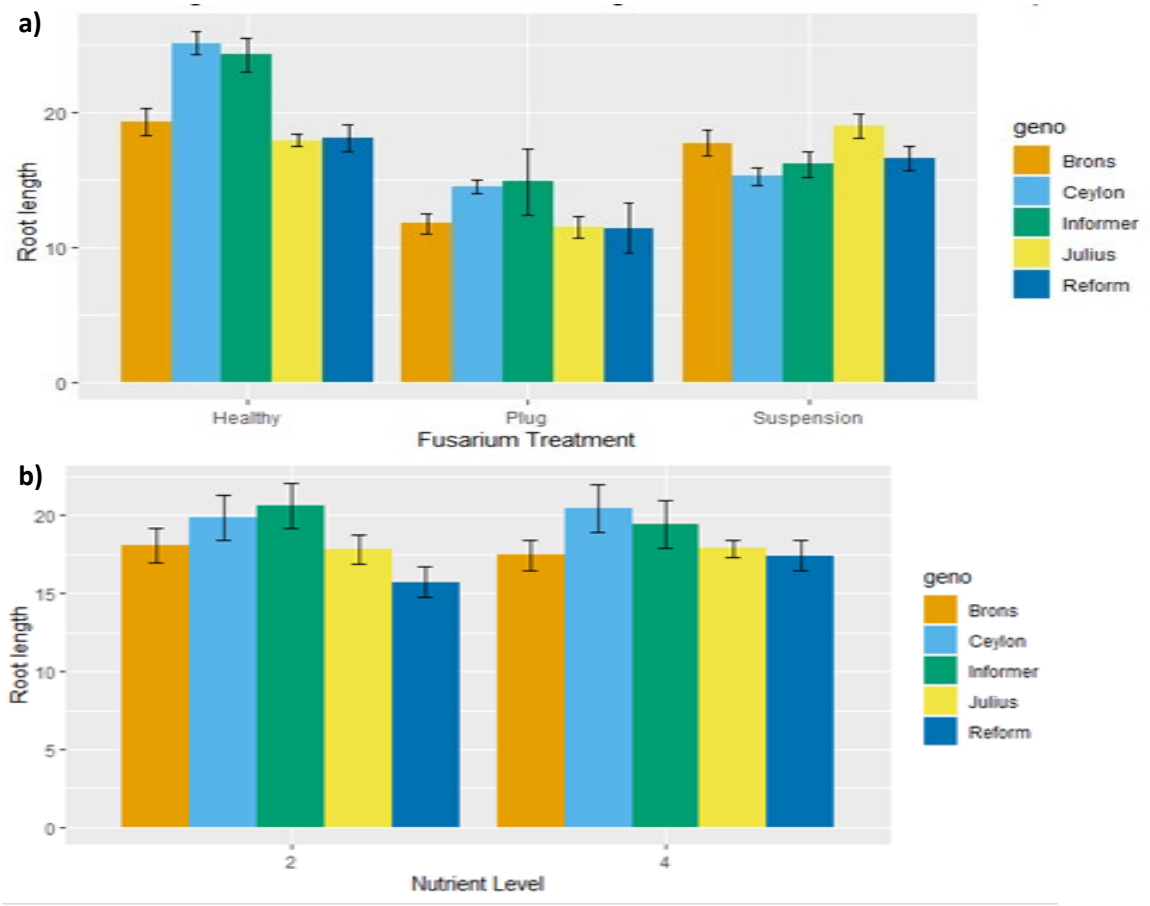


Figure 10. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of Root Length per cm. The error bars display the ± standard error.

3.2.3. Fresh Root Biomass

Significant differences in fresh root biomass were found between the *Fusarium* treatments ($p<0.001$), with both inoculations causing a reduction in biomass compared to the un-inoculated treatment. The agar plug method reduced the biomass more than the conidial suspension method with the fresh shoot biomass. Significant differences in the nutrient applications were also found ($p<0.001$), with the treatment with the higher nutrient level producing more biomass. There was a significant difference in the interaction between the genotype and the *Fusarium* treatment. When inoculated with the agar plug method and inoculated with the conidial suspension method, there was no difference in fresh root biomass weight between the genotypes for every inoculation method. However, significant differences for uninoculated genotypes could be optically discerned in all plants, as shown in Figure 11a. The disparity between the genotypes under two inoculation treatments can be perceived where Brons retained fresher root biomass than the other genotypes when using the agar plug. Still, there was no difference for the conidial suspension treatment (Figure 11a). The uninoculated Ceylon and Informer had higher root biomass than the other genotypes. Ceylon is the only cultivar to increase fresh root biomass when a higher nutrient concentration was used, increasing by 25% compared to the 2ml/l application (Figure 11b).

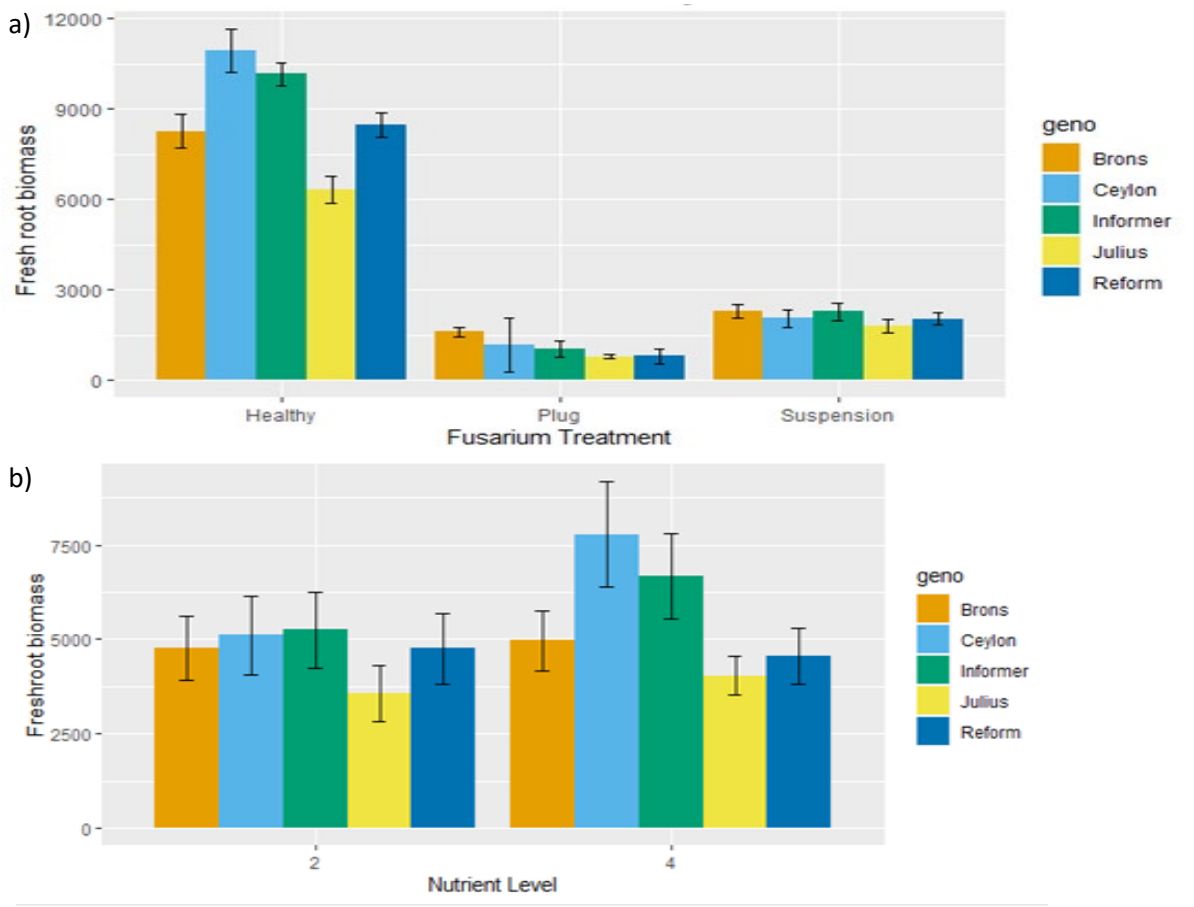


Figure 11. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of fresh root biomass per mg. The error bars display the ± standard error.

3.2.4. Dry Root Biomass

The *Fusarium* treatments displayed a significant difference ($p<0.001$). Compared to the un-inoculated roots, the agar plugs inoculation method caused a more significant reduction in dry root biomass than the conidial suspension method. The nutrient solution with 4ml/l showed higher outcomes than 2ml/l, showing a major disparity . Significant differences were found in the interaction between the genotype and Fusarium treatment ($p>0.001$). It can be seen in Figure 12a that Brons retained a little drier shoot biomass than the other genotypes with both inoculation methods. Differences in the interaction between the genotype and the nutrient level can be seen ($p<0.001$), with Ceylon being the only genotype to show a significant increase in dry root biomass under a 4ml/l application compared to a 2ml/l application (Figure 12b).

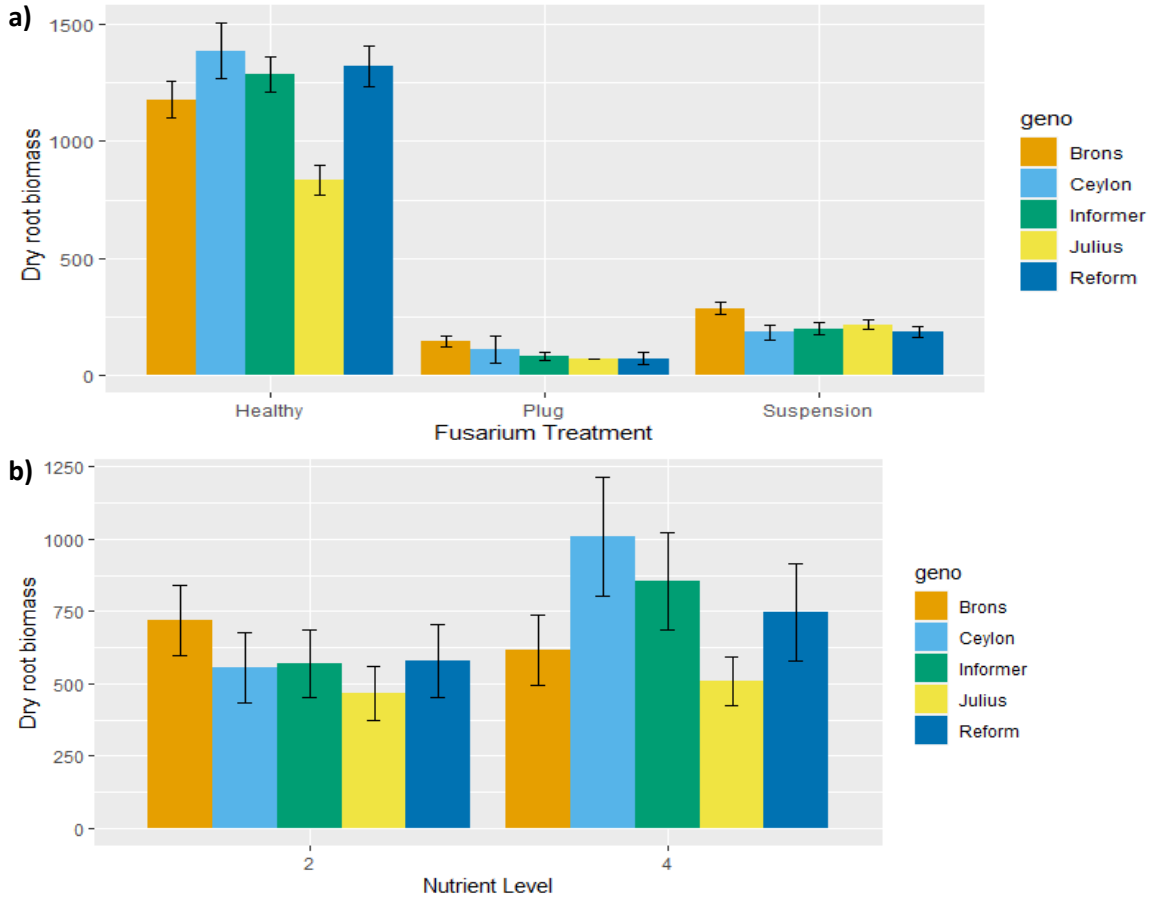


Figure 12. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of dry root biomass per mg. The error bars display the ± standard error.

3.2.5. Nodal Root Number

The *Fusarium* inoculation treatments showed significant differences ($p<0.001$) for the agar plug method lowering the nodal root number more than the suspension treatment compared to the un-inoculated. The variation between nutrient levels shows a significant difference ($p < 0.001$), with the increased nutrient level (4ml/l) returning more nodal root numbers than the 2ml/l *(*Figure 13 a). Julius had much fewer nodal roots when uninoculated but retained the same amount as the other cultivars when inoculated. The interaction between nutrient treatments and genotypes was significant ($p<0.001$). There was no difference in nodal root number when treated with 2ml/l, but Ceylon showed 30% more nodal roots than other genotypes when treated with 4ml/l.

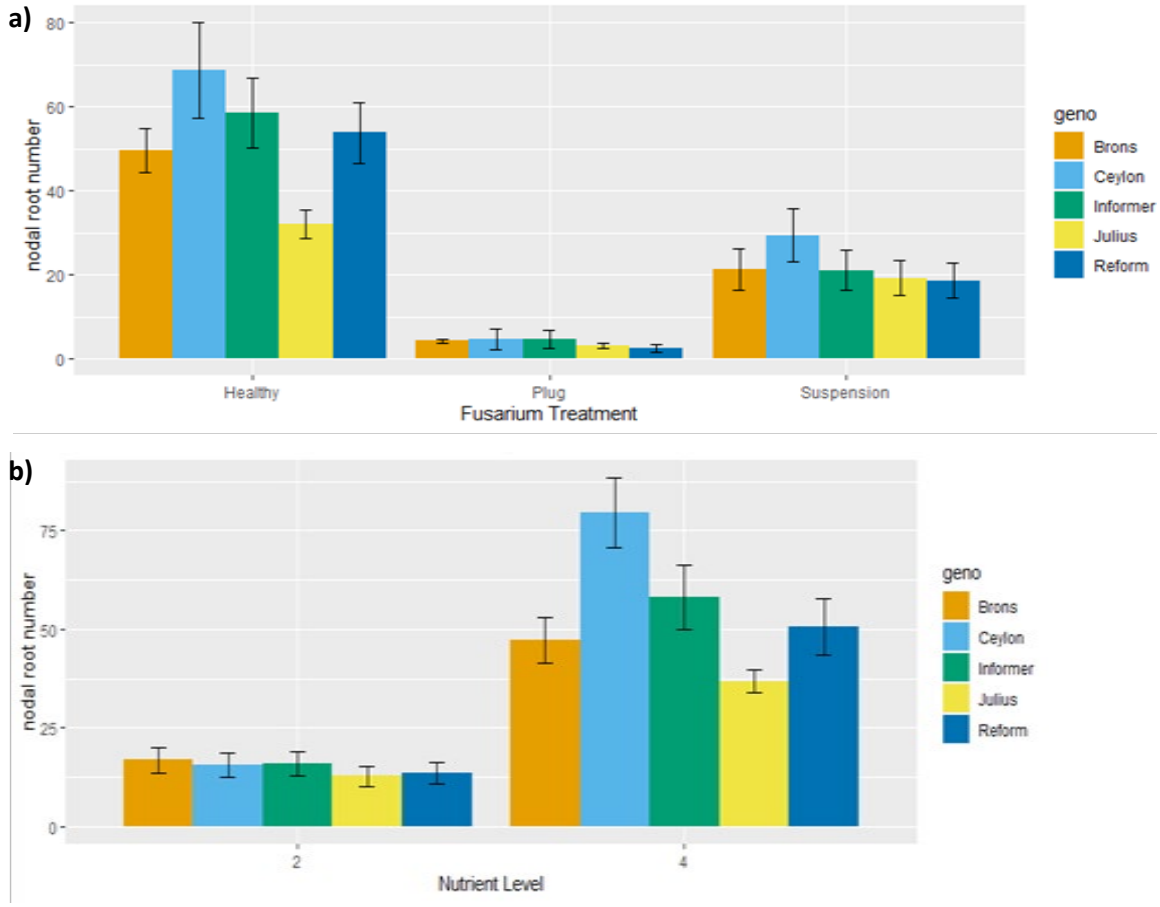


Figure 13. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of nodal root number per plant. The error bars display the ± standard error.

3.2.6. Seminal Root Number

The *Fusarium* inoculation treatments extensively varied ($p<0.001$), with the agar plug method lowering the seminal root number more than the conidial suspension treatment compared to the un-inoculated (Figure 14a). The nutrients with a double dose (4ml/l) retained more seminal root numbers than a single dose (2ml/l); this difference was significant ($p<0.001$). This trend can be seen in all genotypes (Figure 14b). The interaction between the nutrient treatment and the genotype was insignificant. However, there was a significant difference between the genotype and the inoculation treatment in the seminal root. Figure 14a shows Ceylon and Informer produced more seminal roots than the other genotypes when un-inoculated under both nutrient applications.

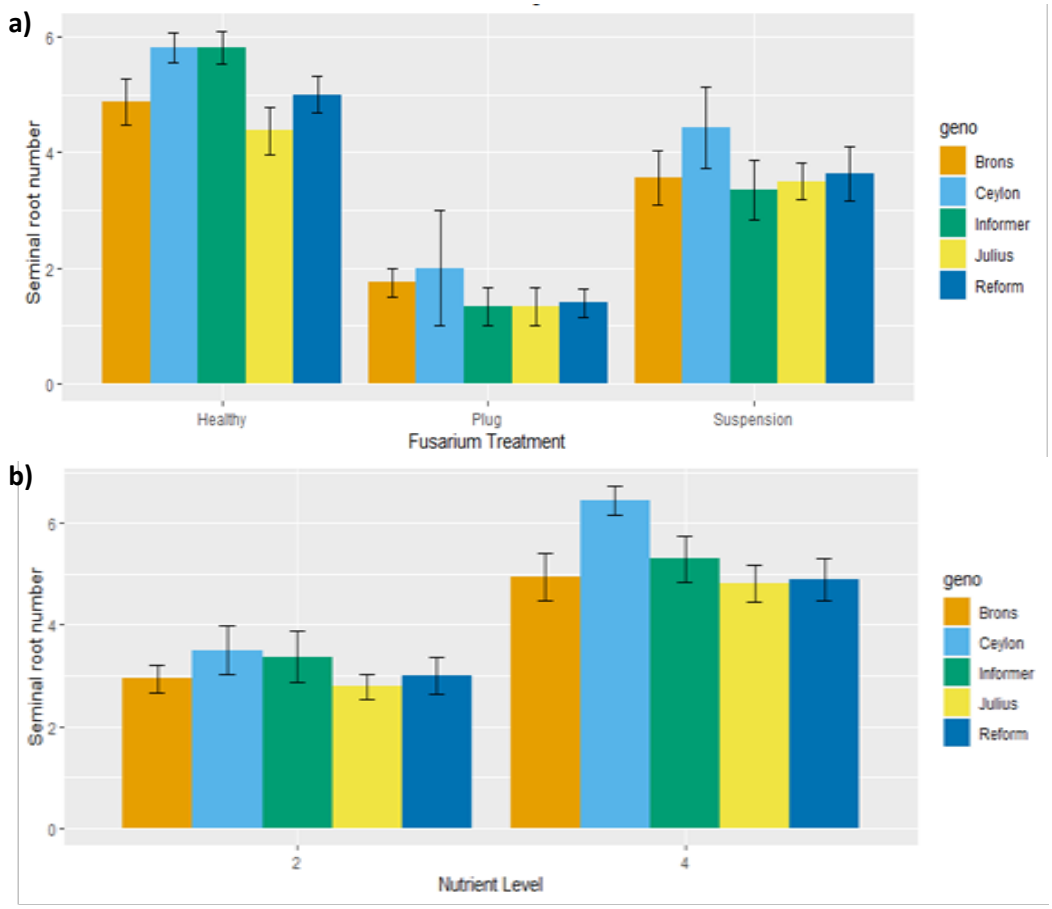
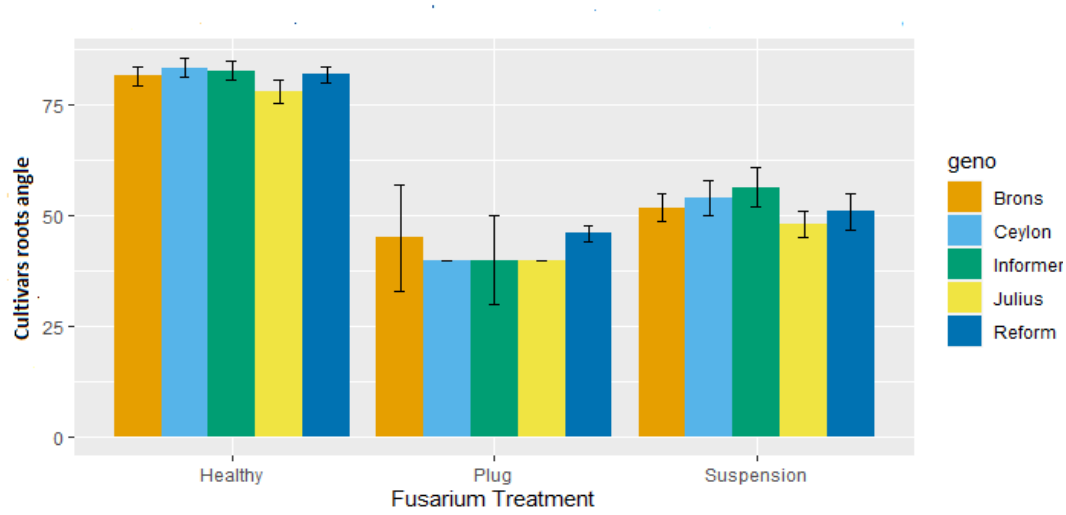


Figure 14. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of Seminal root number per plant. The error bars display the ± standard error.

3.2.7. Root Angle

The *Fusarium* inoculation treatments showed significant differences ($p<0.001$), with the agar plug method reducing the root angle more than the suspension inoculation method, but only by a small amount and not considerably for all genotypes (Figure 15a). There was also a significant difference in the nutrient application ($p<0.001$), with the double dose causing a wider root angle than the single dose, though this can be seen as a minor increase in Figure 15b. The interactions between genotype and Fusarium treatment and nutrient treatment were not significant.

a)



b)

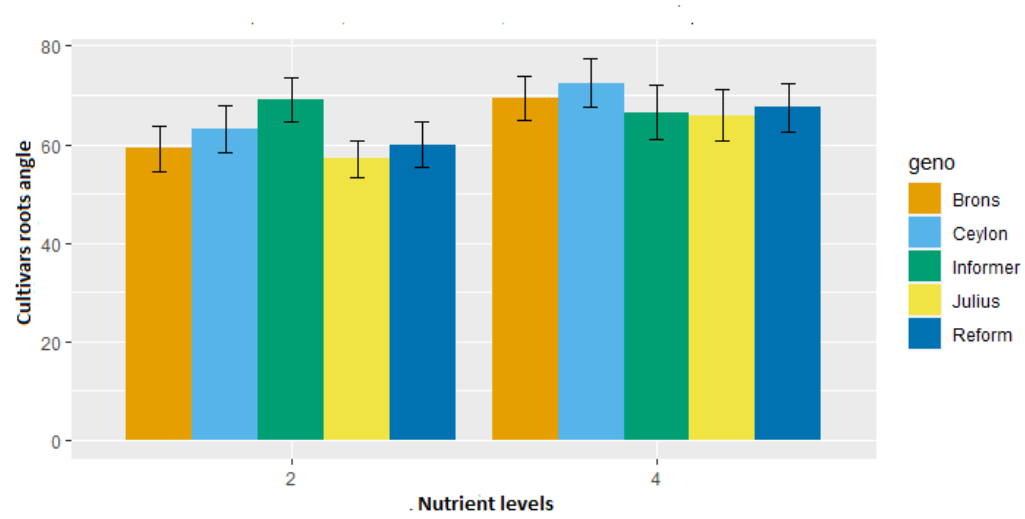


Figure 15. The influence of different Fusarium treatments (a) and nutrient levels (ml/l) (b) on the development of root angle degrees between surface root and main central root per plant. The error bars display the ± standard error.

3.3. Root Cellular Structure

3.3.1. Xylem Number

There was a significant difference in the xylem number between *Fusarium* treatments (p = 0.002). The suspension inoculation produced lower xylem than the un-inoculated roots (figure 16a). However, there was no significant difference in the *Fusarium* treatment and genotype interaction (Figure 16b). There was a significant difference in the nutrient applications where Ceylon showed a higher xylem number under 2 ml/l and Informer showed a higher xylem number under 4 ml/l.

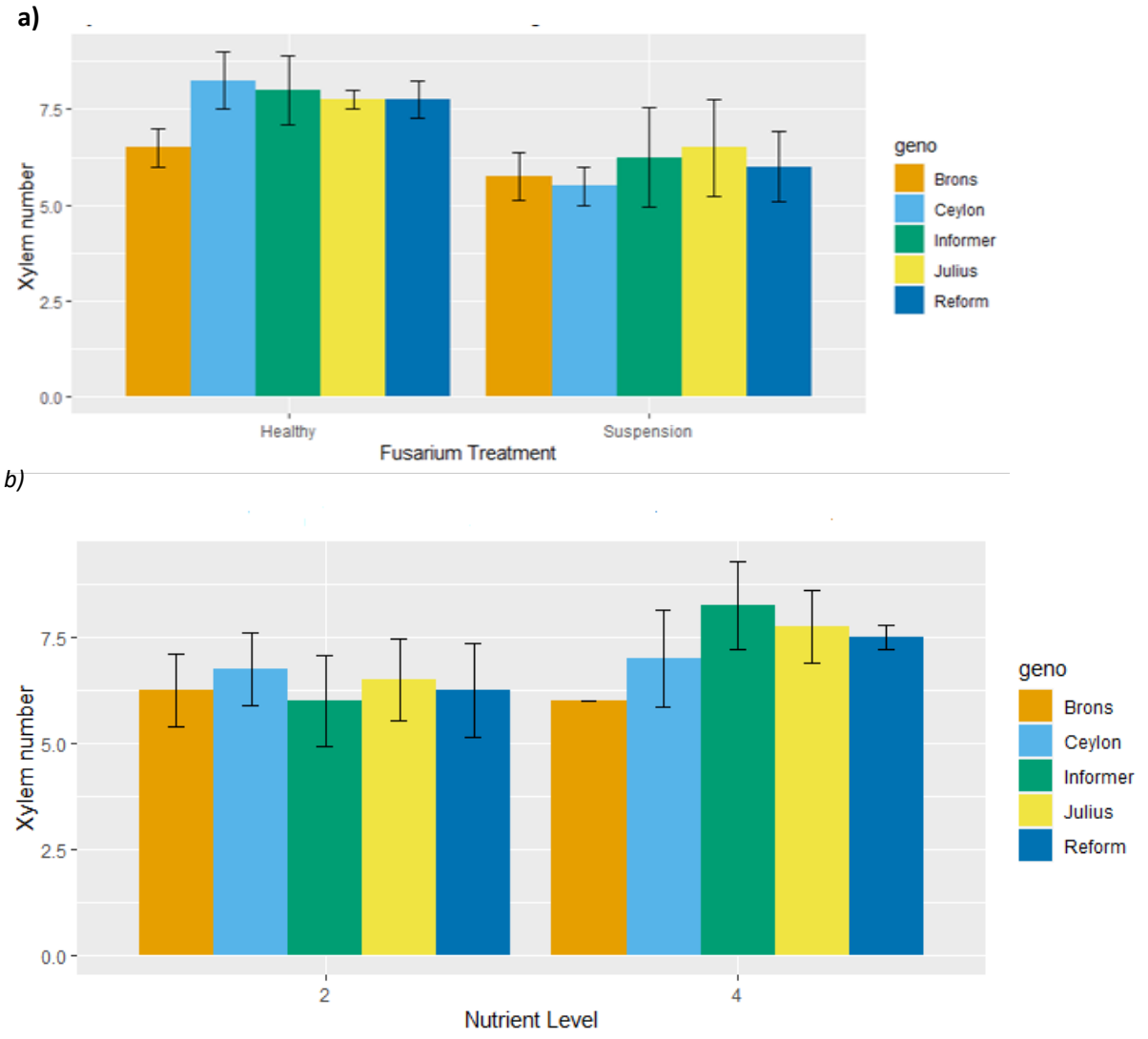


Figure 16. Different Fusarium treatments (a) influence on the development of Xylem number per plant root; and different nutrient levels (ml/l) (b) influence on the development of Xylem number per plant root. The error bars display the ± standard error.

3.3.2. Cortex Layers Number

The *Fusarium* treatments showed a significant difference (p=0.002), with the suspension inoculation showing a lower cortex layers number than in the uninoculated roots. Still, there was no significant difference in the *Fusarium* treatment-genotype interaction. There was no significant difference between the nutrient applications, but a significant difference in the interaction between nutrient application and genotype (p = 0.048) was found. This difference can be seen in Figure 17, showing an increased cortex cell number in Julius when grown with a 2ml/l nutrient application compared to the other genotypes. Still, this difference was not seen between genotypes grown with the 4ml/l application.

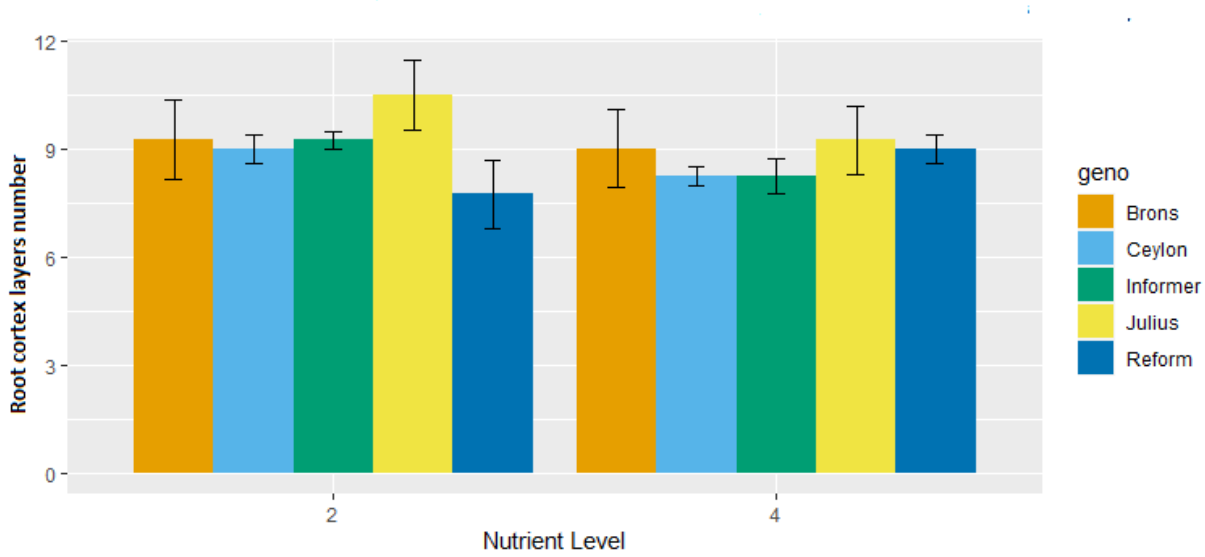
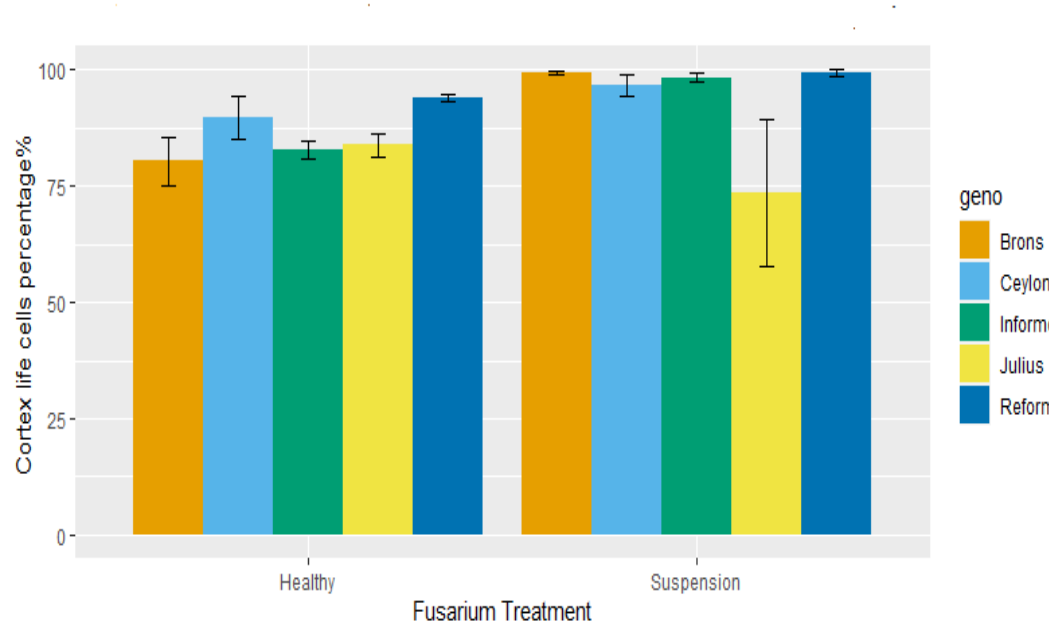


Figure 17. Different nutrient levels (ml/l) influence on the development of cortex cell layer number per plant root. The error bars display the ± standard error.

3.3.3. Live Cortex Cell Percentage

Unlike cortex diameter, which showed no significant difference between variables, there was a noteworthy variance in the live cortex cell percentage. The results show a significant increase in the live cortex cell percentage when inoculated with the conidial suspension compared with the un-inoculated (p = 0.007). In addition, the interaction between *Fusarium* treatment and genotype was significant (p = 0.011) (Figure 18a). Reform has shown a significantly higher number of live cortex cell percentages than Julius when inoculated with the suspension and significantly higher live cortex cell percentages than other uninoculated cultivars. Reform had a more significant live cell percentage than Brons, Informer, and Ceylon under different nutrient levels (Figure 18b).

a)



b)

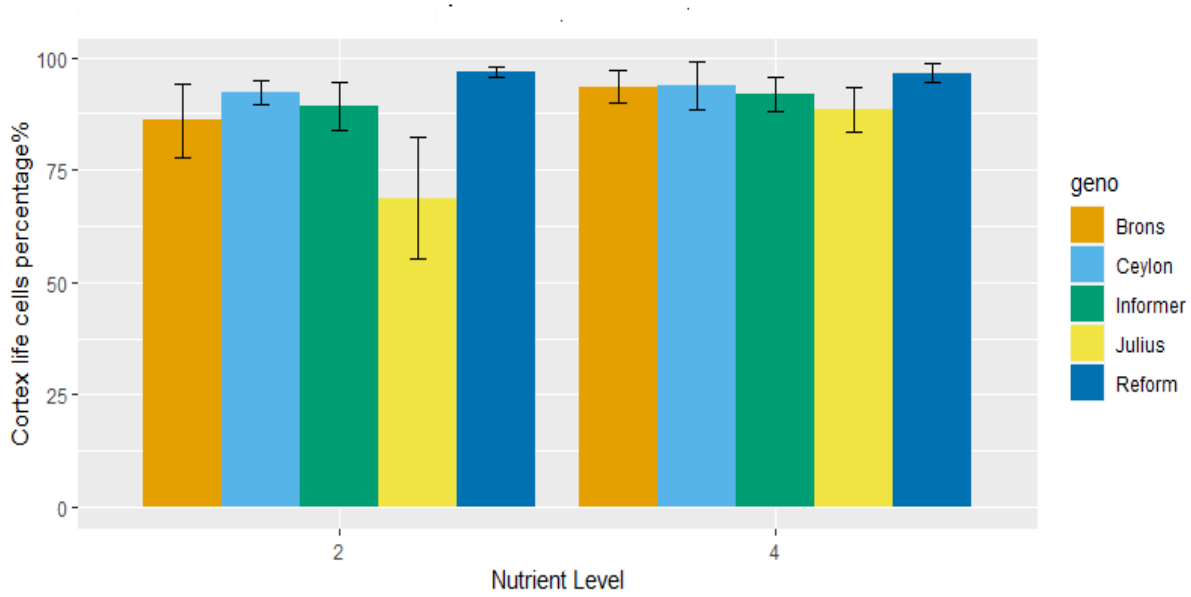


Figure 18. Different Fusarium treatments (a) influence the development of cortex live cells percentage per plant root compared with healthy plant root for five cultivars of wheat; and different nutrient levels (ml/l) (b) influence the development of cortex live cells percentage per plant root for five cultivars of wheat. The error bars display the ± standard error.

The root cross-sections in figure 19 can explain the differences in living cortex cells percentage and between genotypes.

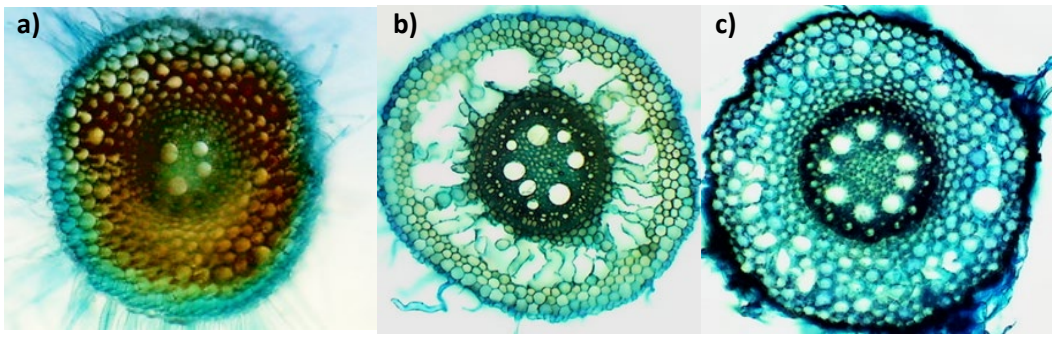


Figure 16. Images showing root cross-section*, inoculated roots in the Julius variety (a), and two uninoculated roots of genotype Brons (b) and Reform (c).*

3.4. Germination ratio

The two types of inoculation methods influenced the germination abilities of the five genotypes. First, as the seeds were inoculated with agar plugs, many seedlings died 1-2 weeks after germination (Figure S1). With the suspension inoculation method, symptoms did not appear until 3-4 weeks after germination (Figure S2). When inoculated (Table 1), a germination ratio shows that Reform maintained its germination better than the other genotypes.

Table 1. The germination abilities of the five genotypes under two types of inoculation help classify the five winter wheat genotypes regarding their susceptibility to the disease infection. Tolerances identified the genotypes roots susceptibility to the disease infection response to sensitivity according to germination ratio

Genotype	Per cent germination reduction under suspension	Per cent germination reduction under agar plug
Julius	0	62.5
Brons	0	50
Reform	0	37.5
Informer	12.5	62.5
Ceylon	12.5	75

4. Discussion

The experiment presented in this thesis compared five genotypes of winter wheat and their performance under two different parameters. The first parameter was two methods of inoculations with the same pathogen, *Fusarium graminearum*. The second parameter was two concentrations of the same nutrient solution.

While there is a difference in root growth between weeks 6-10 (Weaver et al.,1924; Ellis et al., 1980), the uninoculated plants were kept for four weeks longer to compare the effects of the two nutrient concentrations.

4.1. Hypothesis Testing

The living cortex cell percentage confirms the hypothesis that the living cortex cell percentage in an un-inoculated root is smaller than in the inoculated root as a healthy root will develop in a normal life pathway as the stress in the wheat root cortex causes RCS to be triggered in the uninoculated root under the effect of genetic and environmental factors (Schneider & Lynch, 2018). Still, in the roots inoculated with *F. graminearum*, the RCS is inhibited by the response to the infection (Wang, 2015).

The results support that the number of nodal roots will affect the root size and architecture, as shown in (Figure 13a). Ceylon returns with more nodal roots than the other genotypes, which means that more nutrition will be absorbed from the soil to help the plant's immunity system. In addition, these results support previous results that genotypes with more nodal roots tolerate *F. graminearum* and minor inoculation because of their shallow location and horizontal branching and develop later than the other roots (Acharya, 2017).The necrotrophic influence of *F. graminearum*, especially in a dry environment, will dissolve the host root and shoot structure (Wang & Gottwald, 2017).

The results do not confirm the hypothesis that the lower (2ml/l) nutrients application will retain more root biomass, size, and diameter than the higher (4ml/l) nutrients application. Conversely, more root biomass, length, and diameter were developed with the higher dose. Still, the results confirm the root's ability to absorb nutrients, helping the root tissues and architecture tolerate the *F. graminearum* infection (Hossain et al.,2017).

The result supports that inoculating the genotypes with *F. graminearum* could affect root growth, causing fewer seminal roots, decreased root size, and reduced biomass. In comparison with results found by Wang et al. (2015), they referred to root biomass and length reduction but did not specify the most infected root types.

4.2. Comparisons Between Inoculation Methods

A substantial difference in infection of *F. gramineraum* was found comparing the conidial suspension and the agar plug methods. This difference is supported by past research that *F. gramineraum* inoculation with agar plugs was significantly more effective than conidia suspension (Gottwald, 2017). The agar plugs from the PAD medium carry the fungal mycelium, which adheres to the wheat seed as they are put together in the seeds. After seed germination, the surrounding mycelium will penetrate

the host. The agar plug carries the mature pathogen mycelium ready to penetrate the young seedling root cell wall because it is buried beside the seed.

In contrast to the inoculation with conidia suspension, the conidia, washed down by irrigation of the pot away from the seeds bed, will take time to germinate and form the mycelium, then later attack the advanced growing seedling root cell wall. As a result, the conidia will be scattered in the soil or inadequate quantities around the seed. As a result, the conidia will take some time to germinate and establish a mycelium. The conidia will later cause the infection; this can give the seedling much more time to grow and become more vital, allowing the seedling to build up a defence mechanism to stop the disease from spreading and control its symptoms (Bhandari et al.,2018; Wang et al.,2015).

4.3. The flexible capacity of the genotypes resistant to the pathogen

Root cortical senescence (RCS) might show the genotypes' adjustable power by redistributing the nutrients from senescing tissue to other parts and minimizing the root breadth, as conformed by Ben-Noah et al. (2018). They state that the plant develops with reliable performance when carbon catabolism is reduced, causing biotic and abiotic stress factors.

Schneider & Lynch (2018) found that the cortex cell layers play a role in nutrient storage. More cortical layers in un-inoculated roots increase the stored nutrients available for the root's needs. In the treatment with *F. graminearum* conidial suspension inoculation, Brons and Julius returned more cortical layers than the other genotypes. The stored nutrients in cortical layers could help the plant strengthen the immune system and develop a defence mechanism against the pathogen (Schneider & Lynch, 2018). Furthermore, the root diameter improves water use efficiency, nutrients uptake, and resistance to *F. gramineraum,* as shown in our results like in Informer and Ceylon return with great root length and root diameter including Julius, and as it is found by Atta et al. (2013). Furthermore, it was shown that the uninoculated Informer showed an increased root diameter.

On the other hand, inoculated Julius shows negligible effect by conidial suspension on root diameter, indicating a minor impact on water use efficiency, as found by Atta et al. (2013). The xylem number, xylem diameter and other root cellular structure traits may affect the water and nutrients uptake related to drought tolerance according to the vascular bundle capacity in keeping and transporting water and mineral found by Ouyang et al. (2020). It can be realized that the highest xylem number can be recognized in uninoculated Ceylon, and the highest xylem number can be identified in inoculated Julius by the suspension. It was shown that the xylem diameter increased for conidial suspension inoculated in Julius as for uninoculated Ceylon.

The analysed genotypes may build up defence mechanisms such as programmed cell death (PCD); as Dominguez & Cejudo (2014) presented, as the cell walls stop growing because they are dead, there will be no cell senescence causing a space in the cortex. Speculating about programmed cell death could prevent the pathogen from spreading to other cells, as seen in (Figure S3) the brown mass of inoculated cells tissues. Fisol et al. (2021) reported that the necrotic process that *F. gramineraum* mycelium blocks the vascular tissues. This mycelium was causing the plant shoot to wear off and the root to become rotten, as shown in our results (SF4, SF5).

4.4. Traits that influence the genotypes' pathogen interaction

Understanding the plant protection mechanism is necessary to know how Reform retains more Live cortex cell percentage than the other genotypes regarding resistance against *Fusarium*. The germination ratio indicates resistance according to genetic traits and nutrition storage in the seeds, which are the quantities of food minerals components working together for plant development. Therefore, the genotype could employ nutrition storage and resistance genetic traits in defence mechanisms. The pathogen hypha attacks several regions of the roots, such as the epidermis, cortex, endodermis, and vascular tissues (Bhandari et al., 2018). Some studies found a high accumulation of fungal cells in the endodermis, attacking vascular tissues to block them and causing necrotic symptoms (Lahlali et al., 2015; Lionetti et al., 2015; Bhandari et al., 2018). In the experiment regarding cells root structure traits like the number of xylems as (Figure 16a), the different responses of the inoculated genotypes. The pathogen penetrates the host in two ways: the developed hyphae born in the soil or the growing conidia transferred to the soil. It might be that programmed cell death is becoming much more vital in genotype roots if it has thick cortex cell walls, which can be related to how much the cell wall will deposit lignin on their walls (Lionetti et al., 2015). Speculation of molecular deposition, such as lignin, works as a defensive cell wall "fortification" against mycelium penetration. The cell wall's job is to stop the pathogen hypha from spreading towards the vascular trusses, block them, and develop antifungal bodes to prevent the pathogen from emerging papillae (Basinska-Barczak et al. 2020). This cell wall job may be found in Reform and explain why it has a high germination rate after inoculations compared to the other four genotypes studied. It was reported by (Lahlali et al., 2015) that lignin deposition on root cell walls increased its thickness.

4.5. The shoot traits

The shoot traits of the five genotypes were influenced by *F. graminearum*, reducing the characteristics such as leaf number plus fresh and dry biomass weight as it was recognized by (Wang et al., 2015) and trying to resolve by identifying the pathogen's harmful genes expression (Stephens et al., 2008) also with introducing root bacteria strain to control the pathogen infection on the shoot biomass (Henkes et al., 2011). However, Ceylon responded to the higher nutrients application and was returned with more performance on shoot traits such as most enormous leaves numbers, highest fresh biomass weight and dry weight compared to the other genotypes when infected with *Fusarium* with the conidial suspension inoculation method. Still, under the agar plug inoculation method, there was no response from none of the genotypes to nutrients, which means there is a need to study the soil-borne pathogen causing an aggressive infection rather than a transported pathogen conidia to the plantation site.

4.6. The root architecture traits

The root architecture traits, such as nodule and seminal root length, are influenced when interacting with *Fusarium graminearum*, as found and explained by (Moënne-Loccoz et al., 2014). In connection with the experiment, thirty to forty per cent of the nodal roots survived the conidial suspension inoculation. Especially the shallow nodal roots, as they are away from the conidia effect where the conidia washed down in the pot by irrigated water, also received help from nutrient applications to

grow better and become stronger. The result of the interaction may be a reduction in the root architecture traits, as these reductions for root architecture traits are found (Wang & Gottwald, 2017). Informer and Ceylon performed more in the experiment than the other genotypes regarding the root length. Ceylon returns with significantly higher fresh and dry root biomass weight than the different genotypes. In addition, Ceylon has longer roots than the other genotypes under agar plug inoculation, and Brons has the longest roots in the suspension inoculation treatment (Cabrera et al., 2020). According to nutrient treatment with the lower nutrient. Informer showed the longest roots, but Ceylon gave the longest roots with the double nutrient dose (4ml/l). The fresh and dry weights of the root biomass for both inoculation methods showed a considerable reduction. However, Brons statistically appeared differently, indicating a higher root biomass weight than the other genotypes. Ceylon returns more weight under two nutrient concentrations than the other genotypes. Ceylon has the highest seminal root number under the two types of inoculations and the highest nodal root number under conidia suspension inoculation methods than the other genotypes. Regarding genotypes' response to the nutrient applications, it was noticed that Ceylon returned more nodal and seminal roots under 4 ml/l concentration. Wang & Gottwald (2017) showed a similar nodal and seminal root response to nutrition application.

4.7. The root cellular structure traits

The root cellular structure features can suppress susceptibility to penetration of the *F. gramineum* hyphae. The cortex cell walls may play this role in pathogen interaction via enhanced cortical live cell percentage, cortex cell layer numbers, and width, as discussed by Wang et al. (2015). They explained that the cortex cell walls could play a role in stopping the expansion of the pathogen mycelium. In addition, the vascular bundle interacts with the pathogen and may lessen the disease through increased xylem number and diameter (Wang et al., 2015). All those traits have their mission to slow down and prevent the pathogen from spreading hypha. In this scenario, the fatal issue is the time agent necessary for the seedling growth and to build up a defence mechanism after inoculation. It is a speculation of programmed cell death when depositing more lignin at the cell's walls and developing enzymes or antibodies against the pathogen mycotoxin (Wang et al., 2015).

Reform returned a higher cortex-living cells percentage regardless of pathogen inoculation and nutrient treatments than the other genotypes evaluated. Julius has more xylem under the suspension inoculation method than other genotypes. Ceylon and Julius present more xylem in the lower nutrient level than other genotypes. Informer showed more xylem under the higher nutrient treatment than different genotypes. Brons showed a higher xylem diameter for the pathogen inoculation and nutrient application treatments than other genotypes. It was found by Guenther & Trail (2005) that more xylems will help the infected plant survive as the mycelium will not be able to block all the xylem's tubes. As seen in Figure 19a, the cross-section from the inoculated root section of Julius showed infected cortex cells with the pathogen colony as brown deposits on the cortex cells' walls. In the root cross-section of un-inoculated Brons (Figure 19b), empty spaces are seen in the cortex, compared with (Figure 19a), where there are no open areas in the cortex due to the cell's death. The root cross-section of uninoculated Reform (Figure 19c) has fewer cortical tissue losses. Reform showed the highest germination ratio and had the highest percentage of cortical living small-size cells compared to other genotypes. Reform has robust cortical cell walls that prevent pathogen penetration. In the same direction but much deeper in the anatomical and molecular study, Schneider et al. (2021) found the robustness of root cortical cell walls as they contained small cells with a high concentration of lignin

on the cell wall. This trait will prolong the root cortical cells' lives and allow them to keep their storage food longer as a defense mechanism against the pathogen.

4.8. Future Insight into root cortex structure, physiology, and cell walls molecular substances

More research is required to gain insight into root cortical senescence "mortality" (RCS). The RCS includes studies to determine the resilience of cultivars to shifting nutrients from the sensing tissues to other locations and minimising root breathing to extend the life span. This minimised root breathing would help decrease carbon deficiency and assure plant ontogenesis with reliable performance. Understanding the molecular mechanisms of RCS, nutrient absorption, redirection, and root breathing is essential to ensure that plants grow at the proper carbon levels. In addition, we must also examine the molecular insight and the genes responsible for the intercellular flux, which transports nutrients from cell to cell and across the entire living cortex (Schneider and al., 2017). These studies will highlight the pathway to get the optimum cell walls strong enough to stop the pathogen mycelium penetration into root tissues.

There is a need to investigate whether a correlation between the germination ratio and the percentage of cortical living cells throughout seedling development will increase the chance for more lignin on cell walls and improve multiple cortical sclerosis (Schneider et al., 2021). In addition, lignin, suberin, chitin, and cellulose are needed for insight study of producing and depositing mechanisms of the cortex cells wall (Subramaniam et al., 2009).

5. Conclusions

The experiments in this thesis were run to evaluate the susceptibility of five winter wheat genotypes to root rot caused by *F. graminearum*. In addition, two fungal inoculation methods and two levels of nutrient applications were studied.

It is concluded that what gave the seedling enough time was needed for root cell walls to counteract and defend against the pathogen's offensive random ambush. The seedling root cells will use the time to grow and benefit from the nutrients; they will be strong enough to resist conidia inoculation. The agar plug did not show this boost due to the infection of the seedling in the early stage of its germination. Suppose the time factor favours the seedling growth. It will be less susceptible to the cortical cell wall structure or thick enough to resist pathogen hyphae penetration. This time will favour the seedling development and become much more robust. Hence, the disease symptoms are weak in the host or cannot be realized. If the cell wall is thick enough, the pathogen is delayed entering the system, causing reduced symptoms as the pathogen enters a more mature plant. The seedling could employ the time resulting from the difference between the seedling growth time and conidia growth time to advance its development using the supplied nutrients, which can help build a resistance mechanism against the pathogen.

The shoot traits, the root architectural traits and the root cellar structure traits are putative (Danakumara et al., 2021; Voss-Fels et al., 2018), and they complete each other to build up the resistance. Also, these traits help select and design a breeding program to develop a resistance genotype. Future experiments must concentrate on molecular studies, and more insight into the fungus-plant interaction may offer an active interest in comprehending the functionality of the plant resistance to fungal diseases and the lignin deposition mechanism for thickening the root cell walls (Voss-Fels et al., 2018).

Few studies integrate root cellular structure, shoot traits, and architectural root traits in winter wheat. However, the phenotypical research of the three groups of traits consists of fifteen features. Some could be implemented in a long-term breeding strategy based on the experiment quantification results. High narrow-sense heritability estimates the traits of interest to be heritable from one generation to another and used in the breeding program. Some three groups of traits have putative combinations of genetic differences that control the selection response of the characteristics of interest. Some traits were not significantly different in the experiment's three groups, like cortex cells layer number, cortex width, and xylem diameter. Still, some traits, such as root length, root biomass, shoot biomass, root diameter, xylem number and living cell percentage, are quantitative traits that complete each other. Hence, they may have a putative value for the breeders to check if the studied traits have a highly narrow sense of heritability, which is the putative value that makes the cultivar less susceptible to the pathogen.

This experiment showed that the three groups of traits studied – shoot traits, root architecture, and root cellular structure – play a role in influencing pathogen infection. This role has allowed for identifying some characteristics that may help the host plant delay or stop the pathogen from spreading and enable the host plant to acquire more nutrition to build up a resistance mechanism against the saprophytic pathogen.

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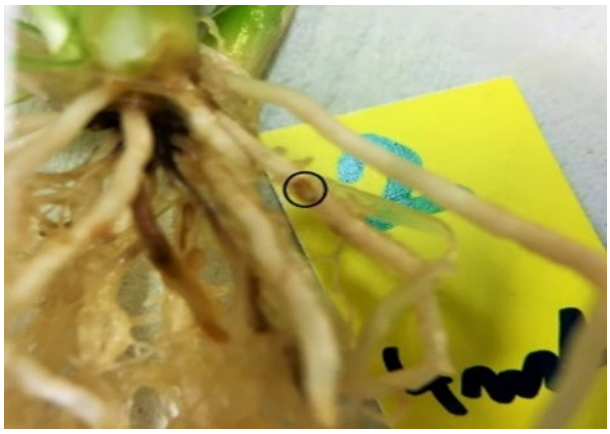
8. Appendix

Element	*Element Concentration in Stock (g/l)*	*Element Concentration in 2 ml/l Application (g/l)*	*Element Concentration in 4 ml/l Application (g/l)*
N	51	1.02	2.04
P	10	0.2	0.4
K	43	0.86	1.72
S	4	0.08	0.16
Ca	3	0.06	0.12
Mg	4	0.08	0.16
Fe	0.17	0.0034	0.0068
Mn	0.2	0.004	0.008
B	0.1	0.002	0.004
Zn	0.03	0.0006	0.0012
Cu	0.015	0.0003	0.0006
Mo	0.0004	8E-06	1.6E-05

Table S1: The minerals component was used as a nutrition solution with two concentrations, 2ml/l and 4 ml/l.

Supplementary Figure S1. The dead seedling is inoculated with an agar plug. The destructive power of pathogen—hypha can be seen in the young seedling.

Supplementary Figure S2. The young seedling inoculated with conidium suspension, we can see it is advanced in its growth and inoculation does not eradicate it.

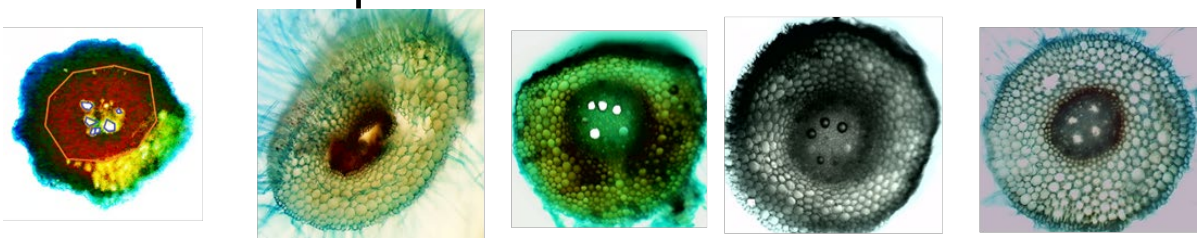


Supplementary Figure S3. The beginning of the inoculation, as in the black circle caused by inoculated seed by conidium suspension.

Supplementary Figure S4. The biotic symptoms of the inoculated seedling. In a slow death under the necrotrophic effect of the pathogen.

Supplementary Figure S5. The inoculation transfer via vascular tissues up to the stem and the leaves, as we could see white spots in the pathogen hypha colony at the bottom of the plant stem.

9. Illustration picture



a) b) c) d) e)

In an illustration of the five genotypes' root cross-sections of the inoculated roots, we can realize how the pathogen blocked the vascular tissues from left to right(a) Julius and (b)Brons when in (c)Reform, (d)Informer, (e)Ceylon gathered around endodermis. Program cell death might be seen in the Reform cortex with brown spots.