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Differential response of *Cucumis melo* to *Fusarium oxysporum* f. sp. *melonis* race 1.2 isolates

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ABSTRACT

Fusarium wilt incited by *Fusarium oxysporum* f. sp. *melonis* (*Fom*) causes severe losses in melon crops. Four physiological races of *Fom* have been identified: 0, 1, 2 and 1.2. In most cases, resistance to race 1.2 has been described as recessive, polygenic and not race specific. However, some evidence of race-specific effects within melon resistance to race 1.2 has been reported. In this work, we study these effects and assess whether they are due to race-specific resistance. Seeds were obtained from 14 melon accessions that exhibit some level of resistance to race 1.2, and from the lines 'Charentais-Fom1' (resistant to races 0 and 2), 'Charentais-Fom-2' (resistant to races 0 and 1), and 'Dinero F_1' (with partial resistance to *Fom* race 1.2). Melon seedlings were artificially inoculated using two different procedures: 'continuous shaking' and 'tray immersion'. Symptom severity was assessed on leaves using a rating scale from 0 (no symptoms) to 4 (death of the plant). Symptoms were recorded weekly over the four-week period following the first appearance of symptoms and the area under the disease progress curve (AUDPC) was calculated. Six *Fom* isolates (3 from pathotype Y and 3 from pathotype W) were used in the inoculation. The less aggressive 'tray immersion' procedure seems to be more appropriate for detecting the typically small resistance factors of this type of polygenic partial resistance. 'Kogane Nashi Makuwa', 'BG-5384', 'Shiro Uri Okayama', 'C-211' and the control, 'Dinero F_1', showed a high level of resistance to all *Fom* isolates. However, some genotype × isolate effects were also detected. 'Baza', when inoculated with isolate *Fom* 9302, and 'Korça, when inoculated with *Fom 37mls.1.2W*, showed resistance levels similar to that of 'Dinero F_1'; this effect was not observed when 'Baza' and 'Korça' were inoculated with other isolates. These results are characteristic of race-specific resistance and offer evidence for the presence of this type of resistance to *Fom* race 1.2 in melons.

1. Introduction

Melon Fusarium wilt, induced by the soilborne fungus *Fusarium oxysporum* f. sp. *melonis* (L&C) Synd. & Hansen (*Fom*), is one of the major biological threats to melon crops around the world (Leary and Wilbur, 1976; Louvet, 1986; Sherf and MacNab, 1986; Gonzalez Torres et al., 1994) and is one of the most difficult diseases to control. Once the soil is infested, the pathogen can persist for very long periods by colonising non-susceptible hosts and by producing durable chlamydospores (Schippers and van Eck, 1981). Four physiological races (0, 1, 2 and 1.2) of *Fom* have been identified based on their reaction to a set of differential genotypes (Risser et al., 1976). Resistance to races 1 and 2 is conferred by the single dominant genes *Fom-2* and *Fom-1*, respectively. Both genes also confer resistance to race 0 (Risser et al., 1976). Recently, Oumouloud et al. (2010) described a recessive resistance to races 0 and 2, controlled by *fom-4* in melon cv. Tortuga.

Partial resistance to race 1.2 was shown to be polygenic and not race-specific (Perchepied et al., 2005; Chikh-Rouhou et al., 2011), while in the breeding line BIZ, two recessive genes were shown to confer full resistance (Herman and Perl-Treves, 2007). However, Chikh-Rouhou et al. (2007) found some evidence for race-specific effects on melon resistance to *Fom* race 1.2, and Sestili et al. (2011) reported differential virulence of two 1.2 strains. Perchepied and Pitrat (2004) reported small race-specific effects on quantitative and polygenic resistance. Moreover, interactions between polygenically controlled traits and the environment are well known. Perchepied and Pitrat (2004) noted that the expression of quantitative trait loci (QTL) involved in the resistance to race 1.2 may depend on factors such as the length of time following infection or the aggressiveness of the race 1.2 isolate.

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This work examines the reaction of 14 melon accessions that have been previously reported as having some level of resistance to race 1.2 (Chikh-Rouhou et al., 2010) when inoculated with 6 different race 1.2 isolates. Following inoculation by one of two different procedures, melon accessions were assessed to determine if a differential response to race 1.2 may be due to race-specific effects, and if the inoculation procedure affected the expression of resistance.

2. Materials and methods

2.1. Plant material

The plant material used in these experiments included 14 melon (*Cucumis melo* L.) accessions that have shown some level of resistance to *Fom* race 1.2 in previous experiments (Chikh-Rouhou et al., 2010), together with the differential genotypes: 'Charentais-Fom-1', which is resistant to races 0 and 2; 'Charentais-Fom-2', which is resistant to races 0 and 1; and the hybrid F_1 'Dinero' (obt. Syngenta), which carries the genes *Fom-1* and *Fom-2* and is resistant to both pathotypes of race 1.2.

2.2. Fungal isolates

F. oxysporum f. sp. *melonis* isolates *Fom 37mls*, *Fom 9302* and *Fom 0502*, belonging to the pathotype 1.2W, and *Fom 0501*, *Fom 0125*, and *Fom 24ml*, belonging to the pathotype 1.2Y, were used for inoculum preparation. All these isolates have been provided by Dr. González-Torres. They have been collected from melon crops of SE Spain (Almeria and Murcia provinces), and classified into the appropriate physiological race, according to their ability to infect a known set of differential melon genotypes and to induce yellowing or wilting symptoms (Risser et al., 1976).

Fungi were grown in potato dextrose broth on a rotary shaker for 10 days at room temperature. Conidia were harvested by filtration through an autoclaved nylon mesh. Spore concentration was determined using a haemocytometer and adjusted to the appropriate density (3×10^6 conidia/mL) by diluting with sterile distilled water.

2.3. Artificial inoculations

Artificial inoculations were carried out using two different procedures: continuous shaking and tray immersion.

2.3.1. Continuous shaking

Ten-day-old melon seedlings, grown in trays containing sterilised sand, were removed from the substrate, washed with tap water and transferred to plastic pots containing 200 mL of 'Hoagland' nutrient solution (Gamborg and Wetter, 1975). Three mL of the conidial suspension were added to each pot. The pots were then placed on an orbital shaker in a growth chamber with 14 h of light per day. Light intensity was 1300 $\mu E/m^2$ s, and temperature was maintained at 26/20 °C (day/night).

2.3.2. Tray immersion

Trays containing ten-day-old melon seedlings grown in sand were dipped into 'Hoagland' nutrient solution containing a suspension of conidia (3×10^6 spores/mL). The trays were then placed in a growth chamber as described above. For both procedures, 10–15 plants were used for each accession–isolate combination.

The severity of symptoms was assessed weekly during the four weeks following the first appearance of symptoms. Symptoms were rated on a scale of 0–4 using the following criteria: (1) no symptoms, (2) beginning of yellowing or wilting on leaves, (3) leaves heavily affected, (4) stem standing and leaves completely wilted and (5) death of plant.

For the statistical analyses, the values of the area under the disease progress curve (AUDPC) were used. The AUDPC integrates both the intensity of symptoms and the time taken between inoculation and expression of symptoms. The AUDPC was calculated according to the formula proposed by Perchepied and Pitrat (2004): $\text{AUDPC} = \sum_i[(x_i + x_{i+1} - 2)/2](t_{i+1} - t_i)$, with i = scoring period 1–4, x_i = mean of the symptom scores for disease, and $t_{i+1} - t_i$ = the number of days between scoring date i and scoring date $i + 1$.

The AUDPCs were analysed using a three-factor analysis, with 10–15 plants/accession, 17 accessions, 6 *Fom* isolates, and 2 inoculation procedures. Mean values were separated using a Tukey b test ($P \leq 0.05$).

3. Results

In all the experiments, the AUDPC values for 'Charentais-Fom1' and 'Charentais-Fom2' were significantly higher than those for most of the tested accessions, while the AUDPC for 'Dinero' was clearly low. These results indicate that all the isolates belong to *Fom* race 1.2. Those classified as pathotype Y induced leaf yellowing, and those classified as pathotype W produced wilting of the plants without previous yellowing.

Mean AUDPC values were significantly higher when the 'continuous shaking' inoculation method was used compared with inoculation by 'tray immersion' (Table 1). In fact most of the plants inoculated using the 'continuous shaking' method died at the end of the experiment regardless of the values of the AUDPC. When the less aggressive inoculation method was utilised, the values of the AUDPC were lower and many plants from different accessions remained alive at the end of the experiment; thus, it was possible to detect differences in the level of resistance.

For both inoculation methods, the accession × isolate interaction was significant (Table 2). That is, the disease levels of some accessions differed significantly among isolates (Tables 3 and 4).

As previously reported, the 'continuous shaking' method proved to be very aggressive. The resistant control, 'Dinero', only behaved as fully resistant when inoculated with the isolate *Fom 0502* (race 1.2W); when inoculated with any other isolate, it showed values of the AUDPC greater than 0. Moreover, accessions that have been

Table 1
Mean values for the area under the disease progress curve (AUDPC) ± SD observed for different accessions of melon using two inoculation methods averaged across groups inoculated with different *Fom* isolates.

Accession	Continuous shaking	Tray immersion
Charentais-Fom1	47.92[a] ± 14.64	38.60 ± 13.04
Charentais-Fom2	44.13[a] ± 12.29	36.48 ± 11.43
Dinero	17.72[a] ± 6.49	4.59 ± 3.34
AOT[b]	32.86[a] ± 12.45	17.76 ± 7.54
C-211	21.25[a] ± 8.23	12.37 ± 15.76
Encin-4078	42.28 ± 13.46	28.48 ± 15.52
CA -311 1C	42.87[a] ± 12.73	23.91 ± 8.57
Baza	26.99 ± 11.23	19.98 ± 13.24
Shiro Uri Okayama	28.81[a] ± 12.66	7.12 ± 10.05
BG-5384	19.12[a] ± 12.11	12.34 ± 11.23
C-181	27.82[a] ± 12.23	20.03 ± 10.09
Kogane Nashi Makuwa	28.80[a] ± 11.45	11.85 ± 13.34
Korça	26.18[a] ± 13.12	20.69 ± 11.13
Mollerusa	31.07[a] ± 14.99	21.29 ± 15.35
Rajado	39.17[a] ± 14.76	24.84 ± 14.01
Mochuelo	34.56[a] ± 11.14	21.59 ± 15.83
NC-44082	31.91[a] ± 12.17	27.75 ± 12.78

[a] Differences between both inoculation methods are significant ($P \leq 0.05$) for each accession.
[b] AOT = Amarillo Oval Tardío.