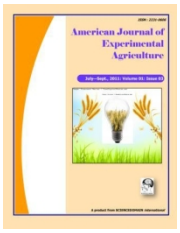

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Evaluation of *Cucumis ficifolius* A. Rich. Accessions for Resistance to Fusarium Wilt

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Short Communication

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ABSTRACT

Aims: Fusarium wilt of melon (*Cucumis melo* L.), which is caused by *Fusarium oxysporum* f. sp. *melonis* is regarded as a severe disease worldwide. Among the races (0, 1, 2 and 1,2), no gene conferringhigh resistance to race 1,2 has been identified in melon Wild *Cucumis* species have been reported to possess resistance to some melon diseases, and some of the methods of overcome to the reproductive barriers in genus *Cucumis* interspecific crosses were reported. We evaluated *C. ficifolius* for novel resistance to *F. oxysporum* f. sp. *melonis* and searched for novel sources of resistance.
Study Design: A total of 10–20 plants were evaluated for each accession. Results were expressed as mean values and standard errors. Disease severity was log-transformed and data were analyzed using least-squares analysis of variance (ANOVA). A post hoc Tukey-HSD test was performed to compare disease severity among the accessions.
Place and Duration of Study: Tokyo University of Agriculture and Technology, and Plant Biotechnology Institute, Ibaraki Agricultural Center between October 2008 and September 2011.
Methodology: Inoculation was conducted using a root dip method. The resistance to each race was evaluated 21 days after inoculation according to a 0–3 disease severity scale (0=no symptoms, 1=small lesions on leaves, 2=leaves strongly affected, 3=plant death).
Results: All inoculated accessions showed susceptibility both in race 0 and 1. In race 2 and 1,2, only the accession PI 273648 showed resistance to both. However, the other accessions showed susceptibility to race 2 and 1,2 and the symptoms were almost severe.
Conclusion: These results suggest that the accession PI 273648 would be a suitable genetic resource to breed the melon cultivar, which has resistance to race 2 and 1,2.

Keywords: Cucumis ficifolius; Cucumis spp.; Fusarium oxysporum f. sp. melonis; genetic resources; resistance.

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1. INTRODUCTION

Wild *Cucumis* species, which belong to the subgenus *Melo*, have been reported to possess resistance to some melon (*Cucumis melo* L.) diseases such as gummy stem blight (Igarashi et al., 1987), powdery mildew (Alvarez et al., 2005; Lebeda, 1984; Pan and More, 1996) and Fusarium wilt (Alvarezet al., 2005; Matsumoto et al., 2011; Pan and More, 1996; Trionfetti-Nisini et al., 2002). Among these diseases, Fusarium wilt of melon, which is caused by *Fusarium oxysporum* Schlechtend. Fr. f. sp. *melonis* (Leach & Currence) Snyder and Hans, is regarded as a severe disease worldwide (Elena and Pappas, 2006; Kurt et al., 2002; Namiki et al., 1998; Zuniga et al., 1997). Once the disease colonizes field, the pathogen survives in the soil for several month because crop residues and roots of most crops grown in rotation increase these persistent pathogenic populations (Banihashemi and Dezeeuw, 1975; Gordon et al., 1989; Zuniga et al., 1997). The causal agent of *F. oxysporum* f. sp. *melonis* is distinguished as four physiological races (0, 1, 2, and 1,2) based on the pathogenicity on a set of differential genotypes (Risser et al., 1976). Among these races, no gene conferringhigh resistance to race 1,2 has been identified in melon (Chikh-Rouhou et al., 2007; Herman and Perl-Treves, 2007; Oumouloud et al., 2009; Perchepied and Pitrat, 2004). Thus, if wild *Cucumis* species are to be used as resistant genetic resources, the evaluation of resistance to each race is necessary. Recently, some of the methods of overcome to the reproductive barriers in genus *Cucumis* interspecific crosses (Chen et al., 1997; Matsumoto et al., 2012; Skálová et al., 2008) and some interspecific hybrid was developed (Chen et al., 1997; Skálová et al., 2008).

To date, although the resistance of some wild *Cucumis* species to each race of *F. oxysporum* f. sp. *melonis* have been reported, the resistance of *C. ficifolius* A. Rich. has rarely been reported (Alvarez et al., 2005; Matsumoto et al., 2011; Pan and More, 1996; Trionfetti-Nisini et al., 2002). Because of genetic variation, *C. ficifolius* can easily be *distinguished from most* wild *Cucumis* species (Chung et al., 2006). Therefore, the evaluation of *C. ficifolius* for resistance would contribute to not only its use as a genetic resource, but also the study of host specificity of *F. oxysporum* f. sp. *melonis*. In this study, we evaluated *C. ficifolius* for resistance to *F. oxysporum* f. sp. *melonis* using each race and explored for novel sources of resistance.

2. MATERIALS AND METHODS

2.1 Fungal Strain and Plant Materials

The fungal strains of *F. oxysporum* f. sp. *melonis*used for inoculation were JCM9289, Mel02221, JCM9288, and Fom142-S1. These strains were classified as race 0, 1, 2, and 1,2, respectively (Matsumoto et al., 2011; Namiki et al., 1998, 2000). Six accessions of *C. ficifolius*, PI 273192, PI 273648, PI 299570, PI 299572, PI 504554 and PI 504558, were obtained from the National Germplasm Resources Laboratory (USDA, Agricultural Research Serviceand Beltsville, Maryland, USA). Three different melon cultivars, ‘Charentais T,’ ‘Doublon’ and ‘CM 17187’ were used for race confirmation of the fungal strains. These cultivars were obtained from the Institut National de la Recherche Agronomique, France. The seeds were sown into sterilized garden soil in plastic trays and grown in a growth chamber at 26ºC–30ºC, and the seedlings with a fully expanded first true leaf were used.

2.2 Artificial Inoculation

Inoculation was conducted using a root dip method (Matsumoto et al., 2011). Each fungal strain was cultured in 100 mL potato dextrose broth (PDB) in 300 mL flasks on a rotary shaker (ca. 120 rpm) for one week at 25ºC. After cultivation, each fungal strain was passed through two-ply gauze. The spore concentration was determined using a haemocytometer and then adjusted to the appropriate density by dilution with sterile distilled water. For artificial inoculation, the seedlings were removed from the sterilized soil and their roots were washed in tap water and then dipped in a conidial suspension (10^7 spores/mL) for 15 s. Inoculated seedlings were transplanted into sterilized garden soil in new plastic pots and cultivated in a growth chamber at 23ºC (16 h photoperiod).

2.3 Evaluation of Disease Index

The resistance to each race was evaluated 21 days after inoculation according to a 0–3 disease severity scale (0=no symptoms, 1=small lesions on leaves, 2=leaves strongly affected, 3=plant death) according to Matsumoto et al. (2011). A total of 10–20 plants were evaluated for each accession or cultivar. Results were expressed as mean values and standard errors. Disease severity was log-transformed and data were analyzed using least-squares analysis of variance (ANOVA). A post hoc Tukey-HSD test was performed to compare disease severity among the accessions. For these analyses, JMP statistical software (ver. 9.0.0; SAS Institute Inc., Cary, NC, USA) was used. When no plant from one accession showed any symptoms (Mean disease severity scale=0), the accession was classified as high resistance.

3. RESULTS AND DISCUSSION

By using JCM9289 as a fungal strain, the inoculated seedlings of ‘Charentais T’ died, and ‘Doublon’ and ‘CM 17187’ were asymptomatic. By using Mel02221 as a fungal strain, the inoculated seedlings of ‘Charentais T’ and ‘Doublon’ died, and ‘CM 17187’ were asymptomatic. By using JCM9288 as a fungal strain, the inoculated seedlings of ‘Charentais T’ and ‘CM17187’ died and ‘Doublon’ were asymptomatic. By using Fom142-S1 as a fungal strain, inoculated seedlings of all three lines, ‘Charentais T,’ ‘Doublon’ and ‘CM 17187’ died.

According to previous reports, ‘Charentais T’ is susceptible to all races, ‘Doublon’ has high resistance to races 0 and 2, and ‘CM 17187’ has high resistance to races 0 and 1 (Risser et al., 1976). Therefore, the fungal strains JCM9289, Mel02221, JCM9288 and Fom142-S1 were confirmed as race 0, 1, 2 and 1,2 from the pathogenicity of the inoculated plants, respectively.

The disease severity of each *C. ficifolius* accession exposed to all races was evaluated. In the inoculations, the significant responses were detected in the races 1, 2, and 1,2, by ANOVA {($F_{5,70}$=9.9777, P<0.01), ($F_{4,45}$=89851.0, P<0.01), and ($F_{5,72}$=17.2249, P<0.01), respectively}. In race 1, two accessions, PI 273648 and PI 504554, showed severe disease severity and the other accessions showed some disease severity (0.5–1.5) (Fig. 1). In race 2 and 1,2 a high resistance accession was observed; PI 273648 showed high resistance to both race 2 and race 1,2 (Fig. 2, 3). However, the other accessions showed symptoms against race 2 and 1,2 and the observed symptoms were almost severe. The disease severity of any other accession was 2.9–3 in race 2 and 2.1–2.6 in race 1,2, respectively (Fig. 2, 3). On the other hand, no significant responses were detected in the race 0 ($F_{4,45}$=1.530,

P=0.2096). All accessions showed severe symptoms, and the mean disease severity was 2.7–3 (Fig. 4).

Little information was available related to the resistance of *C*. *ficifolius* to *Fusarium* wilt. In our previous report, one accession was inoculated to race 1,2 and it exhibited some symptoms (Trionfetti-Nisini et al., 2002). In this study, most of the accessions showed symptoms to all races. Only one accession, PI 273648, showed high resistance to race 2 and race 1,2 (Fig. 2, 3).

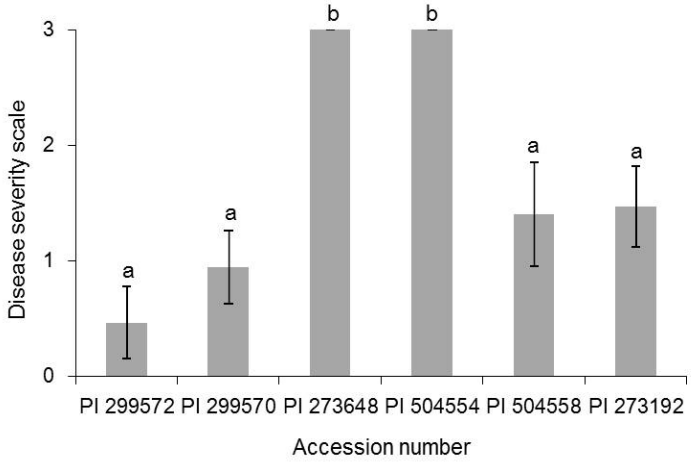

Fig. 1. Response of *Cucumis ficifolius* to *Fusarium oxysporum* f. sp. *melonis* race 1
Same letter indicate a non-significant difference with P < 0.01 by t-test. Error bar = S.E.

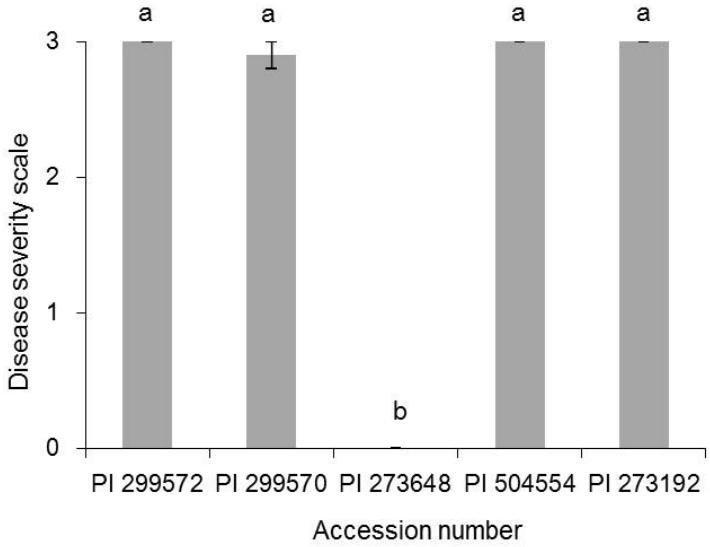

Fig. 2. Response of *Cucumis ficifolius* to *Fusarium oxysporum* f. sp. *melonis* race 2
Same letter indicate a non-significant difference with P < 0.01 by t-test. Error bar = S.E.

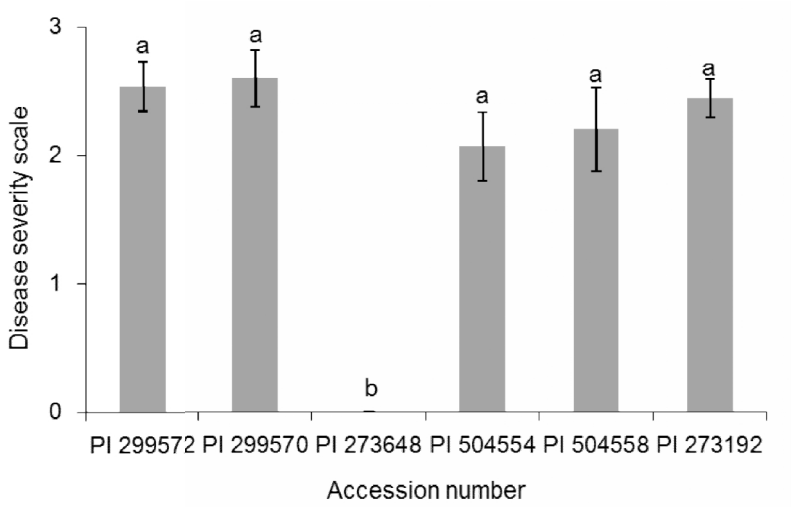

Fig. 3. Response of *Cucumis ficifolius* to *Fusarium oxysporum* f. sp. *melonis* race 1,2
Same letter indicate a non-significant difference with P < 0.01 by t-test. Error bar = S.E.

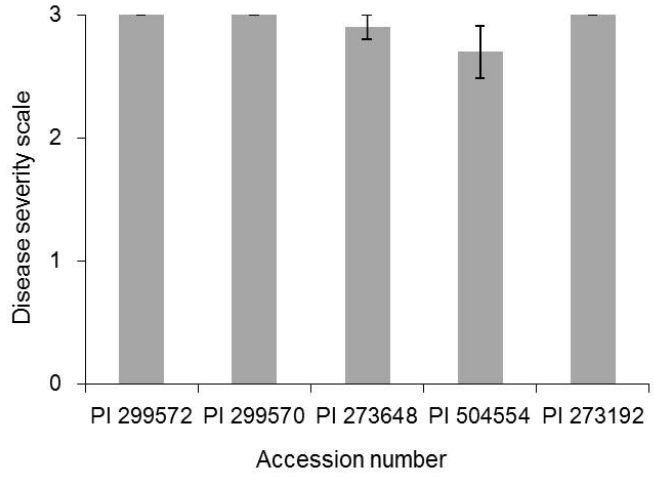

Fig. 4.Response of *Cucumis ficifolius* to *Fusarium oxysporum* f. sp. *melonis* race 0
Non-significant difference was detected between accessions by ANOVA. Error bar = S.E.

To date, the high resistant genes conferring resistance to race 0, 1 and 2 were reported. However, no gene conferringhigh resistance to race 1,2 has been identified in melon (Chikh-Rouhou et al., 2007; Herman and Perl-Treves, 2007; Oumouloud et al., 2009; Perchepied and Pitrat, 2004). Therefore, the line PI 273648 which showed high resistance to race 1,2 might be useful as genetic resources to breed a melon high resistance cultivar to race 1,2. To introduce the resistance of PI 273648 into melon cultivar, overcoming to the reproductive barrier in the interspecific crosses between melon and *C. ficifolius* is necessary, because the reproductive barriers such as pollen-pistil incongruity and hybrid seed abortion has been restricted the interspecific hybrids of gens *Cucumis* (Deakin et al., 1971; Fassuliotis, 1977; Kho et al., 1980; Kishi and Fujishita, 1970; Singh and Yadava, 1984). Recently, the method to overcome pollen-pistil incongruity in the interspecific crosses between wild *Cucumis* species and melon was reported (Matsumoto et al., 2012). Furthermore, some interspecific

hybrids of genus *Cucumis* were developed by overcome to the hybrid seed abortion through embryo culture (Chen et al., 1997; Skálová et al., 2008). In future, introduction of the resistance of *C*. *ficifolius* to melon by developing the interspecific hybrids by such methods would be expected.

4. CONCLUSION

In this study, one accession of *C*. *ficifolius*, PI 273648, showed high resistance to *F*. *oxysporum* f. sp. *melonis* race 2 and 1,2. This accession would be available as a genetic resource to breed melon cultivars which have resistance to race 2 and 1,2.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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