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Studies on the Identification of Resistance to *Fusarium oxysporum* (Schlecht.) in Different Genetic Backgrounds of *Asparagus officinalis* (L.) and Its Defense Responses

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Abstract: Due to the restricted genetic diversity among current asparagus cultivars, wild relatives are of particular interest as a source of resistance. In this study, seventeen cultivars and wild relatives were tested for their resistance to *Fusarium oxysporum* in the seedling test. Two isolates differing in virulence (single spore lines with high and low virulence) were used for testing. Most of the tested genotypes showed high infestation, whereby *Asparagus aethiopicus* caused no browning of the roots or growth depression. This wild relative was evaluated for its defence response after infection with *F. oxysporum*. For comparison, the wild relative *Asparagus densiflorus* and *Asparagus officinalis* cv. Thielim were tested. Like *A. densiflorus*, *A. aethiopicus* showed accumulation of hydrogen peroxide as a defence mechanism, while *Asparagus officinalis* cv. Thielim showed no storage of hydrogen compounds.

Keywords: *Asparagus officinalis*; *Asparagus aethiopicus*; *Fusarium oxysporum* resistance; hypersensitive response; distance analysis

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1. Introduction

Asparagus officinalis is cultivated on about 1.55 million ha worldwide, with the highest production in China, Europe, and Peru [1]. The human use of asparagus species other than *A. officinalis* is limited. Once mentioned as medicinal plants, some are now used as ornamentals, e.g., *Asparagus plumosus*. Only a few are still important medicinal plants, such as *Asparagus racemosus* in India [2]. Other wild species are considered a delicacy and are collected from the wild [2]. Cultivation efforts were only moderately successful [3]. Asparagus crown and root rot, caused by pathogenic soil fungi of the genus *Fusarium* are considered to be the main component of asparagus decline syndrome. Several *Fusarium* species are present in asparagus fields, but not all of them are pathogens of asparagus [4,5]. According to the study of Andrzejak and Janowska [4], only 50% of the collected field isolates are pathogens of asparagus. In infested plants, mainly *F. oxysporum*, *F. culmorum*, *F. proliferatum* [4,6,7], and *F. redolens* [6,8,9] were found. *F. oxysporum* is the most common and widespread species [5,6]. Studies have shown that the older the asparagus plantation, the higher the *Fusarium* infestation [4,6]. This can be explained by its ability to survive for a long time in plant debris and soil [6,10–12], which makes its control very difficult [6,11]. There are some studies on the use of fungicides and biological control agents to control Fusarium crown and root rot, but the results are mostly inconclusive or contradictory, especially in field trials. To make matters worse, the pathogen is ubiquitous [13–15]. Therefore, breeding asparagus cultivars resistant to *F. oxysporum* is an important strategy to control root and crown rot in asparagus production. The viability of *F. oxysporum* is enhanced by higher humidity and higher temperatures [16]. This is particularly important in the context of climate change. Studies by Delgado-Baquerizo et al. [17] indicate that temperature is one of the most important environmental factors for the global spread of potential plant pathogens and that their numbers are likely to increase in most regions. To date, resistance

to *F. oxysporum* has been experimentally confirmed only in one wild species. Resistance to *A. densiflorus* was first mentioned by Lewis and Shoemaker in 1964 and has been validated several times [18–22]. He et al. [19] demonstrated hypersensitive cell death. However, this resistance has not yet been transferred into cultivars due to strong crossing barriers [23]. To date, several attempts to produce interspecific hybrids between *A. officinalis* and asparagus wild relatives have been described. *A. officinalis* × *A. tenuifolius*, *A. officinalis* × *A. maritimus*, and *A. officinalis* × *A. prostratus* are examples of interspecific F1 hybrids [24–26]. Many other crosses, such as *A. officinalis* × *A. densiflorus*, *A. officinalis* × *A. stipularis*, and *A. officinalis* × *A. acutifolius* have failed [24,27–29]. Despite these difficulties, the wild relatives are of particular interest, because of the restricted genetic diversity in recent asparagus cultivars [30,31]. The aim of this study was to find resistance to *F. oxysporum* using a seedling resistance assay and to investigate the defence mechanisms in more detail.

2. Materials and Methods

2.1. Fungal Isolates and Cultures

Two single-spore lines of *Fusarium oxysporum* were used for resistance tests, both are pathogenic and produce visual symptoms of damage but differ in virulence (Foxy II and Foxy III). For comparison, Foxy II expresses low and Foxy III high virulence [18]. The original material was isolated from asparagus spears in experimental fields at the JKI in Quedlinburg (Germany) in 2011. All isolates used in this study were cultured on a sterile potato dextrose agar (PDA) for 14 days at 22 ± 2 °C in the dark. The addition of 200 mg/L streptomycins to PDA suppressed contaminants without affecting the growth of *F. oxysporum*. For the microscopic determination of hypersensitive reaction, only Foxy III was used, which was prepared by the same method.

2.2. Plant Material and Growing Conditions

Eleven asparagus cultivars, two landraces, and four wild relatives were tested for their resistance to Foxy II and Foxy III (Table 1). Asparagus seeds were surface disinfected with 3% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water for 1 min. The disinfected seeds were soaked in water at 36 °C for 24 h, then sown in autoclaved sand, and cultivated at 20 °C. Seedlings with a shoot length between 4 and 10 cm and a well-developed main root with first lateral roots were selected. The roots were cleaned of sand before use in the seedling resistance test. For the microscopic determination of hypersensitive response, seedlings from the same lot of *A. densiflorus*, *A. aethiopicus*, and *A. officinalis* cv. Thielim were grown under the conditions described above.

The results of the distance analysis were obtained from the modified data set of Nothnagel et al. [32]. For this work, they were re-analysed in a new combinations.

2.3. Seedling Resistance Test

Resistance to *Fusarium oxysporum* was tested on the basis of post-infection symptoms, according to Kathe et al. [18], with some modifications. Fungal conidia were harvested by scraping the spores with distilled water and a Drigalski spatula. The roots were immersed for 5 min in the conidial suspension (1×10^6 conidia/mL) and carefully shaken. The plants were then incubated for 14 days at 22 °C with a photoperiod of 16 h in covered square bioassay dishes (245 × 245 × 24 mm) on moistened filter paper. Disease symptoms such as brown lesions and asparagus root reduction were evaluated using LemnaTec Scanalyzer PL (LemnaTec GmbH, Aachen, Germany). The software was calibrated on a symptom basis to distinguish between the healthy and infected root tissue. The pixels of the calibrated classes used for statistical evaluation were measured. In addition to absolute root length, the browning of roots was also studied as a symptom. To measure the root length, lines were drawn through each root (see Figure S1). Finally, to determine the absolute root length, the pixels of these lines were summed.

Table 1. *Asparagus* species evaluated for resistance to *Fusarium oxysporum*.

Code	Asparagus Species	Cultivar/ Wild Relative [1]	Seed Origin [2]	Accession	Geographic Origin
1	*A. amarus* DC.	Wild relative (Pop.)	CRA		Italy
2	*A. aethiopicus* L.	Wild relative (Pop.)	GG	3333-3	Malaga
3	*A. densiflorus* (Kunth) Jessop	Wild relative (Pop.)	ISR		Israel
4	*A. stipularis* Forssk.	Wild relative (Pop.)	IBZ		Ibiza
5	*A. officinalis* L.	Landrace (Pop.)	WUR	CGN25609	Armenia
6	*A. officinalis* L.	Landrace (Pop.)	WUR	CGN25611	Armenia
7	*A. officinalis* L.	cv. Argenteuil (OP)	RZ		French
8	*A. officinalis* L.	cv. Grolim (F_1)	LIM		The Netherlands
9	*A. officinalis* L.	cv. Ivancicky (OP)	IPK	ASP 1	unknown
10	*A. officinalis* L.	cv. Leistungsauslese (OP)	DS		Germany
11	*A. officinalis* L.	cv. Ramada (F_1)	SWS		Germany
12	*A. officinalis* L.	cv. Ramires (F_1)	SWS		Germany
13	*A. officinalis* L.	cv. Ravel (F_1)	SWS		Germany
14	*A. officinalis* L.	cv. Ruhm von Braunschweig (OP)	IPK	ASP 31	Germany
15	*A. officinalis* L.	cv. Schwetzinger Meisterschuss (OP)	SWS		Germany
16	*A. officinalis* L.	cv. Start (OP)	IPK	ASP 3	unknown
17	*A. officinalis* L.	cv. Thielim (F_1)	LIM		The Netherlands

[1] Pop.: Population, OP: Open pollinated, F_1: Hybrid. [2] CRA: Research Institute for Vegetable Crops (Italy), ISR: Volcani Centre Bet Gagan (Israel), LIM: Limgroup B.V. (The Netherlands), SWS: Süd-West-Saat (Germany), RZ: Rijk Zwaan Zaadteelt en Zaadhandel B.V. (The Netherlands), IBZ: ornamental and wild asparagus from Ibiza (Spain), IPK: Leibniz Institute of plant genetics and crop plant research (Germany), DS: Deutsche Spargelzucht (Germany), GG: Gruson Gewächshäuser Magdeburg (Germany), WUR: Wageningen University & Research.

A complete screening was performed with the genotypes listed in Table 1. Because very little material was available for some genotypes, especially wild species, and old landraces, and the germination rate of these genotypes was also very low in some cases, there were some failures. These are listed in Table S1. To demonstrate that one screening provides clear information about the susceptibility or resistance of a genotype, a second screening was performed for a portion of the genotype (partial screening). To demonstrate this, Table S2 in Supplementary Material compares the complete screening with the partial screening for four genotypes.

2.4. Staining Procedure and Microscopic Determination of Hypersensitive Response

A. densiflorus, *A. aethiopicus*, and cv. Thielim were tested for hydrogen peroxide accumulation in response to infection with *F. oxysporum*. Three seedlings per *Asparagus* species were infected. According to He et al. [19], the hyphal-sandwich root inoculation was used for inoculation. Between two- and ten week old seedlings were cleaned of sand and blotted dry. The roots were placed between two pieces of PDA containing a hyphae of Foxy III. Uninoculated PDA strips were used as the controls. For incubation, the treated seedlings were placed in a moistened and covered square BioAssay dish (245 × 245 × 24 mm) in a growth cabinet at 22 °C with a 16-h photoperiod. The method described by Serfling et al. [33] was used for staining. The roots of the test plants were sampled immediately before inoculation and after 8, 12, 24, 48, and 72 h of incubation. A 3,3′-Diaminobenzidine (DAB) solution was used to detect hydrogen peroxide (H_2O_2) accumulation in the inoculated roots. Calcofluor white M2R was used to stain the fungal structures. Three 1 cm root pieces per asparagus species were tested and examined under the microscope.

The microscopy of the roots and fungal structures was performed using the Axioskop 50 microscope equipped with an Axiocam 305 color and the ZEN 3.0 (blue edition) software package (Carl Zeiss AG, Jena, Germany). Fungal structures stained with Calcofluor white M2R were observed using a filter set 02 (excitation filter G 365, beam splitter FT 395, and

barrier filter LP 420). The evaluation of the DAB staining was performed using the Axio Imager.A1 microscope equipped with an Axiocam MRc and the AxioVision SE64 Rel. 4.9 software package (Carl Zeiss AG, Jena, Germany).

2.5. SSR Markers and Distance Analysis

The total genomic DNA was isolated from 100 mg juvenile cladophyll tissues of one plant per accession according to the protocol of Porebski et al. [34] and finally adjusted to 10 ng/µL. For SSR analysis, 25 PCR primer pairs (Table S3) were used, as described by Mercati et al. [35], along with a universal labelling strategy according to Oetting et al. [36]. The unlabelled forward primer extended by a 19 bp M13 sequence was combined with the fluorescence-labelled M13 universal primer and the reverse primer of an original length. Amplifications were performed in 6 µL total volume, containing 12.5 ng plant DNA, 1 × concentrated buffer solution (with MgCl2 and dNTPs), 0.8 µM primers, and 0.2 Units MyTaq DNA polymerase (Bioline, London, GB). A GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Forster City, CA, USA) was programmed as follows: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, followed by a final step of 72 °C for 7 min. The amplification products of two independent PCR amplifications with IRDye700 and IRDye800 labelled M13 primers were mixed. DNA fragments were separated using an LI-COR 4300 automatic sequencer (LI-COR Biosciences, Lincoln, NE, USA). The fragment sizes were calculated by comparison with IRDye700 and IRDye800 labelled 50–350 bp size ladder. Polymorphic bands were recorded as a 1/0 matrix and used for distance analysis according to Nei and Li [37] with the program Treecon (Scanalytics, Milwaukee, WI, USA). Cluster analysis was performed using the unweighted pair group method with the arithmetic mean (UPGMA) algorithm. The confidence level for each branch point was determined by bootstrap analysis with 100 iterations.

2.6. Statistical Analysis

The program R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for the statistical analysis of the percentage data of diseased tissue and pixel data of absolute root lengths. When a normal distribution and homogeneity of variance were present, an independent sample t-test was used to test for significant differences between the control and infected plants ($p < 0.05$). If this was not the case, the Mann-Whitney–U test was used.

3. Results

3.1. Resistance Test

A total of 17 genotypes were tested, including four wild relatives, two landraces, and eleven cultivars. As part of the seedling resistance test, the root reduction and root browning after infection with F. oxysporum were examined.

Both used Foxy isolates were pathogenic to asparagus, with different virulence. As described by Kathe et al. [18], Foxy II was less virulent than Foxy III.

The roots of plants infected with *F. oxysporum* (Foxy II and Foxy III) showed reduced root growth compared to uninfected plants. In addition, lateral root growth was also obviously reduced. In the following, only the results for Foxy III are shown (Figures 1 and 2). Foxy II has a similar effect on the root length but to a lesser extent. There are no significant differences between the control and Foxy III in the wild relatives *A. densiflorus* and *A. aethiopicus*, in the landrace CGN25609, and the *A. officinalis* cvs. Leistungsauslese, Schwetzinger Meiserschuss, and Ivancicky (Figure 2). All other genotypes show a significant difference between the absolute root length of control and Foxy III (*, Figure 2).

Figure 1. *A. officinalis* cv. Ramada 14 dpi with *F. oxysporum*. (**a**) Control; (**b**) Foxy III.

Infection with *F. oxysporum* was indicated mainly by elongated brown spots and by the discoloration of entire root sections. In severe infection symptoms, purple exudates were released sometimes on the filter paper, and/or the roots disintegrated partially. In rare cases, a white mycelium formed on the infected root. Differences in virulence between Foxy II and Foxy III were indicated by visibly fewer browning symptoms and a smaller infected area in Foxy II.

The wild species *A. aethiopicus* and *A. densiflorus* differ from the other genotypes tested. Both show thickened shoot roots as seedlings that turn green after 14 days of incubation (Figure 3b,c). This resulted in an increased discoloration rate due to the evaluation method. However, the images in Figure 3b,c show that neither the control nor the infected seedlings showed any signs of infection. For comparison, the susceptible *A. officinalis* cv. Thielim is shown in Figure 3a. This obviously shows the difference between the uninfected and infected root and the typical browning symptoms.

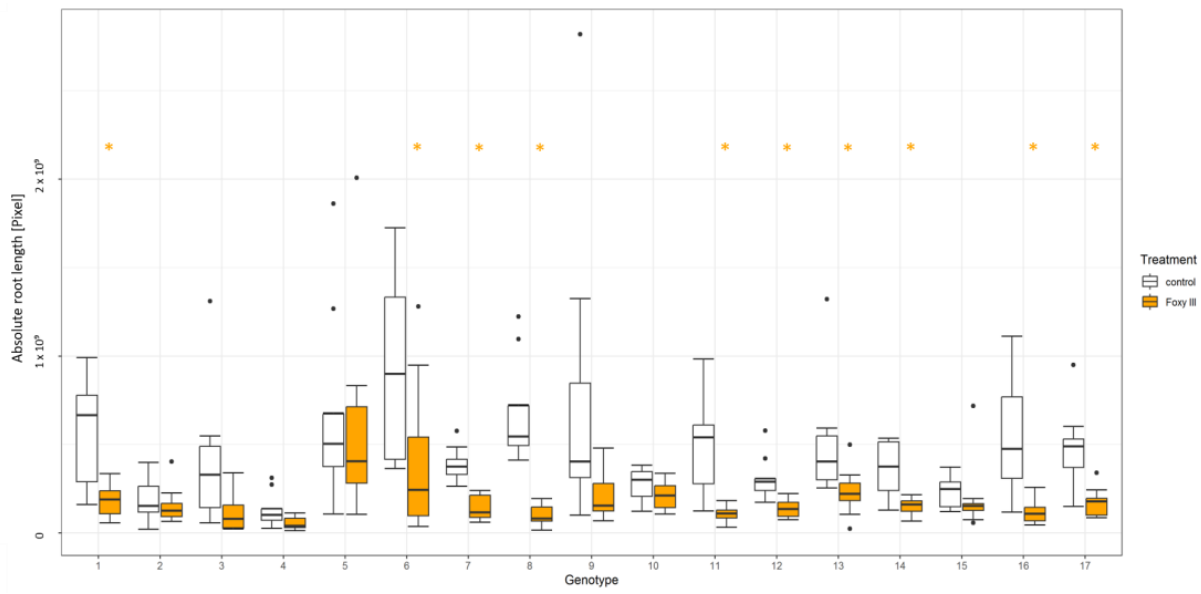


Figure 2. Comparison of absolute root growth 14 dpi with Foxy III of the 18 asparagus genotypes (Table 1) in pixel. * Indicates significance between control (white) and Foxy III (orange) for each genotype ($p < 0.05$).

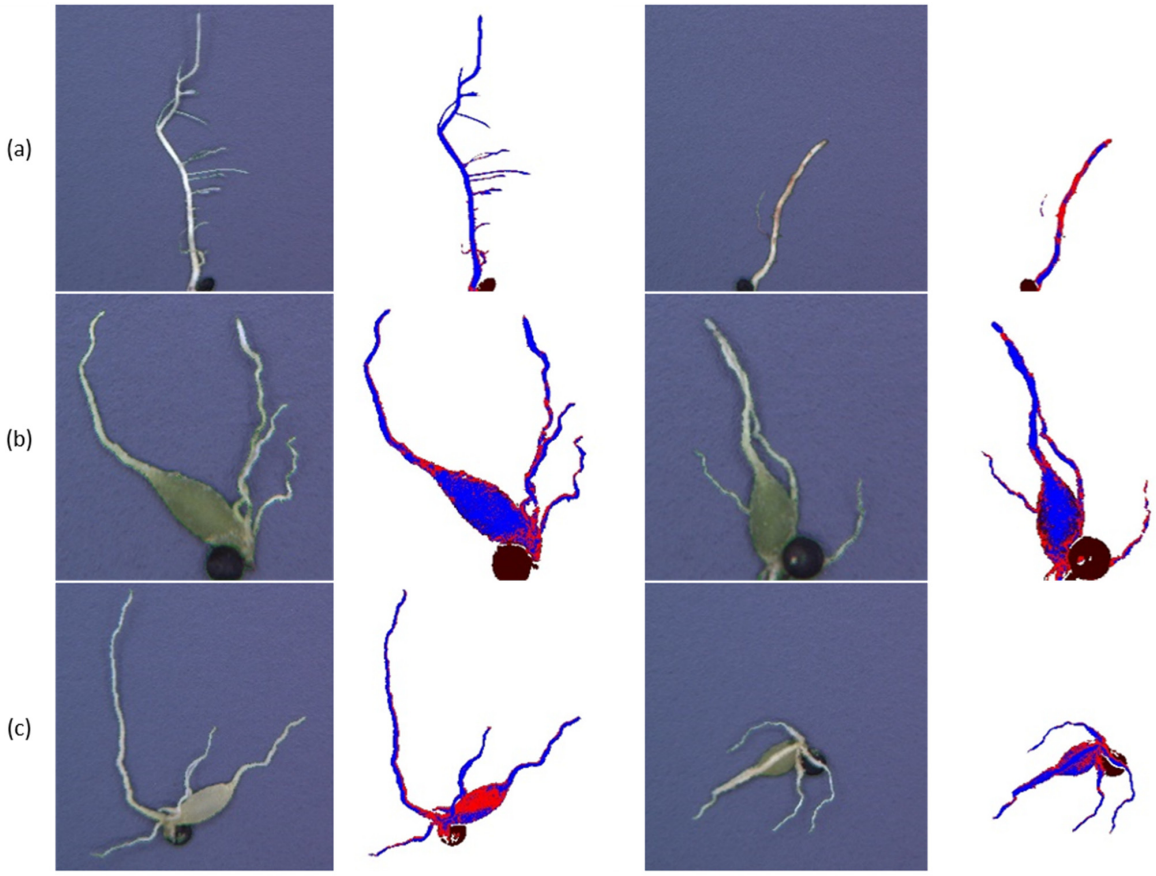


Figure 3. Analysis of diseased tissue caused by *F. oxysporum* (Foxy III, 14 dpi) using the digital image analysing system of LemnaTec. Left is the control and right is the infected seedlings, each with the original image and the generated colour class image. (**a**) *A. officinalis* cv. Thielim; (**b**) *A. aethiopicus*; (**c**) *A. densiflorus*.

All tested cultivars showed susceptibility to both *F. oxysporum* isolates (Foxy II and Foxy III). However, there were some differences between the cultivars. For example, *A. officinalis* cv. Schwetzinger Meisterschuss had a significantly higher susceptibility than *A. officinalis* cv. Argenteuil (Figure 4, Genotype 15 and 7). The severity of infection ranged from 9% to 42% for Foxy II and 13% to 77% for Foxy III among the tested cultivars (Figure 4).

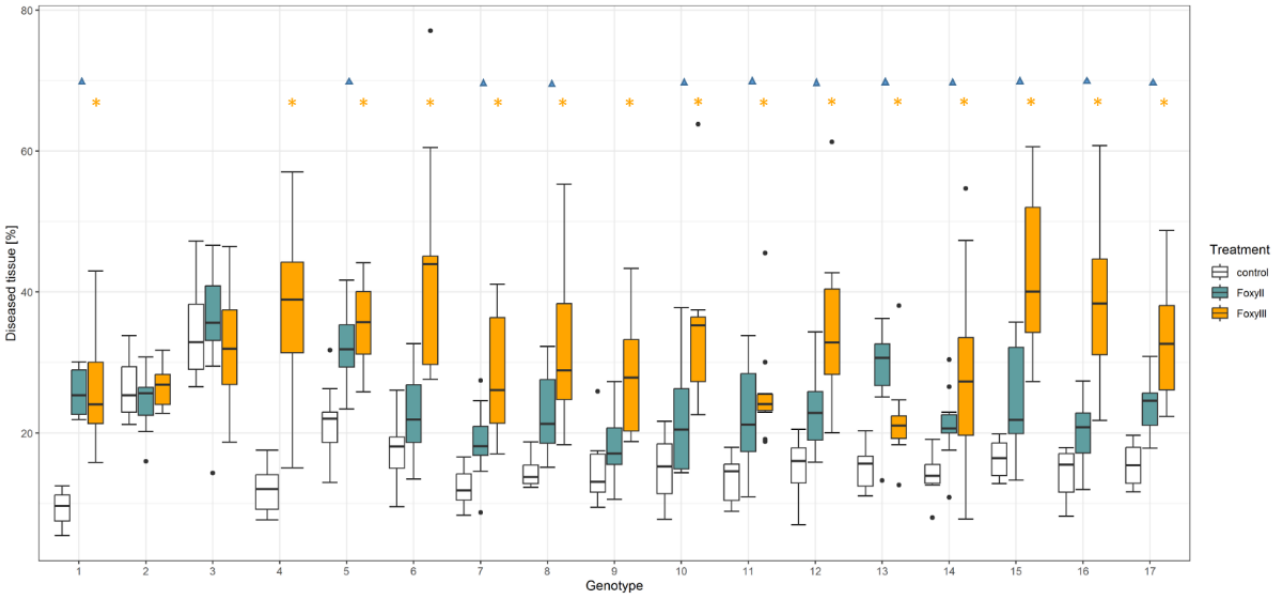


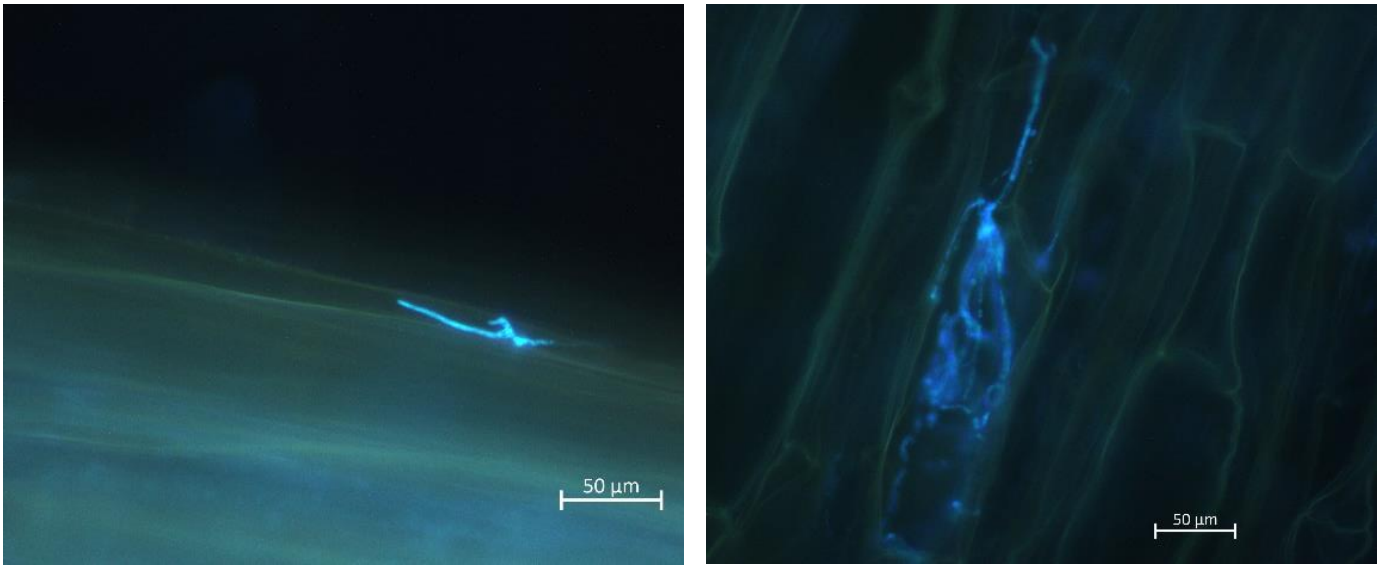
Figure 4. Resistance test against *Fusarium oxysporum* isolates Foxy II and Foxy III, 14 dpi. Percentage of diseased tissue for the 17 asparagus genotypes (Table 1) shown is the complete screening. * Indicates significance between the control and Foxy III for each genotype ($p < 0.05$). $^{\Delta}$ Indicates significance between control and Foxy II for each genotype ($p < 0.05$).

In addition, the wild relatives *A. amarus* and *A. stipularis* showed susceptibility to both *F. oxysporum* isolates after two weeks of incubation. For the wild relatives *A. densiflorus* and *A. aethiopicus,* there was no significant difference on average between the infected root and the control (Figure 4, Genotype 2 and 3). However, not all tested plants of these genotypes were free of symptoms. Of the 40 *A. aethiopicus* seedlings tested, three showed visible signs of infection. In addition, of the 10 *A. densiflorus* seedlings tested, two showed visible symptoms.

One screening already provided a meaningful result on the susceptibility or resistance of the genotypes tested. Although there were significant differences between the complete and partial resistance screening in some comparisons (Table S2), it was found that the susceptibility or resistance statement never changed for the genotypes tested twice.

3.2. Microscopic Analyses

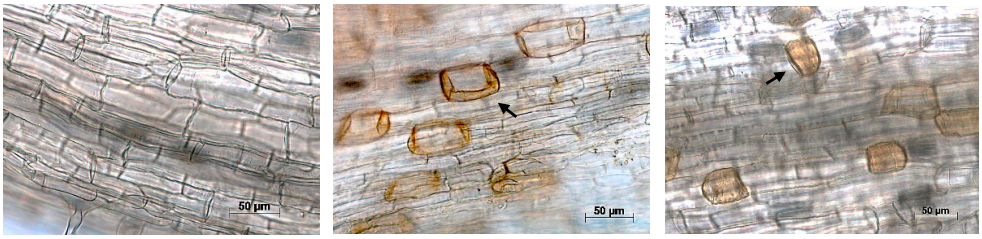
After the resistance test showed a resistance of *A. aethiopicus* to *F. oxysporum*, the exact defence mechanism was investigated. Since it is known that *A. densiflorus* reacts with a hypersensitive response to invading fungi [19], this mechanism was tested for *A. aethiopicus*. To verify that the fungus had indeed invaded the root, the fungus was stained with the fluorescent dye Calcoflour White. As can be seen in Figure 5, *F. oxysporum* invades and spreads in the cells of susceptible *A. officinalis* cv. Thielim.



(**a**) (**b**)

Figure 5. Fungal structures (Foxy III) in the susceptible genotype *A. officinalis* cv. Thielim 72 hpi. Fungal cell walls stained using Calcofluor White M2R. (**a**) Growing hypha; (**b**) Mycelium spreading from a cell.

Staining with 3,3′-Diaminobenzobenzidine (DAB) shows that hydrogen peroxide (H_2O_2) has accumulated in the root cells of *A. densiflorus* and *A. aethiopicus* at 8 hpi. The stained areas increase with incubation time. A difference in the rate of H_2O_2 accumulation was observed. Thus, the storage of H_2O_2 in *A. densiflorus* WAS apparently faster than in *A. aethiopicus* (Figure 6, arrows in Figure 6b,c). By contrast, no DAB-stained areas were found in the susceptible genotype *A. officinalis* cv. Thielim (Figure 6a).



(**a**) (**b**) (**c**)

Figure 6. DAB stain of hydrogen peroxide on *F. oxysporum*-infected roots. Arrows in (**b**,**c**) mark enhanced hydrogen peroxide concentration stained by 3,3 Diaminobenzidine (**a**) *A. officinalis* cv. Thielim 12 hpi; (**b**) *A. densiflorus* 12 hpi; (**c**) *A. aethiopicus* 12 hpi.

3.3. Phylogenetic Relationship

A new potential source of resistance was identified in *A. aethiopicus*. Because of the strong crossing barriers mentioned in the introduction, the determination of the genetic distance is important to develop strategies for resistance transmission. Twenty-five microsatellite loci, distributed over all ten *A. officinalis* chromosomes, were used to estimate the genetic distance of 26 *Asparagus* accessions. On average, 8.9 (3–17) alleles were amplified per tested SSR. The 222 polymorphic bands were recorded as a 1/0 matrix and used for cluster analysis. Two main clusters, which represent the Eurasian clade and the African clade, corresponded to their geographic origin.

The results showed that *A. densiflorus* and *A. aethiopicus* are closely related in a subgroup and that both wild relatives have a large genetic distance from the *A. officinalis* cultivars, forming their own subclade (Figure 7).

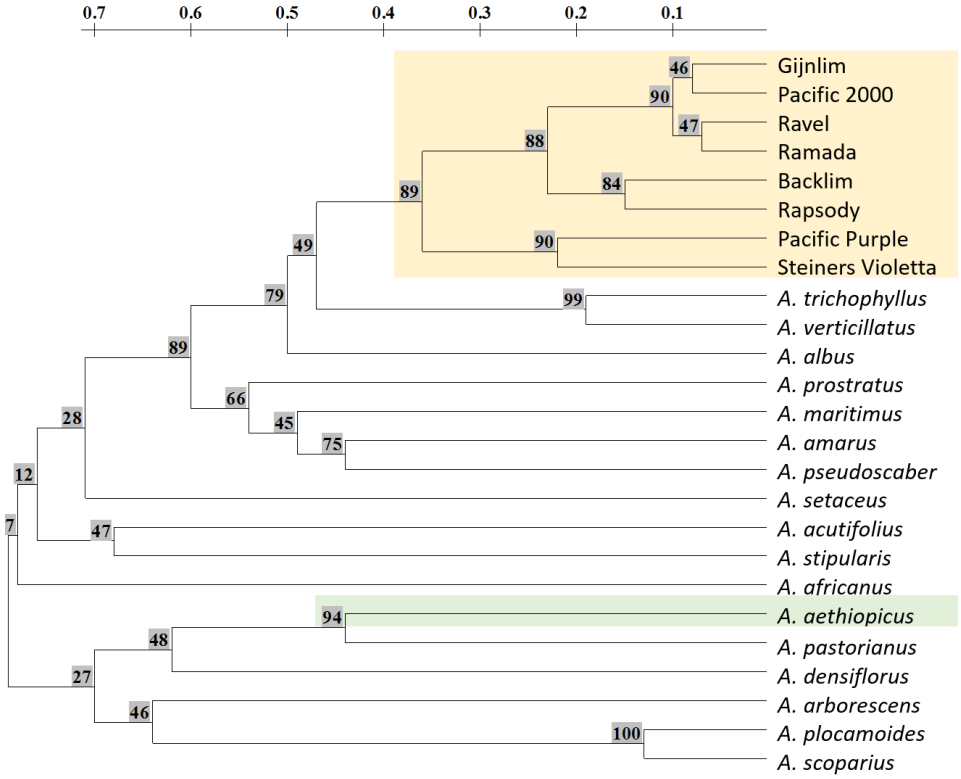


Figure 7. Determination of the phylogenetic distance between *A. aethiopicus* (green) and *A. officinalis* (cultivars; yellow) using 222 bands from SSR analysis. Numbers at the branches indicate bootstrap values in percent.

4. Discussion

Our study identified a new potential source of resistance to *F. oxysporum* in *A. aethiopicus*. In the seedling resistance test, this wild relative showed almost no susceptibility to the *Fusarium* isolates used (Foxy II and III). Moreover, it reacted rapidly after inoculation with *F. oxysporum* (Foxy III) with an accumulation of hydrogen peroxide.

As reported in the results, there was a problem with the increase in the discoloration rate in the wild species *A. aethiopicus* and *A. densiflorus* due to the method of evaluation (Figure 3). This was problematic for comparing these wild species to the other genotypes. Because the digital image analysis was performed with the same configuration, camera settings, and symptom definition for all genotypes. Changing these settings in favour of the wild species resulted in a significant misinterpretation of the other genotypes. Therefore, the described configuration was used even though it misinterpreted the fact that the wild species were infected.

In the resistance test, the browning of roots proved to be a better selection criterion than the reduction in root length. It is known that *F. oxysporum* has a negative effect on root growth [5,38]. This was also shown in our study. However, in our experiment, it was shown that this was not a suitable trait for the selection of resistant and susceptible genotypes. Some genotypes that were classified to be susceptible in the resistance test did not show a significant reduction in root lengths, such as landrace CGN25609 and *A. officinalis* cv. Leistungsauslese and *A. officinalis* cv. Schwetzinger Meisterschuss (Figure 2). Mostly this was because they had a high diversity in root growth even when uninfected.

All tested *A. officinalis* cultivars were susceptible in the resistance test, as were the wild relatives *A. amarus* and *A. stipularis* (Figure 4). The cultivars showed little variation in susceptibility, which was manifested by a more or less severe browning of the roots. This probably reflects the restricted gene pool of asparagus, especially in cultivars [30,31]. In contrast, the wild relatives *A. densiflorus* and *A. aethiopicus* showed resistance to both tested *Fusarium oxysporum* isolates. In the case of *A. densiflorus*, this result was consistent with

previous studies [19,20]. Both wild species react rapidly after infection with a hypersensitive response, which is shown microscopically by an accumulation of H_2O_2 (Figure 6b,c). A difference in the rate of H_2O_2 accumulation was observed; the storage of H_2O_2 is apparently faster in *A. densiflorus* than in *A. aethiopicus*. According to our results, the resistance of the two wild species was based on the same mechanism. However, it is not yet possible to say anything more definite to verify these preliminary observations. For this, it needs further detailed tests. The rapid production of ROS, including H_2O_2, in response to infection, is a typical feature of resistance in plants [39]. This leads to rapid and localized cell death, which inhibits the spread of *F. oxysporum* to healthy tissues [19,39].

To better assess and strategically transfer resistance to cultivars, it is important to determine the genetic distance. For the distance analysis, more wild species were used than in the resistance test to present the most accurate analysis of relationships. This was conducted in order to see if the classical crossing and embryo rescue were promising or if protoplast fusion will be necessary. This fact is demonstrated by the interspecific hybrids of Ito et al. [27], who found a correlation between phylogenetic relativity and the potential for interspecific hybridization [27]. The results of the close relationship between *A. aethiopicus* and *A. densiflorus* indicate that *A. aethiopicus*, similar to *A. densiflorus*, is very difficult to cross-breed [28,29]. Therefore, it is important to search for other sources of resistance. This is because the probability of transferring resistance to *F. oxysporum* increases with multiple opportunities. In particular, if resistance was found in more closely related wild species, inclusive of native wild *A. officinalis*, the probability of a successful cross would increase significantly. These analyses support the goal of transferring Fusarium resistance into cultivated asparagus.

The next important step is to develop a greenhouse test to analyse resistance behaviour at later plant stages. The aim is to determine whether resistance in the seedling also occurs in the adult plant. The development of an in vitro assay is also needed to find as many sources of resistance to *F. oxysporum* as possible. This would especially facilitate the testing of wild species, which are sometimes very difficult and time-consuming to propagate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020158/s1, Table S1: Overview of the resistance tests carried out in the complete and partial screening. x represents complete runs (control, Foxy II, and Foxy III with 10 seedlings each). Deviations are defined more precisely. Table S2: Resistance test against *Fusarium oxysporum* isolates Foxy II and Foxy III, 14 dpi. Comparison of the percentage of diseased tissue measured in complete and partial resistance screening for *A. amarus*, *A. aethiopicus*, *A. officinalis* cv. Ravel, and *A. officinalis* cv. Start. Asterisks behind the average and SE show significant differences between the complete and partial resistance screening ($p < 0.05$). $^{\Delta}$ indicates significance between control and Foxy II or Foxy III for each genotype ($p < 0.05$). Table S3: SSR primer used for the distance analysis. Figure S1: Analysis of root length evaluated with LemnaTec Scanalyzer PL. *A. officinalis* cv. Grolim 14 dpi. (**a**) Control; (**b**) Foxy III.

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Data Availability Statement: Rating and marker data can be obtained from us on personal request for scientific use.

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