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Screening a variable germplasm collection of *Cucumis melo* L. for seedling resistance to *Macrophomina phaseolina*

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Abstract

We evaluate the seedling resistance to charcoal rot caused by *Macrophomina phaseolina* in ninety-seven *Cucumis melo* accessions, from different geographical origins and five F1 generations, derived from crosses of five accessions selected for their resistance. Artificial inoculations with the toothpick method, previously reported to be useful for predicting shoot resistance, were performed, and plants were scored using a scale of disease severity. The average disease severity was calculated for each accession and was used to cluster the accession in five reaction classes. The screening revealed that sources of natural resistance to this fungus are limited. However, seedlings of seven accessions of different botanic groups displayed a resistant response to the stem inoculation, one cantaloup from Israel, one *conomon* accession from Korea, two wild *agrestis* and one *acidulus* from Africa, and two *dudaim* accessions from Middle East. The response of the F1 progenies varied from susceptibility to high resistance, the latter in progenies from the two *agrestis* wild types. These results suggest differences in the genetic basis of the resistance in the different selected sources. The resistant accessions are suggested to be screened under field conditions to confirm the level of resistance at adult plant stage and under stressful conditions.

Keywords melon; charcoal rot; soilborne fungus; germplasm; resistance.

Introduction

Melon (*Cucumis melo* L.) is an important cucurbit of growing importance in international markets. Nearly 31 million tons of melons were produced worldwide in 2012 being China, Turkey and Iran the major producers (Food and Agriculture Organization 2014).

Due to continuous cropping, soilborne pathogens are an increasing problem, resulting in reduced yields and fruit quality. Among them, *Macrophomina phaseolina* (Tassi) Goidanich is one of the most serious and potentially damaging fungus worldwide. It is a destructive pathogen that causes charcoal rot (Salari et al. 2012). It has a broad host range and is capable of attacking and infecting more than 500 cultivated and wild plant species throughout the world (Khan 2007; Radwan et al. 2014). The fungus has been reported worldwide, but it is economically more important in subtropical and tropical countries with

a semi-arid climate (Wrather et al. 2001; Purkayastha et al. 2006).This pathogen has often been detected causing outbreaks in warm and hot areas under dry weather conditions.

In Brazil, this pathogen is consistently isolated from roots and stems of melons (Andrade et al. 2005; Dantas et al. 2013) and associated weeds (Sales Jr et al. 2012). In the last few years *M. phaseolina* has been also one of the main fungus isolated from roots of collapsed watermelon and melon plants in several regions of America, Europe and Asia, such as Texas and California (Bruton et al. 1987; Aegerter et al. 2000), Honduras and Chile (Bruton and Miller 1997; Jacob et al. 2013), Spain (García-Jiménez et al. 1993), Israel (Cohen et al. 2012) and Iran (Salari et al. 2012), being the leading cause of the drastic reduction in cucurbits cultivation in some of these countries (Krikun et al. 1982).

Macrophomina phaseolina is seed-borne and seed-to-seedling transmission has been documented (Kaur et al. 2012). Generally this pathogen can cause a range of symptoms, such as seedling blight, pre and post-emergence damping-off, bleaching of stems, gum exuding from stems as the bleached areas turn drier, stem and root rot, leaf blight and death near the crown of the plant and wilting of the plant. In fields, the pathogen commonly infects melon stems soon after planting, but the extended lesions that result in the wilting of the plant occur late in the growing season and are especially severe under high temperature and drought conditions (Watson and Napier 2009). *M. phaseolina* is considered to be difficult to control due to its heterogeneous host specificity and to the specialized resistance structures that can survive for more than 10 months under dry soil conditions. The severity of the disease is directly related to the population of viable *sclerotia* in the soil (Khan 2007).

The chemical control of *M. phaseolina* in intensive horticultural systems, similarly to other soilborne pathogens, was based for years on the use of methyl bromide (Noling and Becker, 1994). The restriction on the use of this fumigant increased the risks for soilborne pathogen outbreaks and has resulted in efforts to develop chemical and non-chemical environmentally user-friendly alternative control methods (Stapleton 2000; Ambrósio et al. 2009; Cohen et al. 2012; Dantas et al. 2013; Chamorro et al. 2015). One of the most feasible measures of control is the use of resistant varieties, which has the advantage of being safe for the environment, easy to adopt when resistance is available, and that can be used complementarily to other methods of control. In this sense, the screening of germplasm collections for resistance to this fungus is necessary to identify useful sources to control this disease.

C. melo is a highly diverse species, originally thought to originate in Africa. However, recent data suggests that melon may be of Asian origin (Sebastian et al. 2010). Several recent papers dealing with the variability of the species confirm the previously proposed taxonomic subdivision into two subspecies, subsp *melo* and subsp *agrestis* (Pitrat 2008; Esteras et al. 2013). The huge intra-specific variability reported in melons has not yet been exploited for resistance to *M. phaseolina*.

Few reports describe the screening of cucurbit germplasm against this pathogen. Salari et al. (2012) reported the seedling screening of Iranian melon landraces against *M. phaseolina* and other soilborne pathogens (*Monosporascus. cannonballus* Pollack & Uecker and *Rhizoctonia. solani* J.G. Kühn) under greenhouse conditions. None of the tested melon cultivars was immune to all the soilborne pathogenic fungi. However, two of the landraces were moderately resistant to the three fungi, both showed low levels of stem damage after infection with *M. phaseolina*. These melon cultivars are promising sources of resistance to *M. phaseolina*, but it is necessary to find higher levels of resistance. Another recent study has

focused on the screening of watermelon germplasm (Cohen et al. 2014) using soil naturally infested with *M. phaseolina* from northeastern Israel, and has resulted in the selection of four accessions with promising resistance.

In this context, the current study was conducted to screen for resistance to *Macrophomina phaseolina* a collection of *C. melo* of diverse origins, representing the species diversity. We analyzed the seedling responses of melon germplasm to different isolates of the pathogen from Brazil, where this fungus is now one of the main problems of melon, and Spain, where this pathogen is still a potential problem. Several sources of both subspecies, *melo* and *agrestis,* with quite high levels of seedling resistance that can be used to develop resistant cultivars, were selected.

Materials and methods

Melon germplasm

Two screening assays were performed, in 2013 and 2014. A total of 97 melon accessions (Table 1) from different geographical origins and representing the different botanical groups of the species were tested for seedling resistance to *M. phaseolina.* The first assay was performed in 2013 in Brazil, including 33 accessions from the genebank collection of the Department of Plant Sciences of the Universidade Federal Rural do Semi-Árido (UFERSA, Brazil). These Brazilian accessions have been morphological and molecularly characterized recently (Dantas et al. 2014) being tentatively classified in the *momordica* and *conomon* (subspecies *agrestis*), and in the *chate*, *ameri* and *cantalupensis* (subspecies *melo*) botanical groups within *C. melo*. This collection was screened along with 12 additional accessions (`Amaral`, `Edisto 47`, `Gulf Coast`, `HBJ`, `Olimpic`, `PMR 5`, `PMR 6`, `Védrantais`, `WMR-29`, `PMR 45`, `MR-1` and `PI 414723`), mostly reference commercial cultivars and breeding lines of the *cantalupensis*, *reticulatus*, *inodorus* and *momordica* groups.

The second assay was performed in Spain, screening part of the core collection of melon maintained by the Cucurbits Breeding group of the Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV) of the Universitat Politècnica de València (UPV, Spain). Fifty-two accessions of the COMAV´s core collection were selected, representing most of the botanical groups of the species, but trying to include a high number of variable accessions from Northern Africa, Eastern Europe, Western and Central Asia and India. In the Spanish assay we included 6 control accessions selected from those tested in Brazil (AC-13, AC-16, AC-24, AC-25, AC-26 and PI414723) (Table 1).

In the experiment performed in 2014 in Spain five F_1 generations, derived from crosses of 5 accessions, selected for their resistance, with susceptible cultivars, were assayed (Table 2).

Isolates of *Macrophomina phaseolina*

Three Brazilian isolates of *M. phaseolina* were used. These isolates were obtained from roots of melon plants with symptoms of charcoal root rot caused by *M. phaseolina* collected from two different commercial fields in Rio Grande do Norte (Me 248), Ceará (Me 250), and one experimental field of UFERSA (Me 249). The three isolates (Me 248, Me 249 e Me 250) were deposited in the culture collection

of plant pathogenic fungi of UFERSA, Brazil and COMAV, Spain. They were selected for this work on the basis of a preliminary assessment of pathogenicity. One isolate from Spain (isolated from infected soybean roots), was also tested.

Inoculation conditions

The experiment performed in 2013 in Brazil was conducted from May to August, under greenhouse conditions. The average air temperature was 33.6 °C and average humidity 39.8 %. The forty-five melon accessions (Table 1) were inoculated with the three Brazilian isolates (Me 248, Me 249 and Me 250) of *M. phaseolina*. A total of fifteen plants per accession (five per each fungal isolate) were tested.

The experiment performed in 2014 in Spain was conducted from May to October under greenhouse conditions. The average air temperature was 28 °C and average humidity 65%. The fifty-eight melon accessions and the five derived F_1 generations (Tables 1 and 2) were inoculated with the most aggressive Brazilian isolate of *M. phaseolina* (Me 248). Fifteen plants per accession were used. All the genotypes with moderately or highly resistant response in the first inoculation round were tested in two additional independent inoculations, using the same conditions and fifteen plants per accession.

One genotype selected for its highly resistant response against Me 248 in Spain and a highly susceptible control (Can-NyIsr and Flex-KhiIrak) were tested with the four isolates (Me 248, Me 249, Me 250 and Soy Spain) using fifteen plants per genotype and isolate.

Seeds of the tested melon accessions were germinated in commercial substrate previously autoclaved. The plants were manually irrigated daily to drainage with tap water and were not fertilized during the experiment. The inoculation technique used in both cases was a modification of the toothpick method used by Scandiani et al. (2011) with *Fusarium* spp. This method has proved to be useful for discriminating levels of aggressiveness among isolates of *M. phaseolina* and other fungal pathogens, and for detecting resistance rankings comparable with those obtained using infested soils (Keeling 1982; Bramel-Cox et al. 1988; Diourte et al. 1995; Mertely et al. 2005). To obtain inoculum using the toothpick method, 12 mm long toothpicks were placed, with the sharpened end up, in holes made in a 90 mm diameter filter paper. The toothpicks were then placed in a Petri dish and autoclaved for 30 min, for two days with an interval of 24 hours, at 121ºC. Twenty mL of melted PDA (potato-dextrose-agar) + streptomycin sulfate was added to each toothpick-containing Petri dish. Once solidified, the PDAS plates were inoculated with five mycelial plugs (6 mm in diameter) of one isolate of *M. phaseolina* and then were incubated at 28±2˚C in the dark for 8 days. Seedlings were inoculated 14 days after planting by inserting a toothpick tip overgrown with mycelia and microsclerotia of the corresponding isolate in each hypocotyl, 1 cm above the soil. Non infested and autoclaved toothpicks were used as negative controls. Seedlings were kept in the greenhouse for 30 days. All the experiments were performed with a completely randomized design.

Symptoms scoring

Thirty days after each inoculation, disease severity was assessed using a modified version of the scale described by Ravf and Ahmad (1998), where, 0=symptomless, 1=less than 3% of shoot tissues infected, 2=3 to 10% of shoot tissues infected, 3=11 to 25% of shoot tissues infected, 4=26 to 50% of shoot tissues infected and 5=more than 50% of shoot tissues infected.

The average disease severity was calculated for each cultivar and was used to classify the cultivars in five reaction classes: 0=immune (I); 0.1 to 1.0=highly resistant (HR); 1.1 to 2.0=moderately resistant (MR); 2.1 to 4.0=susceptible (SU) and 4.1 to 5.0=highly susceptible (HS) (Salari et al. 2012).

INSERT TABLE 1

INSERT TABLE 2

Statistical analysis

Data from the Brazilian and Spanish assays were analyzed using ANOVA separately for each isolate. The ANOVA was performed with the PROC GLM of SAS® (Sas Institute, 2000). We used the methodology described by Scott-Knott (1974) for grouping treatment averages.

Results

Screening of the Brazilian collection

The three Brazilian isolates of *M. phaseolina* induced typical symptoms of stem rot in all the assayed accessions, indicating that there was no immunity to this pathogen in this germplasm collection, when inoculated using the toothpick method (Table 3).

The aggressiveness of the three fungal isolates was different. The isolate Me 248 appeared to be the most aggressive. No significant differences were found among accessions in the response to Me 248 according to the method of Scott-Knott (1974) ($F = 1.30$; $p > 0.05$) (Table 3), being most of them highly susceptible (93.3%). However, we were able to distinguish different symptom levels among accessions in response to the inoculations with isolates Me-249 ($F = 1.76$; $p < 0.05$) and Me-250 ($F = 1.71$; $p < 0.05$).

Some Brazilian landraces and reference cultivars were classified as highly resistant (AC-09 and ´PMR 45´) or moderately resistant (AC-13, AC-16, AC-27, AC-31, ´Gulf Coast´, ´HBJ´ and ´Olimpic) to Me249. Also some others were highly resistant (AC-26) or moderately resistant (AC-12, AC-24, AC-25 and ´MR-1`) to Me 250. Despite some accessions showed certain levels of resistance to the toothpick inoculation with one isolate of *M. phaseolina*, no one was resistant to the three isolates and all were susceptible to the most aggressive Brazilian isolate Me 248.

Some of the accessions that were susceptible to Me 248, but displayed different levels of resistance to the other two isolates (AC-13, AC-16, AC-24, AC-25, AC-26 and PI 414723) were selected to be tested in the Spanish trial.

INSERT TABLE 3

Screening of a melon core collection

We selected the most aggressive Brazilian isolate, Me-248, to screen the core collection of melons conserved at COMAV-UPV in Spain. In this assay, seven accessions selected in the Brazilian trial were included as controls (AC-13, AC-16, AC-24, AC-25, AC-26 and PI 414723). In general, these accessions displayed symptoms that were less severe in the Spanish than in the Brazilian trial (Table 4). The lower average temperature in the Spanish trial (28 versus 33.6ºC) might explain these results as the virulence of *M. phaseolina* is influenced by the temperature regime (Fang et al. 2011). Even so, all these control accessions ranged from susceptible to highly susceptible to M-248 (Table 4), confirming the aggressiveness of the isolate and the accuracy of the screening procedure in both assays.

Similarly to the Brazilian assay, no immunity was found to the toothpick inoculation and all melon accessions developed different levels of stem rot during the course of the experiment. However, in this assay we found statistical differences in symptoms severity among the accessions ($F=16.28$; $p< 0.01$), that were allocated into four groups by the method of Scott-Knott (1974) (Tables 4 and 5). Most accessions were highly susceptible (24.1%) or susceptible (55.2%), but some groups were classified as moderately resistant (8.6%), and seven accessions (12.1%) were highly resistant, one cantaloup and 6 exotic accessions from Africa, Asia and Eastern Europe (Can-NyIsr, Dud-CUM296Georg, Dud-QPMAfg, Ac-TGR1551Zimb, Con-Pat81Ko, Ag-15591Ghana and AgC38Nig). The resistance of these accessions was confirmed in two additional inoculation rounds (Table 5).

INSERT TABLE 4

INSERT TABLE 5

We selected the resistant cantaloup Ca-NyIrs to test its response to all the *M. phaseolina* isolates (Me 248, Me 249, Me 250 and Soy Spain) (Fig. 1), using Flex-KhiIraq as susceptible control. Can-NyIsr accession was highly resistant to all isolates tested, with an average symptom severity below 1 in all cases. The Flex-KhiIrak accession was highly susceptible to all Brazilian isolates (average scores of 5), Me 248, Me 249 and Me 250. However, the Spanish isolate from soybean was the least aggressive one, also in this highly susceptible genotype.

INSERT FIGURE 1

We also selected the most resistant accessions to cross them with susceptible genotypes. Four F1 generations derived from the crosses of Dud-QPMAfg, Con-Pat81Ko, Ag-15591Ghana, and Ag-C38Nig with the susceptible In-PsPiñSp were evaluated along with their parents against the Me 248 isolate. The two F1 derived from the African *agrestis* accessions were highly resistant (with average severity of symptoms below 1) and no significant differences were observed between the response of the F1 generation and the corresponding highly resistant parental (Fig. 2). However, the F1 progenies of the *conomon* and *dudaim* accessions were moderately resistant (with average severity of symptoms between 1 and 2), being the resistance of these F1 intermediate between the corresponding susceptible and highly resistant parentals.

We also tested the F1 progeny from Can-NyIsr x Flex-KhiIrah. In this case the F1 generation was as susceptible as the highly susceptible parent, with symptom scores between 4 and 5 (Fig. 3).

INSERT FIGURE 2

INSERT FIGURE 3

Discussion

Macrophomina phaseolina, the causal agent of charcoal rot, is one of the most serious and potentially damaging fungus worldwide. After the phase out of methyl bromide, its control has become increasingly troublesome (Islam et al. 2012; Kaur et al. 2012; Chamorro et al. 2015). The identification of sources of resistance to this fungus can facilitate the management of this emerging disease in melons. Several methods have been described to evaluate *M. phaseolina* resistance in different crops, including growth chamber, greenhouse and field experiments in which seedlings and/or adult plants are scored. These studies report the occurrence of the resistance response at different plant developmental stages (Grezes-Besset et al. 1996; Khan and Shuaib 2007; Salari et al. 2012; Twizeyimana et al. 2012).

Although evaluations in naturally infested soils are employed to confirm the resistance of the selected material, the variability among fields with different soil characteristics, non-uniform inoculum distribution and microflora, and the variability across locations and seasons with different weather and management patterns, make these tests unappropriated for routine screening assays (Nischwitz et al. 2004; Roustaee et al. 2011; Kaur et al. 2012). Artificial inoculation methods that induce lesions similar to those produced under natural infections are used to avoid inconsistent results between field experiments. These facilitate the assessment of large germplasm collections in breeding programs in a rapid and uniform way (Sharmishtha et al. 2006; Twizeyimana et al. 2012).

The toothpick method is one of the most usually employed artificial inoculation protocol to initiate uniform *M. phaseolina* infections (Mughogho and Pande 1984; Bramel-Cox et al. 1988; Diourte et al. 1995; Mertely et al. 2005; Shekhar et al. 2006). It has been frequently employed to perform an easy assessment of isolate aggressiveness (Shekhar et al. 2006). Phenotypic as well as genetic variation in the pathogen population, even from the same geographical region, has been documented, adding difficulties to the implementation of successful management strategies (Almeida et al. 2003; Purkayastha et al. 2006; Kaur et al. 2012; Mahmoudi and Ghashghaie 2013; Almeida et al. 2014). The use of highly aggressive isolates is recommended to optimize the results of screening assays (Mahmoudi and Ghashghaie 2013). In the current study, the use of the toothpick method allowed us the selection of the highly aggressive Brazilian isolate Me 248 to be used in further screenings. It was also useful to confirm the low virulence of the Spanish isolate form soybean. This is consistent with the fact that isolates of *M. phaseolina* tend to be more aggressive towards the host species from where they were isolated than towards other host species (Diourte et al. 1995).

The toothpick method does not completely reproduce the natural infection processes (Mughogho and Pande 1984). For example, this and other methods, where inoculum is introduced into the plant by causing tissue wounding, can break down some stem resistance barriers, such us structural barriers, and increase disease severity (Hutcheson 1998; Kaur et al. 2012). However, it has been used efficiently to screen for resistant sources by reducing field testing expenses and length of time for evaluations. Also some authors report good agreement between the seedling response to toothpick, and other similar stem inoculation methods, and the response of plants under natural conditions (Keeling 1982; Grezes-Besset et al. 1996; Twizeyimana et al. 2012).

In fact, it was observed from the present study that stem inoculation with *M. phaseolina* at seedling stage caused high disease severity to a large number of accessions, but allowed us to select a subset of genotypes with different levels of resistant response. The results of our screening with the melon core collection revealed that seven accessions displayed high seedling resistance to the aggressive isolate of *M. phaseolina* Me 248. These accessions belong to different botanical groups of the melon species (*cantalupensis*, *conomon*, *acidulus*, wild *agrestis* and *dudaim*) providing resistance in different genetic backgrounds. These results add new sources to those reported by Salari et al. (2012), who, using one isolate of *M. phaseolina* from Iran and a seedling screening method with culture discs, reported that two Iranian landraces cultivars, namely `Sfidak khatdar´and `Sfidak bekhat` were moderately resistant to the disease.

The Can-NyIsr accession was the only resistant accession belonging to the *cantalupensis* group (that includes many commercial market classes), being rated as highly resistant to all four isolates tested. This accession has been previously reported as relatively tolerant to *Fusarium oxysporum* f. sp. *melonis* (Burger et al. 2003) and resistant to powdery mildew (*Sphaerotheca fuliginea* Race 1) (Cohen 1993; Cohen et al. 1996). Also, a relatively high level of resistance to the most aggressive isolate Me 248 was found in the Asiatic *conomon* and *dudaim* accessions, Con-Pat81Ko, Dud-CUM296Georg and Dud-QPMAfg, the former previously reported to be resistant to *Monosporascus cannonballus* and *Acremonium cucurbitacearum* (Iglesias et al. 2000; Dias et al. 2004). The lowest index of disease severity to Me 248 was found in the African wild *agrestis* AgC38Nig and Ag-15591Ghana, the latter reported as source of resistance to gummy stem blight caused by fungus *Didymella bryoniae* (Wolukau et al. 2007).

The different behavior of the F1 generations derived from these selected resistant sources suggests different mode of inheritance of the resistance. For example, resistance derived from Can-NyIsr seem to be recessive, as the F1 generation behave as the susceptible parental. In contrast, the highly resistant behavior of the F1 generations derived from the African *agrestis* sources suggest dominance of the resistance genes. Further studies with segregant populations are needed to determine the genetic control of each resistance. If the existence of different gene/allelles is confirmed in the different sources, their use could result in a more durable resistance.

The toothpick method successfully distinguished differences in seedling response to the stem rot caused by *M. phaseolina* among melon genotypes. Considering the aggressiveness of the inoculation method, and the fact that seedlings, in general, offer less resistance to the attack of pathogens than adult plants (Bedendo 2011), this resistance could contribute to improve the response against this pathogen of adult plants in field conditions. In naturally infested fields, the plant wilting symptoms occur late in the growing season, usually within 1 to 2 weeks of harvest. However, it is known that the pathogen commonly

infects melon seedlings early after planting (Davis et al. 2009). Therefore, stem resistance to the fungal attack at seedling stage could delay and/or reduce the severity of field infections.

However, the accessions found resistant here will need to be screened under field conditions to confirm the level of resistance at adult plant stage and to evaluate their response under stressful conditions that increase disease incidence, such as water stress, a heavy fruit load, high temperatures and /or saline conditions (Roustace et al. 2011).

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Table 1 Origin and taxonomic classification of the melon accessions evaluated against *Macrophomina phaseolina* (Brazilian landraces (AC)[†], commercial cultivars, breeding and reference lines (in bold) and COMAV´s core collection)[††].

Accession code/name	Origin	Botanical group	Accession code/name	Origin	Botanical group	Accessions code/name	Origin	Botanical group
AC-06[a]	Brazil	*cantalupensis*	**`Amaral`[a]**	Holanda	*inodorus*	Am-GalaTun/Galaoui[*b]	Tunisia	*ameri*
AC-27[a]	Brazil	*cantalupensis*	**`Edisto 47`[a]**	França	*cantalupensis*	Am-KhaIran/Khatoni[*b]	Iran	*ameri*
AC-36[a]	Brazil	*cantalupensis*	**`Gulf Coast`[a]**	USA	*cantalupensis*	Am-SarakIran/Sarakhs[*b]	Iran	*ameri*
AC-44[a]	Brazil	*cantalupensis*	**`HBJ`[a]**	USA	*cantalupensis*	Am-HasanTur2/Hasanbey PI169310[b]	Turkey	*ameri*
AC-33[a]	Brazil	*ameri*	**'Olimpic'[a]**	Japan	*cantalupensis*	Am-HasanTur3/ Hasanbey PI176947[b]	Turkey	*ameri*
AC-13[ab]	Brazil	*chate*	**`PMR 5`[a]**	USA	*cantalupensis*	Flex-Co20Ind/Snakemelon[b]	India	*flexuosus*
AC-14[a]	Brazil	*chate*	**`PMR 6`[a]**	USA	*cantalupensis*	Flex-KhiIrak/Khiar[b]	Irak	*flexuosus*
AC-01[a]	Brazil	*momordica*	**`Védrantais´[a]**	França	*cantalupensis*	Flex-Co24Irak/Snakemelon[b]	Irak	*flexuosus*
AC-02[a]	Brazil	*momordica*	**`WMR-29`[a]**	USA	*cantalupensis*	Flex-AcukTur/Acuk[b]	Turkey	*flexuosus*
AC-04[a]	Brazil	*momordica*	**`PMR 45`[a]**	USA	*reticulatus*	Flex-AryaInd/Arya[b]	India	*flexuosus*
AC-09[a]	Brazil	*momordica*	**'MR-1'[a]**	Índia	*momordica*	Flex-SnakeSA/Snakemelon[b]	Saudi Arabia	*flexuosus*
AC-15[a]	Brazil	*momordica*	**'PI 414723'[ab]**	India	*momordica*	Dud-CUM296Georg/ CUM296[b]	Georgia	*dudaim*
AC-16[ab]	Brazil	*momordica*	In-PsPiñSp/Piñonet[b] Piel de sapo	Spain	*inodorus*	Dud-QPMAfg/Queen´s pocket melon[*b]	Afganistan	*dudaim*
AC-18[a]	Brazil	*momordica*	In-AsliTun/Melon Asli[b]	Tunisia	*inodorus*	Chate-CarIta/Carosello[b]	Italy	*chate*
AC-19[a]	Brazil	*momordica*	In-MaazTun/Maazoon[b]	Tunisia	*inodorus*	Mom-KhaInd/Kharbuja[b]	India	*momordica*
AC-22[a]	Brazil	*momordica*	In-WTTur/Winter type PI 169329[b]	Turkey	*inodorus*	Mom-PI124Ind/ PI 124112[b]	India	*momordica*
AC-23[a]	Brazil	*momordica*	In-kirkTur/Kirkagac PI 169333[b]	Turkey	*inodorus*	Mom-FPInd/Faizabadi Pont[*b]	India	*momordica*
AC-25[ab]	Brazil	*momordica*	In-CV1Tun/Melon Jaune[b]	Tunisia	*inodorus*	Mom-MR1Ind/MR1[b]	India	*momordica*
AC-26[ab]	Brazil	*momordica*	In-HamiChi/Hami melon[b]	China	*inodorus*	Ac-TGR1551Zimb/TGR1551 PI482420[b]	Zimbabwe	*acidulus*
AC-28[a]	Brazil	*momordica*	In-KirkTur2/ Kirkagac PI169322[b]	Turkey	*inodorus*	Con-GMJa/Ginsen Makuwa[b]	Japan	*conomon*
AC-29[a]	Brazil	*momordica*	Can-NYIsr/Noy Israel[b]	Israel	*cantalupensis*	Con-Pat81Ko/Pat 81[b]	Korea	*conomon*
AC-34[a]	Brazil	*momordica*	Can-PSUSA/Persian Small Type[*b]	USA	*cantalupensis*	Con-CUM188Jap/Omaru Gin Makuwa[b]	Japan	*conomon*
AC-39[a]	Brazil	*momordica*	Can-PresFran/ Prescott Fond Blanc[b]	France	*cantalupensis*	Chi-VellInd/PI 164320[b]	India	*chito*
AC-41[a]	Brazil	*momordica*	Can-VedFran/Vedrantais[b]	France	*cantalupensis*	Ag-15591Ghana/PI 185111[b]	Ghana	wild *agrestis*
AC-45[a]	Brazil	*momordica*	Am-NanaGeorg/ Melon Nanatri[b]	Georgia	*ameri*	Ag-C38Nig/Co38[b]	Nigeria	wild *agrestis*
AC-11[a]	Brazil	*conomom*	Am-6053Iran/ PI140632[b]	Iran	*ameri*	Ag-WChInd/Wild chibbar[b]	India	wild *agrestis*
AC-12[a]	Brazil	*conomom*	Am-AfrMor/Afr-c-1[b]	Morocco	*ameri*	La-SousIran/Souski[*b]	Iran	*indet landrace*
AC-35[a]	Brazil	*conomom*	Am-KorcaRus/Korca[b]	Russia	*ameri*	La-AcurTur/Acur PI344343[b]	Turkey	*indet landrace*
AC-42[a]	Brazil	*conomom*	Am-ChandAfg/ Chandalack PI 276660[b]	Afghanistan	*ameri*	La-PopEthi (11)/ PI 193495[b]	Ethiopia	*indet landrace*
AC-43[a]	Brazil	*conomom*	Am-TokTaj/Tokash[b]	Tajikistan	*ameri*	La-ErizoSp/Erizo[b]	Spain	*indet landrace*
AC-08[a]	Brazil	*indet landrace*	Am-AltimTur/Al Timbas PI 169£331[b]	Turkey	*ameri*	La-Bol (5)/Bol84[b]	Bolivia	*indet landrace*
AC-24[ab]	Brazil	*indet landrace*	Am-CV3Tun/Ananas[b]	Tunisia	*ameri*			
AC-31[a]	Brazil	*indet landrace*	Am-UrfaTur/Urfa PI 174150[b]	Turkey	*ameri*			

[†]Genebank collection of the Department of Plant Sciences of the Universidade Federal Rural do Semi-Árido (UFERSA, Brazil). [††]The COMAV´s collection was established on the framework of a previous project MELRIP 2007-2010 (Esteras et al. 2009; 2013) and was multiplied by the COMAV´s Cucurbits Breeding Group (www.comav.upv.es). Genotypes labelled with *were kindly provided by M. Pitrat. PI and CUM genotypes were kindly provided by NPGS USDA and IPK Gatersleben genebanks, respectively. Genotypes labelled with [a] were tested in Brazil, [b] were tested in Spain and [ab] were tested in Brazil and Spain.

Table 2 F1 generations derived from crosses between accessions resistant and susceptible to *Macrophomina phaseolina*, and evaluated for their response to this pathogen in Valencia/Spain.

Botanical group	Accessions
wild agrestis x inodorus	F_1 (Ag-15591Ghana/PI 185111) X (In-PsPiñSp/Piñonet)
conomon x inodorus	F_1 (Con-Pat81Ko/Pat81) X (In-PsPiñSp/Piñonet)
wild agrestis x inodorus	F_1 (Ag-C38Nig/Co38) X (In-PsPiñSp/Piñonet)
dudaim x inodorus	F_1 (Dud-QPMAfg/Queen´s pocket melon) X (In-PsPiñSp/ Piñonet)
cantalupensis x flexuosus	F_1 (Can-NyIsr/Noy Israel) X (Flex-KhiIrak/Khiar)

Table 3 Average symptoms severity and reaction class of melon accessions inoculated with three *Macrophomina phaseolina* Brazilian isolates (Me 248, Me 249 and Me 250) using the toothpick method, in Mossoró/Brazil.

Accession	Me 248		Me 249		Me250	
	Average	Reaction	Average	Reaction	Average	Reaction
AC-01	5.0a[ǂ]	HS	4.2b	HS	4.2b	HS
AC-02	5.0a	HS	3.8b	SU	3.8b	SU
AC-04	5.0a	HS	3.4b	SU	4.2b	HS
AC-06	5.0a	HS	4.6b	SU	3.4b	SU
AC-08	5.0a	HS	4.6b	HS	4.4b	HS
AC-09	5.0a	HS	1.0a	HR	4.4b	HS
AC-11	5.0a	HS	4.0b	SU	3.8b	SU
AC-12	5.0a	HS	3.2b	SU	1.2a	MR
AC-13[ǂǂ]	**5.0a**	**HS**	**1.8a**	**MR**	**4.0b**	**SU**
AC-14	5.0a	HS	4.0b	SU	4.2b	HS
AC-15	5.0a	HS	5.0b	HS	5.0b	HS
AC-16	**5.0a**	**HS**	**1.8a**	**MR**	**4.8b**	**HS**
AC-18	5.0a	HS	4.8b	HS	4.4b	HS
AC-19	4.4a	HS	4.2b	HS	4.2b	HS
AC-22	3.8a	SU	4.2b	HS	4.8b	HS
AC-23	5.0a	HS	4.6b	HS	3.6b	SU
AC-24	**3.8a**	**SU**	**4.4b**	**HS**	**1.6a**	**MR**
AC-25	**5.0a**	**HS**	**4.2b**	**HS**	**1.4a**	**MR**
AC-26	**4.4a**	**HS**	**4.6b**	**HS**	**0.6a**	**HR**
AC-27	4.6a	HS	1.6[a]	MR	3.4b	SU
AC-28	4.4a	HS	4.4b	HS	3.8b	SU
AC-29	5.0a	HS	3.8b	SU	4.2b	HS
AC-31	4.4a	HS	1.6[a]	MR	5.0b	HS
AC-33	5.0a	HS	4.0b	SU	4.8b	HS
AC-34	5.0a	HS	5.0b	HS	4.4b	HS
AC-35	5.0a	HS	3.2b	SU	3.4b	SU
AC-36	5.0a	HS	4.0b	SU	4.6b	HS
AC-39	5.0a	HS	4.0b	SU	3.2b	SU
AC-41	5.0a	HS	3.6b	SU	4.6b	HS
AC-42	5.0a	HS	4.4b	HS	3.8b	SU

AC-43	5.0a	HS	5.0b	HS	4.2b	HS
AC-44	4.6a	HS	3.6b	SU	3.2b	SU
AC-45	5.0a	HS	4.4b	HS	4.2b	HS
'Amaral'	5.0a	HS	3.2b	SU	4.2b	HS
'Edisto 47'	4.4a	HS	3.2b	SU	3.6b	SU
'Gulf Coast'	3.6a	SU	1.8a	MR	4.8b	HS
'HBJ'	5.0a	HS	2.0a	MR	4.6b	HS
'MR-1'	4.2a	HS	4.6b	HS	1.8a	MR
'Olimpic'	4.6a	HS	1.4a	MR	3.4b	SU
PI 414723	**4.4a**	**HS**	**3.6b**	**SU**	**3.6b**	**SU**
'PMR 45'	4.2a	HS	0.8a	HR	3.4b	SU
'PMR 5'	5.0a	HS	3.6b	SU	3.4b	SU
'PMR 6'	5.0a	HS	3.4b	SU	3.4b	SU
'Védrantais'	5.0a	HS	3.6b	SU	4.0b	SU
'WMR 29'	4.2a	HS	3.2b	SU	3.6b	SU
Average	4.28		3.72		3.75	
F[††:]	1.30 (p>0.05)		1.76 (p<0.05)		1.71 (p<0.05)	

HR: Highly resistant [0.1-1.0]; MR: Moderately resistant [1.1-2.0]; SU: Susceptible [2.1-4.0]; HS: Highly susceptible [4.1-5.0]. [†]Averages in a column followed by the same letter do not differ ($p<0.05$) according by the Scott-Knott cluster (1974). Each number is the mean of five plants. [††]Estimate of value F de Snedecor. [†††]Accessions marked with bold letters were also included in the Spanish trial (Table 4)

Table 4 Average symptoms severity and reaction class of melon accessions inoculated with one *Macrophomina phaseolina* Brazilian isolate (Me 248), using the toothpick method, in Valencia-Spain. Accessions classified as susceptible or highly susceptible have been included.

Highly Susceptible [4.1-5.0]		Susceptible [2.1-4.0]			
In-HamiChi	4.01[ŧ]d	Can-VedFran	2.23b	Am-GalaTun	3.00c
Am-KorcaRus	4.24d	La-ErizoSp	2.33b	Flex-AryaInd	3.07c
PI 414723[ŧŧŧ]	**4.43d**	Am-AltimTur	2.36b	Am-AfrMor	3.09c
La-SousIran	4.44d	In-kirkTur2	2.36b	Flex-SnakeSA	3.13c
Can-PSUSA	4.56d	Con-CUM188Jap	2.37b	**AC-13**	**3.41c**
Am-ChandAfg	4.60d	Mom-PI124Ind	2.41b	Flex-Co24Irak	3.44c
Flex-Co20Ind	4.60d	Am-CV3Tun	2.44b	Ag-WChInd	3.57c
Am-UrfaTur	4.61d	In-kirkTur	2.46b	Mom-FPInd	3.57c
Mom-MR1Ind	4.67d	Chi-VellInd	2.51b	Am-HasanTur3	3.61c
Flex-AcukTur	4.75d	Con-GMJa	2.60b	Am-6053Iran	3.64c
Am-NanaGeorg	4.76d	In-WTTur	2.68b	In-MaazTun	3.66c
Flex-KhiIrak	4.83d	Mom-KhaInd	2.70b	Am-HasanTur2	3.74c
La-AcurTur	4.90d	**AC-25**	**2.71b**	Chate-CarIta	3.87d
Am-KhaIran	4.94d	Am-TokTaj	2.78b	**AC-16**	**3.94d**
		Am-SarakIran	2.90b	**AC-24**	**3.96d**
		In-PsPiñSp	2.96c	**AC-26**	**3.96d**

$F^{ŧŧ} = 16.28\ (p < 0.01)$

[ŧ]Averages in a column followed by the same letter do not differ ($p < 0.05$) according by the Scott-Knott cluster (1974). Each number in the mean of fifteen plants. [ŧŧ]Estimate of value F de Snedecor. [ŧŧŧ]Accessions marked with bold letters were also included in the Brazil trial (Table 3).

Table 5 Average symptoms severity and reaction class of melon accessions to one *Macrophomina phaseolina* Brazilian isolate (Me 248) inoculated by the toothpick method, in Valencia-Spain. Accessions classified as moderately or highly resistant have been included.

Highly Resistant [0.1-1.0]		Moderately Resistant [1.1-2.0]	
Ag-15591Ghana	0.10[ŧ]a	Can-PresFran	1.12a
Dud-CUM296Georg	0.11a	In-CV1Tun	1.40a
Ag-C38Nig	0.25a	La-PopEthi	1.62a
Can-NYIsr	0.44a	In-AsliTun	1.88a
Con-Pat81Ko	0.61a	La-Bol(5)	1.97a
Dud-QPMAfg	0.61a		
Ac-TGR1551Zimb	0.69a		
$F^{ŧŧ} = 16.28\ (p < 0.01)$			

[ŧ]Averages in a column followed by the same letter do not differ ($p<0.05$) according by the Scott-Knott cluster (1974). Average of the three round of inoculations (the first screening and the two independent inoculation rounds two confirm the resistance, each round with fifteen plants) is shown for each accession.
[ŧŧ]Estimate of value F de Snedecor.

Table 6 Average symptoms severity and reaction class of five F1 hybrids to one *Macrophomina phaseolina* Brazilian isolate (Me 248) inoculated by the toothpick method, in Valencia-Spain

Highly Resistant [0.1-1.0]		Moderately Resistant [1.1-2.0]		Highly Susceptible [4.1-5.0]	
F1: Ag-15591Ghana x InPsPiñSp	0.13†a	F1: Con-Pat81Ko x In-PsPiñSp	1.50a	F1: Can-NyIsr x Flex-KhiIrak	5.0b
F1: Ag-C38Nig x In-PsPiñSp	0.61a	F1: Dud-QPMAfg x In-PsPiñSp	1.92a		
$F^{††} = 16.28$ ($p < 0.01$)					

†Averages followed by the same letter do not differ ($p<0.05$) according by the Scott-Knott cluster (1974). Average of the three assays (the first screening and the two independent rounds two confirm the resistance, each one with fifteen plants) is shown for each accession††. Estimate of value F de Snedecor.

Fig. 1 Comparison of the resistance found in two melon accessions to four isolates (Me 248, Me 249, Me 250 and Soy Spain) of *Macrophomina phaseolina* inoculated by the toothpick method, in Valencia-Spain. Average severity: Highly resistant [0.1-1.0]; Moderately resistant [1.1-2.0]; Susceptible [2.1-4.0]; Highly susceptible [4.1-5.0].

Fig. 2 Comparison of the resistance of melon accessions and progenies derived from crosses with susceptible controls to Me 248 isolate of *Macrophomina phaseolina* inoculated by the toothpick method, in Valencia-Spain. Highly resistant [0.1-1.0]; Moderately resistant [1.1-2.0]; Susceptible [2.1-4.0]; Highly susceptible [4.1-5.0]. A= (Ag-15591Ghana x In-PsPiñSp); B= (Con-Pat81Ko x In-PsPiñSp); C= (Ag-C38Nig x In-PsPiñSp); D= (Dud-QPMAfg x In-PsPiñSp) and E= (Can-NyIsr x Flex-KhiIrak). $F=7.18$ ($p < 0.01$).

Fig. 3 Symptoms of *Macrophomina phaseolina* in stems of the susceptible control Flex-KhiIrak (in the middle), of the resistant accession Noy Israel (on the right) and their corresponding F1 hybrid (on the left) inoculated by the toothpick method, in Valencia-Spain with isolate Me 248.