Identification of Sources of Quantitative Resistance to *Fusarium oxysporum* f. sp. *medicaginis* in *Medicago truncatula*

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Abstract

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The resistance of 267 *Medicago truncatula* accessions was determined against the soilborne pathogen *Fusarium oxysporum*, one of the major constraints of forage and grain legumes worldwide. The initial screening of the collection revealed a wide range of disease response from completely resistant to highly susceptible to one strain of *F. oxysporum* f. sp. *medicaginis*. As a result, 26 accessions were identified as resistant, 9 as susceptible, and all other accessions as partially resistant. The phenotype of 12 resistant accessions was confirmed in two independent experiments on a subset of 23 accessions. Quantification of *F. oxysporum* f. sp. *medicaginis* within plant tissue indicated that the resistance level of the accessions is correlated with the amount of *F.*

oxysporum f. sp. *medicaginis* within its shoot. Inoculation with a different *F. oxysporum* f. sp. *medicaginis* isolate indicated that the resistance phenotype was stable because accession response to both *F. oxysporum* f. sp. *medicaginis* strains followed similar trends. However, grouping accessions according to their geographic origin did not reveal foci of resistance, which supports the idea that resistance arose from independent events. The identification of 12 resistant accessions will be useful for further cellular and molecular studies to unravel the basis of resistance to *F. oxysporum* in this model species and to transfer resistance to legume crop.

Fusarium wilt, caused by the soilborne ascomycete fungus *Fusarium oxysporum* Schldl., is one of the major constraints of forage and grain legumes worldwide (16,20,26). Several races and pathotypes have been identified for most legume-infecting *formae speciales* based on their virulence on subsets of differential hosts and their molecular and genetic characterization (20). Thus, four races have been identified thus far for the pea (*Pisum sativum* L.) pathogen *F. oxysporum* f. sp. *pisi* (20,25,26) and eight races and two pathotypes for the chickpea (*Cicer arietinum* L.) pathogen *F. oxysporum* f. sp. *ciceris* (23). By contrast, no physiological specialization has been described thus far for *F. oxysporum* f. sp. *lentis* or *F. oxysporum* f. sp. *medicaginis* that infect lentil (*Lens culinaris* Med.) and alfalfa (*Medicago sativa* L.), respectively (11,20). As for many soilborne pathogenic fungi, control of this disease is difficult and requires the integration of several disease management methods, among which utilizing host resistance is the most effective method (27). Fortunately, monogenic resistance to *F. oxysporum* has been described for most legume crops (20) and used to develop resistant cultivars to control the disease (20). However, this qualitative resistance can be rapidly overcome by the emergence of new pathogen variants such as in chickpea and pea (3,37). Breeding programs that incorporate resistance sources with quantitative and polygenic mechanisms would improve the durability of resistance. However, this requires a better understanding of the molecular and cellular basis of the resistance in legumes to direct breeding strategies and contribute to a more efficient and durable breeding.

Barrel medic (*M. truncatula* L.) is an important pasture legume (7) that has recently emerged as a model plant species for struc-

tural and functional genomics (6,14). *M. truncatula* is closely related to cultivated alfalfa and to other legumes such as pea, chickpea, faba bean (*Vicia faba* L.), and lentils. This close phylogenetic relationship increases the value of *M. truncatula* as a resource for understanding the important agronomic traits of grain and forage legumes, including their resistance to diseases (35). As such, this model legume has been already used to dissect the genetic, cellular, or molecular basis of resistance to six legume pathogens or pests (1,4,5,9,12,31–33,36,38–40). A recent study also used *M. truncatula* to provide valuable information on molecular and cellular aspects of the *M. truncatula*–*F. oxysporum* interaction in the context of susceptibility. However, this research did not identify resistant *M. truncatula* genotypes (34). To understand the molecular basis underlying resistance, it is important to identify and characterize resistant accessions. To this aim, we screened a wide collection of *M. truncatula* accessions of worldwide origin to identify resistance to *F. oxysporum* f. sp. *medicaginis*.

Materials and Methods

Fungal isolates and culture conditions. *F. oxysporum* f. sp. *medicaginis* isolates MIAE00930 (strain 605) and MIAE01129 (strain 179.29) were obtained from the Microorganismes d'Intérêt AgroEnvironnemental (MIAE) collection of UMR Agroecology, INRA Dijon, France. Strain 179.29 is a reference strain of *F. oxysporum* f. sp. *medicaginis* of unknown origin while strain 605 was originally collected in Greece. Both strains are equally pathogenic on *M. truncatula* (34). Thus, strain 605 was used in all experiments while strain 179.29 was used to test the stability of the resistance phenotype on a subset of *M. truncatula* accessions between *F. oxysporum* f. sp. *medicaginis* isolates. The fungal strains were routinely stored as microconidial suspensions at –80°C with 30% glycerol. For microconidia production, cultures were grown in potato dextrose broth (Difco) at 28°C with shaking at 170 rpm (8).

Plant material and growth conditions. A collection of 267 accessions of *M. truncatula* of worldwide distribution, kindly provided by the United States Department of Agriculture–Agricultural Research Service germplasm collection, was used in this study. The collection contained mainly wild accessions (78%) originating from Mediterranean countries, although many cultivars and breeding materials developed in Australia and additional countries were also included.

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*The e-Xtra logo stands for “electronic extra” and indicates that a supplementary table is available online and that Figure 1 appears in color online.

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M. truncatula seed were wrapped in wet filter paper in a petri dish, stratified for 2 days at 4°C in the dark, and incubated at 20 ± 2°C until germination. Once germinated, seedlings were transferred to pots (36 cm² by 8 cm) containing sterile vermiculite (1 to 3 mm diameter) and grown in a controlled environment chamber under a photoperiod of 16 h of light and 8 h of darkness. Temperatures were maintained at 25°C during the day and 22 ± 2°C at night, with 200 µmol m⁻² s⁻¹ of illumination. Plants were watered every 3 days with tap water and weekly with Hoagland nutrient solution (19).

Inoculation and disease assessment. Twenty-day-old *M. truncatula* seedlings (two to three leaves) were inoculated following a modified version of the dip technique described by Haglund (17). For this, roots were removed from vermiculite, cleaned, and dipped for 30 min in a suspension containing microconidia at 5 × 10⁶/ml of water. Control plants were immersed in sterile water. Seedlings were planted in individual 50-ml tubes containing autoclaved vermiculite and maintained in above mentioned growth chamber.

The screening of the entire collection was performed once on five seedlings per *M. truncatula* accession using the 605 isolate. The resistance reaction of 18 of the 26 highly resistant accessions of *M. truncatula* was validated and contrasted with the reaction of two representative partially resistant accessions and three susceptible accessions in two independent experiments (Table 1). For these validation experiments, inoculations were performed with the same *F. oxysporum* f. sp. *medicaginis* strain (strain 605) on five seedlings per accession and experiment. Preliminary statistical tests (one-way analysis of variance [ANOVA]) did not detect significant differences between these repetitions; thus, the results obtained were combined before further analysis.

To evaluate the stability of the resistance, a representative subset of the *M. truncatula* collection was challenged with strain 179.29 in two independent experiments, each using 10 seedlings per accession and experiment. This subset comprised 12 *M. truncatula* accessions: 4 resistant (W6 5976, PI 577616, PI 577618, and PI 577629), 5 partially resistant (PI 197358, PI 283662, PI 577619,

W6 5964, and W6 5978), and 3 susceptible (PI 239879, PI 516927, and PI 577607).

For all experiments, *F. oxysporum* f. sp. *medicaginis* symptoms were rated every 3 days from 8 to 35 days post inoculation (dpi) on a scale from 1 to 10, corresponding to the following phenotypes: 1 = healthy plant (no symptoms), 2 = first leaf chlorosis, 3 = <25% of the leaves showing symptoms (both leaf chlorosis and necrosis), 4 = <50% of the leaves with symptoms, 5 = <75% of the leaves showing symptoms, 6 = between 75 and 100% of the leaves with symptoms, 7 = 100% of the leaves with symptoms, 8 = 100% of the leaves with necrosis, 9 = initiation of stem and petiole drying and collapsing, 10 = dead plants. These data were used to calculate the area under the disease progression curve (AUDPC) for each plant, as described previously (3).

Quantification of *F. oxysporum* f. sp. *medicaginis* in planta. In planta quantification of *F. oxysporum* strain 605 was performed by real-time quantitative polymerase chain reaction (qPCR). For this, 12 *M. truncatula* seedlings were grown and inoculated with strain 605 as described above. After 8 days, the shoots of nine seedlings were harvested, weighed, and frozen under liquid nitrogen before storing at -80°C. DNA was extracted from frozen tissue according to the cetyltrimethylammonium bromide protocol (30) and quantified in triplicate with a Nanodrop-1000 spectrophotometer (Thermo Scientific). All DNA samples were then adjusted to a concentration of 10 ng µl⁻¹. The amount of *F. oxysporum* f. sp. *medicaginis* DNA within each accession was then estimated by qPCR on a Step One Real-time PCR system (Applied Biosystems) following the method previously described (22) on three independent samples per accession, each sample consisting of the shoots of three plants pooled together. In parallel, the remaining three seedlings were maintained in the growth chamber and use to evaluate *F. oxysporum* f. sp. *medicaginis* disease development, as described above.

Statistical analysis. To analyze the significance of the differences in *F. oxysporum* f. sp. *medicaginis* disease rating and in the amount of *F. oxysporum* f. sp. *medicaginis* DNA between *M. trun-*

Table 1. Disease rating of a representative selection of *Medicago truncatula* accessions to *Fusarium oxysporum* f. sp. *medicaginis* isolate MIAE00930 (strain 605)^x

Accessions	Class ^y	Disease index			
		10 dpi	20 dpi	30 dpi	AUDPC ^z
W6 5976	R	1.17 ± 0.08 a	1.36 ± 0.17 a	2.16 ± 0.42 a	16.1 ± 5.29 a
PI 577600	R	1.02 ± 0.00 a	1.61 ± 0.24 a	2.06 ± 0.23 a	17.8 ± 4.64 a
PI 577629	R	1.03 ± 0.09 a	1.38 ± 0.20 a	2.56 ± 0.38 a	21.1 ± 4.46 a
PI 577640	R	1.48 ± 0.20 a	1.78 ± 0.41 a	1.93 ± 0.44 a	23.2 ± 10.30 a
PI 577618	R	1.22 ± 0.14 a	1.82 ± 0.17 a	3.02 ± 0.22 a	31.8 ± 3.23 a
PI 577646	R	1.25 ± 0.22 a	1.54 ± 0.23 a	3.38 ± 0.30	34.5 ± 5.30 a
PI 577616	R	1.31 ± 0.19 a	2.31 ± 0.30 a	3.23 ± 0.20	38.3 ± 6.03 a
PI 577630	R	1.19 ± 0.11 a	2.24 ± 0.24 a	3.60 ± 0.37	41.9 ± 5.77 a
PI 197358	R	1.34 ± 0.16 a	2.80 ± 0.36 a	3.51 ± 0.40	43.3 ± 7.50 a
PI 535552	R	1.57 ± 0.24 a	2.03 ± 0.34 a	4.00 ± 0.32	46.9 ± 7.51 a
PI 577645	R	1.94 ± 0.30 a	2.22 ± 0.32 a	3.58 ± 0.33	48.1 ± 8.21 a
PI 197360	R	1.74 ± 0.20 a	3.11 ± 0.22	3.43 ± 0.22	50.0 ± 5.34
PI 442892	R	2.35 ± 0.31	2.74 ± 0.38 b	3.54 ± 0.37	54.6 ± 10.53
PI 535554	R	2.43 ± 0.47	2.74 ± 0.40 a	3.54 ± 0.32	56.5 ± 10.26
PI 577619	R	2.26 ± 0.43	2.61 ± 0.43 a	3.88 ± 0.31	56.7 ± 9.97
PI 577641	R	2.34 ± 0.28	2.95 ± 0.35	3.83 ± 0.24	61.3 ± 8.50
PI 384636	R	2.36 ± 0.33	3.67 ± 0.55	3.95 ± 0.55	65.9 ± 14.57
PI 516923	R	2.88 ± 0.46	3.56 ± 0.41	4.46 ± 0.23	76.6 ± 9.79
PI 442895	P	2.75 ± 0.43	4.10 ± 0.33	4.24 ± 0.28	84.5 ± 8.16
PI 283661	P	3.40 ± 0.35	3.70 ± 0.29	4.74 ± 0.40	86.0 ± 8.07
PI 239878	S	5.03 ± 0.47 b	7.62 ± 0.46 b	8.17 ± 0.38 b	181.3 ± 13.27 b
PI 577607	S	6.37 ± 0.29	8.95 ± 0.32	9.49 ± 0.24	216.6 ± 7.37
PI 516927	S	6.58 ± 0.50	8.46 ± 0.37 b	9.44 ± 0.31	221.0 ± 11.99

^x Accessions were chosen following the screening of the whole collection against this *F. oxysporum* f. sp. *medicaginis* isolate in order to validate the resistance phenotype of most of the resistant accessions originally detected (Supplementary Table S1). Data are means ± standard error of 15 replicates; dpi = days post inoculation. Letter indicates that the accession is not significantly different from the (a) resistant PI 577600 or (b) susceptible PI 239878 reference accessions as determined by analysis of variance with contrast analysis at *P* = 0.05.

^y Previous classification of accessions according to the result of the screening of the entire collection. R = resistant, P = partial resistance, and S = susceptible.

^z Area under the disease progression curve.

catula accessions, all data were subjected to a one-way ANOVA. Whenever this test was statistically significant ($P \leq 0.05$), contrast analysis (Scheffe) assessing the differences of the means between each accession with the resistant accession PI 577600 or the susceptible PI 239878 were performed to separate accessions according to their disease ratings. A Scheffe mean comparison test was also performed to separate accessions according to the amount of *F. oxysporum* f. sp. *medicaginis* DNA they contained.

Results

On highly susceptible accessions such as PI 239878 and PI 577607, initial symptoms appeared on the first leaf around 7 dpi and sequentially reached the later-formed leaves until the whole plant withered and died. This sequential appearance of disease symptoms allowed us to evaluate the plants according to the 1-to-10 disease scale described above (Fig. 1A). Three types of disease progressions were observed (Fig. 1B). Susceptible accessions, including PI 577607 and PI 239878, showed symptoms evolving rapidly, with disease ratings higher than 5 at 10 dpi and almost dead at 20 dpi (Fig. 1B). Resistant accessions such as PI 577600 and P I577618 showed almost no symptoms over the evaluation period, with disease ratings less than 4 at 30 dpi. Intermediate reactions, including the accessions W6 5978, W6 5964, and A17, showed intermediate symptom development, with disease ratings comprised between 4 and 8 at 30 dpi (Fig. 1B). Classification of accessions according to their AUDPC values showed a wide variety of responses from resistant to susceptible following a Gaussian distribution of the accessions indicative of the presence of quantita-

tive mechanisms (Fig. 1C). Comparison of the AUDPC values obtained for each accession allowed us to separate three broad categories of disease responses and set the limits of these groups (Fig. 1C). Contrast analysis identified 26 accessions that were not significantly different from the resistant accession PI 577600 (Supplementary Table 1). Among them, eight accessions (3%), including the resistant reference PI 577600, developed almost no symptoms and were characterized by AUDPC values lower than 25 and disease indexes <1.3 at 10 dpi, <2 at 20 dpi, and <3.5 at 30 dpi. These accessions were considered to be highly resistant (Fig. 1C) while the other 18 accessions (6.7%), showing AUDPC values between 25 and 50 and disease indexes <2 at 10 dpi, <4 at 20 dpi, and <6 at 30 dpi were considered to be resistant. The contrast analysis also identified nine accessions (3.4%) that were not significantly different from the susceptible accession PI 239878. These accessions were characterized by AUDPC values higher than 175 and disease indexes >4 at 10 dpi, >7 at 20 dpi, and >7.9 at 30 dpi and were considered to be susceptible. Finally, 232 accessions (86.9%) showed AUDPC values between 50 and 150 and were considered to be partially resistant/susceptible (Fig. 1C).

Grouping of *M. truncatula* accessions as wild or breeding material showed moderate levels of resistance within both categories. Wild accessions were slightly more susceptible, with higher mean AUDPC values (AUDPC = 104.5) than cultivated or breeding accessions (AUDPC = 92.7) (Fig. 2A). Because the collection contains accessions of *M. truncatula* from many different countries, wild accessions were classified according to their geographic origins to test whether resistance was focused on a specific geo-

Fig. 1. Assessment of *Fusarium oxysporum* f. sp. *medicaginis* disease symptoms in the *Medicago truncatula* collection. **A**, Photographs represent the evolution of disease symptoms induced by *F. oxysporum* f. sp. *medicaginis* isolate MIAE00930 (strain 605) on susceptible *M. truncatula* accessions. Numbers within each photograph indicate its respective disease rating value based on a disease index scale ranging from 1 (healthy plant) to 10 (dead plant). **B**, Evolution of disease symptoms of different *M. truncatula* accessions differing in their susceptibility to strain 605. **C**, Distribution of *M. truncatula* accessions upon inoculation with strain 605 according to area under the disease progression curve (AUDPC) values calculated from the evaluation of disease ratings.

graphic location or whether it occurred independently worldwide. With the exception of accessions from the Middle East (Turkey and Lebanon) that tended to be more susceptible to strain 605, with mean AUDPC values of 146.5 and 235, respectively, accessions from all other countries presented moderate levels of resistance, with mean AUDPC values ranging from 88 for Tunisia to 118.6 for Morocco (Fig. 2B).

The resistant phenotype of 18 accessions was contrasted with the phenotype of two partially resistant and three susceptible accessions in two independent evaluations of their disease response to strain 605 (Table 1). This assessment confirmed the highly resistant phenotype to this strain of *F. oxysporum* f. sp. *medicaginis* for 12 of the 18 resistant accessions. These 12 accessions had AUDPC values lower than 50, and disease ratings were not statistically different from disease ratings on PI 577600, the resistant reference ($P \leq 0.05$; Table 1). The other seven accessions showed varying level of partial resistance, with a disease index <4 at 20 dpi and <5.5 at 30 dpi and an AUDPC values higher than 50, which was not significantly distinct from the AUDPC values observed for the partial resistant accessions PI 442895 and PI 283661 ($P > 0.05$). In turn, the AUDPC values of these partially resistant accessions were largely and significantly lower than the 181.31 AUDPC value of the susceptible reference PI 239878 ($P < 0.05$; Table 1).

To determine whether observed resistance was due to resistance mechanisms per se or to tolerance mechanisms, the amount of

strain 605 was quantified at 8 dpi within shoots of seven *M. truncatula* accessions, including two resistant (PI 577618 and W6 5976), two partially resistant (PI 283661 and A17), and three susceptible (PI 239878, PI 516927, and PI 577607). At this early stage, we could detect low amounts of *F. oxysporum* f. sp. *medicaginis* DNA in all samples. The comparison of the amount of strain 605 in these accessions indicated that susceptible accessions accumulated more *F. oxysporum* than the resistant ones (Fig. 3A). The relative amount of *F. oxysporum* DNA in the DNA samples of the susceptible accessions ranged from 0.32% in PI 516927 to 0.41% in PI 239878. In contrast, the relative amount of *F. oxysporum* DNA was 0.04 and 0.1% in the resistant accessions W6 5976 and PI 577618, respectively. Partially resistant accessions accumulated an intermediate quantity of *F. oxysporum* DNA of 0.14 to 0.18% (Fig. 3A). Significant differences were observed between each group by one-way ANOVA ($P < 0.001$). Interestingly, a positive correlation was found when plotting the relative amount of strain 605 DNA per sample DNA according to AUDPC value of each accession, as estimated by the linear regression curve ($r^2 = 0.77$; Fig. 3B).

Fig. 2. Assessment of disease response to *Fusarium oxysporum* f. sp. *medicaginis* of the *Medicago truncatula* collection after classification of accessions according to **A**, type of material and **B**, geographical origin. Histograms represent the mean area under the disease progression curve (AUDPC) value for each group calculated from the evaluation of disease ratings. Vertical bars are standard errors. Numbers indicate the number of *M. truncatula* accessions.

Fig. 3. In planta quantification of *Fusarium oxysporum* f. sp. *medicaginis* within plant tissue. **A**, The histogram represents the proportion of *F. oxysporum* f. sp. *medicaginis* DNA within plant DNA sample extracted from the shoot of different *Medicago truncatula* accessions 8 days after inoculation with strain 605 quantified by quantitative polymerase chain reaction assay (26). **B**, The graph represents the distribution of the amount of *F. oxysporum* f. sp. *medicaginis* DNA within plant DNA sample according to the area under the disease progression curve (AUDPC) value of the sample. Positive correlation between both parameters was detected by drawing a linear regression curve ($r^2 = 0.77$). Horizontal and vertical lines are the standard errors.

To test whether the resistance response was maintained against other *F. oxysporum* f. sp. *medicaginis* isolates, we assessed disease development on a subset of 12 selected accessions following inoculation by isolates 605 and 179.29 (Fig. 4). Strain 179.29 was less aggressive than strain 605. Thus, disease severity, estimated by the AUDPC values, only reached 64.2 for the most susceptible accession, PI 577607, with strain 179.29, which was much lower than the AUDPC of 216.6 obtained for this accession when inoculated with strain 605 (Fig. 4). Nevertheless, plant response to both isolates followed a similar trend; the most resistant accessions to strain 605 were also the most resistant to strain 179.29, and the most susceptible accessions to strain 605 were most susceptible to strain 179.29. Only one accession, W6 5964, had a differential response between isolates showing partial resistance to strain 605 but a high resistance response to strain 179.29.

Discussion

Resistance against *F. oxysporum* f. sp. *medicaginis* was assessed in a large *M. truncatula* collection and revealed a wide range of disease responses from completely resistant to highly susceptible, with most *M. truncatula* accessions showing a moderate to low level of resistance to the pathogen. In addition, we identified 12 completely resistant accessions that may be highly valuable for breeding programs and useful in improving our understanding of the resistance mechanisms leading to *F. oxysporum* resistance in legumes.

F. oxysporum f. sp. *medicaginis* have been described to be the causal agent of both vascular wilt and root and crown rot in alfalfa (11,24). Fusarium wilt disease causes a series of external symptoms, including vein clearing, leaf epinasty, wilting, chlorosis, necrosis, and abscission leading to the complete plant wilting and death (28). By contrast, root and crown rot is characterized by cortical necrosis, stellar necrosis, and vascular browning of the root (10). As expected from previous studies (2,34), inoculation with two *F. oxysporum* f. sp. *medicaginis* isolates induced a combination of the typical Fusarium wilt symptoms in the susceptible and partially resistant accessions of *M. truncatula*, which indicates that the *F. oxysporum* f. sp. *medicaginis* isolates used here cause Fusarium wilt and not root rot (Fig. 1A).

The detection of clear Fusarium wilt symptoms allowed the development of our methodology based on plant symptoms to evaluate the *M. truncatula* collection. Most previous studies on virulence of *F. oxysporum* f. sp. *medicaginis* were performed on alfalfa (2,11,13). In these studies, disease rating was performed on adult plants according to the degree of tap root internal discoloration (11). This method requires the transversal section of the tap root, which is very difficult to perform on plants with thin root systems such as *M. truncatula* seedlings. Thus, evaluation of the leaf symptoms are preferred (34). In a previous study, Ramirez-Suero et al. (34) monitored the susceptibility to *F. oxysporum* f. sp. *medicaginis* of eight lines of *M. truncatula* in a miniature hydroponic culture system. The disease symptoms of inoculated *M. truncatula* plants were scored using a visual scale of 0 to 5 according to appearance of chlorosis and necrosis on cotyledons and first leaves, which allowed identification of one line (F83005.5) with moderate resistance to *F. oxysporum* f. sp. *medicaginis* (34). Although this method was successful in establishing susceptibility on very young plantlets, it was very difficult to apply to older seedlings. Indeed, past the seedling establishment period, *M. truncatula* showed very profuse growth, whereas Fusarium wilt symptoms are a more progressive event. Thus, a single inoculated plant often shows a mixture of phenotypes, including healthy, yellowing, chlorotic, necrotic, and dead leaves. In addition, cotyledon necrosis is often observed on older healthy plants, which impedes the use of this scale on older plants (*data not shown*). We adapted a new scale with a higher number of categories (from 1 to 10) to account for the presence of symptoms and the extent of diseased leaves, and also the later stages of disease progression, including the stem external discoloration and withering that precede plant death (Fig. 1A).

The screening for *F. oxysporum* f. sp. *medicaginis* resistance of the *M. truncatula* collection with this modified scale revealed that it contained sufficient genetic variation to detect a wide range of responses to *F. oxysporum* f. sp. *medicaginis*, from complete resistance to high susceptibility (Table 1). Interestingly, we established a correlation between the amount of *F. oxysporum* f. sp. *medicaginis* within plant tissue and the overall level of resistance or susceptibility of the accession (Fig. 3). Similar correlations between the extent of colonization and resistance level have been previously described in several species, including tomato, watermelon, and chickpea (15,21,41). This suggests that resistance phenotypes for *F. oxysporum* are generally due to the presence of mechanisms that reduce or limit colonization of the pathogen (41). Results presented here on *M. truncatula* support this hypothesis.

On the other hand, the *M. truncatula* collection is composed of wild and cultivated *M. truncatula* accessions collected throughout the world, although most wild accessions came from countries of the Mediterranean area, including Algeria, Morocco, and Tunisia. Resistance in wild accessions has been found from most locations, including Morocco, Tunisia, Greece, and Italy (Fig. 2B). Resistant and susceptible accessions were often found in nearby areas. This would indicate that resistance to *F. oxysporum* f. sp. *medicaginis* did not originate from a precise world region, which is confirmed by the analysis of accessions' responses to *F. oxysporum* f. sp. *medicaginis* according to their geographic origin (Fig. 2B). This was similar to the situation found by McPhee et al. (29) in pea against two different races of *F. oxysporum* f. sp. *pisi*.

Resistance to legume-infecting *F. oxysporum* has been considered qualitative, with either a monogenic inheritance, including resistance to races 1, 5, and 6 of *F. oxysporum* f. sp. *pisi* and races 3 and 5 of *F. oxysporum* f. sp. *ciceri*; or oligogenic, including resistance to races 1A, 2, and 4 of *F. oxysporum* f. sp. *ciceri* conferred by two to three genes (20). Previous studies on the tetraploid alfalfa indicated that resistance to *F. oxysporum* f. sp. *medicaginis* in this plant species is also qualitative and controlled by at least two genes, one completely dominant (FW_1) and one with an additive effect (FW_2) (18). In the present study, we detected a continuity of responses from highly resistant to highly susceptible, with most accessions presenting moderate to low levels of resistance to *F. oxysporum* f. sp. *medicaginis*. Such continuity would support the existence of quantitative resistance mechanisms in the *M. truncatula* collection. This is similar to recent observations in a pea collection in response to one isolate of *F. oxysporum* f. sp. *pisi* race

Fig. 4. Comparison of the response of 12 selected accessions to two isolates of *Fusarium oxysporum* f. sp. *medicaginis*. Solid and open bars indicate plants inoculated with strains 605 and 179.29, respectively. Data are means area under the disease progression curve (AUDPC) values calculated from the evaluation of disease ratings of 10 replications. Vertical lines are the standard errors.

2 (3). However, genetic analysis of the *M. truncatula* accessions would be needed to confirm this hypothesis.

In the present study, two different isolates of *F. oxysporum* f. sp. *medicaginis* were used to confirm the resistance phenotypes of selected accessions. Both isolates were pathogenic on susceptible accessions such as PI 577607 and PI 516927, although the second isolate (strain 179.29) was less aggressive than strain 605 (Fig. 4). Variation in aggressiveness between *F. oxysporum* f. sp. *medicaginis* isolates was observed when comparing isolates of different geographic origin (11,24). Despite the aggressiveness difference of these strains, they induced a similar pattern of infection when inoculating the same accessions (13,24,34; Fig. 4). The similar plant responses to different *F. oxysporum* f. sp. *medicaginis* isolates could indicate a lack of physiological specialization of *F. oxysporum* f. sp. *medicaginis*, although molecular characterization of these isolates and additional testing of *F. oxysporum* f. sp. *medicaginis* isolates would be needed to ascertain this hypothesis.

This screening of a large collection of *M. truncatula* for resistance to *F. oxysporum* f. sp. *medicaginis* identified a variety of responses that will be aid in characterizing defense mechanisms that participate in *F. oxysporum* resistance at the cellular and molecular level. In particular, the identification of 12 resistant accessions will be a valuable tool to confer resistance to *F. oxysporum* in legume crops.

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