Brazilian melon landraces resistant to *Podosphaera xanthii* are unique germplasm resources.

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Abstract

P. xanthii is the most important causal agent of powdery mildew in melon, a crop ranked within the most economically important species worldwide. The best strategy to face this fungus disease, which causes important production losses, is the development of genetically resistant cultivars. Genetic breeding programs require sources of resistance, and a few ones have been reported in melon, mostly in Momordica and Acidulus horticultural groups. However, the existence of many races that reduces the durability of the resistance makes necessary to find new resistant genotypes with different genetic backgrounds.

In this work, Brazilian germplasm, together with a set of Indian landraces, and the COMAV´s (Institute for the Conservation and Breeding of Agricultural Biodeviversity) melon core collection, representing the whole variability of the species, were assessed for resistance against some common races in Spain and Brazil and genotyped with a 123-SNP genotyping platform to study the molecular relationships of the resistant accessions. In the first experiment, carried out in Valencia (Spain) in 2013, seventy-nine melon accessions were evaluated using artificial inoculation. Five accessions selected as resistant were also evaluated against races 1, 3, and 5 in Mossoró (Brazil, 2015) and against race 3.5 in Valencia (2016) under greenhouse conditions, and under four field conditions in Brazil. The accessions, AL-1, BA-3, CE-3, and RN-2, within the Brazilian collection, presented resistance against all the races of *P. xanthii* assayed in all conditions tested. Al-1, CE-3 and RN-2 were molecularly more similar to wild *agrestis* and Acidulus melons from Asia and Africa, while BA-3 grouped with Momordica types. Molecular analysis also confirmed that these new Brazilian sources of resistance differ from those previously reported, constituting interesting materials for encourage genetic breeding programs, especially in Brazil and Spain.

Keywords

Cucumis melo, powdery mildew, screening, genetic resistance, diversity

Introduction

Melon (*Cucumis melo* L; 2n = 2x = 24) is one of the most important cultivated crops worldwide, with a harvested area of about 1.2 million hectares and a production of 29.5 million tons in 2014. China is the largest producer with 48.9% of the total production, followed by Turkey, Iran and Egypt (5.8%, 5.1%, and 3.5%, respectively). Brazil and Spain, where this study was carried out, are the 7th and 11th world producers, with 857 and 567 thousand tons, respectively (SIDRA/IBGE 2016; FAOSTAT, 2016). Therefore, due to its economic importance, it is necessary to guarantee an efficient production with high quality standards through crop improvement.

Cucurbit powdery mildew is the most severe and widespread fungal disease of cucurbits, causing growth reduction, premature desiccation of the leaves and detriment of the quality and marketability of the fruits (McGrath and Thomas, 1996). Two are the causal agents of this disease in Cucurbits, *Podosphaera xanthii* (Castag.) U. Braun & N. Shish. (Shishkoff, 2000), mainly in subtropical and tropical areas and greenhouse crops, and *Golovinomyces orontii* (Castagne) Heluta (Braun and Cook, 2012), which is more frequent in temperate and cold areas.

In southeastern Brazil, both species have been detected in greenhouse conditions, but *P. xanthii* is more prevalent, causing economically important damages in melon crops (Aguiar *et al*., 2012). However, in northeast Brazil and Spain, only *P. xanthii* has been observed (Sales-Júnior *et al*., 2011; Álvarez and Torés, 1997). High variable pathogenicity and virulence are observed for the two species, which has led to the description of a large number of pathotypes and races (Bertrand *et al*., 1992; McCreight *et al*., 2005; Lebeda and Sedláková, 2006; McCreight, 2006; Lebeda *et al*., 2007, 2008, 2016; Sedláková *et al*., 2014), based on the response to powdery mildew of differential melon genotypes.

The development of resistant cultivars has been reported to be much more effective than chemical treatments which usually lead to fungicide resistances (Hollomon and Wheeler, 2002), and can cause environmental and human-health problems. In addition, genetic breeding is also compatible with other methods of control in an integrated disease management. The first step to develop resistant cultivars in breeding programs is the identification of sources of resistance in suitable germplasm.

Melon is considered the most polymorphic species within the *Cucumis* genus (Burger *et al*., 2010), showing a great morphological and physiological variation distributed worldwide. According to the morphological observations of Jeffrey (1980, 2005) and Stepansky *et al.*,

(1999), the species is taxonomically divided into two subspecies, subsp. *melo* and subsp. *agrestis*. Pitrat (2008) grouped melons into 15 widely accepted horticultural groups, molecularly studied by Esteras *et al.*, (2013) and Leida *et al.*, (2015), (Inodorus, Cantalupensis, Reticulatus, Adana, Chandalak, Ameri, Chate, Flexuosus, and Dudaim (in subsp. *melo*), and Momordica, Conomon, Chinensis, Makuwa, Acidulus and Tibish (in subsp. *agrestis*). This melon classification has been recently updated (Pitrat, 2016). This huge diversity within the species has been explored for resistance to several races of *P. xanthii* and *G. orontii*.

In the 30´s, the Indian melon accession LJ 525 was found to be resistant to *P. xanthii* race 1, and was used to introgress the *Pm-1* gene in the cultivar PMR 45 (Jagger and Scott, 1937). Since then, many melon resistance sources to powdery mildew (Bohn *et al*., 1980; Bohn and Whitaker, 1964; Harwood and Markarian, 1968; Thomas, 1986; Kenigsbuch and Cohen, 1989; Pitrat, 1991; Fukino *et al.*, 2006; McCreight *et al*., 2012), mostly belonging to the Momordica group coming from India (Dhillon *et al*., 2012), have been described. Resistance sources have been also identified in Acidulus melons from India and Africa (McCreight, 2003; Gómez-Guillamón *et al.*, 2006; Yuste-Lisbona *et al*., 2008, 2010) and in some accessions of the Flexuosus group (reviewed in Dogimont, 2010-11; Liu *et al*., 2010; Dhillon *et al.*, 2012). In most of them, the genetic control of their resistance to different powdery mildew races has been also studied, by using both traditional and molecular tools. The last melon gene list (Dogimont, 2010-11) includes the most updated information regarding resistance genes and resistance germplasm sources for powdery mildew in the species. In total, 19 cucurbit powdery mildew resistant genes (Pitrat, 2002; Wang *et al*., 2011; Yuste-Lisbona *et al*., 2011; Zhang *et al*., 2013; Ning *et al*., 2014) and 4 QTLs associated with this trait (Perchepied *et al*., 2005; Fukino *et al*., 2008, Ning *et al*., 2014) have been reported. Besides, MLO genes have also been suggested to mediate in this resistance being considered as susceptibility factors for the disease (Iovieno *et al*., 2015). Recently, Natarajan *et al.* (2016) published a melon map containing 390 SNPs (Single Nucleotide Polymorphisms) and 45 InDels in genes associated to disease-defense responses. In this study, Next-Generation Sequencing (NGS) technologies were used to re-sequence a powdery mildew-susceptible genotype, and the cultivars Edisto 47 and PMR 5, and the breeding line MR-1, resistant to different races of this pathogen.

The great genetic diversity found in melon for the resistance to *P. xanthii* responds to the high worldwide genetic variability found for this pathogen, of which at least 45 races have been

already recorded (McCreight *et al*., 2012). In southwestern Spain races 1, 2, 4 and 5 were reported (Del Pino *et al*., 2002), although more recently Torés *et al*. (2009) also reported the presence of the race 3.5. In Brazil, the races of *P. xanthii* found on melon crops were 0, 1, 2F, 3, 4 and 5, with prevalence of races 1 and 2F (Reis and Buso, 2004; Fazza, 2006). In more recent trials conducted in northeastern Brazil, the races 1, 2F, 3, 3.5 and 5 have been identified (unpublished data). The presence of so many races is a substantial problem for the breeding process, as the variation in the pathogen population reduces the durability of resistance (Hosoya *et al*., 2000; Kuzuya *et al*., 2004). Therefore, together with the identification of new sources of resistances, it is important to study the prevalent races in a particular region in order to establish specific breeding programs. Molecular analysis can also assist the phenotyping process, allowing an accurate characterization of the germplasm used in each assay, avoiding redundancies and providing molecular tags for further characterization of the resistance. In melon, a large collection of SNPs is available in the Melogene database (http://www.melogene.net/) due to several transcriptome sequencing and re-sequencing projects carried out (González-Ibeas *et al*., 2007; Blanca *et al*., 2011, 2012). SNPs are excellent markers as they are very polymorphic and have been demonstrated to be effective for the establishment of genetic relationships among melon genotypes in previous studies (Blanca *et al*., 2011, 2012; Esteras *et al*., 2013, Leida *et al*., 2015; Nimmakayala *et al*., 2016).

The objectives of this work are the screening against different *P. xanthii* isolates from Brazil and Spain of a large germplasm collection of *C. melo*, representing the species diversity, including a new collection of Brazilian germplasm unexplored to date for resistance (Dantas *et al*., 2015), and the study of the molecular relationships of the new resistant sources with previously described resistant genotypes.

Materials and Methods

Melon germplasm

A set of 40 Brazilian accessions collected from 9 states of the country, previously phenotyped for vine and fruit traits (Dantas *et al*., 2015), were screened for resistance to *P. xanthii* (Table 1 and Table S1, Supporting information). These Brazilian accessions are maintained in the genebank of the Department of Plant Sciences of the Universidade Federal Rural do Semi-Árido (UFERSA, Brazil). A set of 26 accessions from the COMAV´s core collection (Esteras *et al*., 2013; Leida *et*

al., 2015) was included as reference genotypes, representing most of the melon horticultural groups, but mainly those in which powdery mildew resistance has been previously reported (Acidulus, Flexuosus, Conomon, and wild *agrestis* types). Additionally, 13 Indian accessions (wild *agrestis*, Momordica, and Acidulus), previously explored by Dhillon *et al*. (2009), Fergany *et al*. (2011) and Roy *et al*. (2012), along with several powdery mildew race differential genotypes were included in this research (Table 1 and Table S1). The molecular analysis has been done using all the accessions phenotyped for powdery mildew resistance and the whole COMAV´s core collection, built in the framework of a previous project which includes 176 accessions representative of all melon varieties and from diverse origins, including cultivated, feral and wild types (Table S1).

Isolates of *P. xanthii*

The species identification of the powdery mildew isolates was done by examination of conidia for the presence of fibrosin bodies and the production of forked germ tubes (Kable and Ballantyne, 1963; Ballantyne, 1975). Powdery mildew races were identified attending to the reactions of ten differential genotypes (McCreight, 2006; Lebeda *et al*., 2016). Three artificial inoculation assays, two in Spain (Valencia 2013 and Valencia 2016) and one in Brazil (Brazil 2015), were performed. In Valencia 2013 an isolate of *P. xanthii* collected in a susceptible Piel de Sapo plant (In-PsPiñSp) grown in the greenhouse and multiplied in Piel de Sapo melon plants, was used. The three isolates used in Brazil 2015 were kindly supplied by Sakata Seed Sudamerica Ltda as belonging to races 1, 3 and 5 of *P. xanthii*. An additional isolate, previously identified as race 3.5 and multiplied in Piel de sapo plants grown at greenhouse, was employed in the last assay, Valencia 2016.

Cultivation, inoculation and evaluation of the response to *P. xanthii* in greenhouse

A total of 79 accessions, including the whole Brazilian collection, and the selected accessions from COMAV and Indian germplasm, were evaluated in 2013 for resistance to the isolate of *P. xanthii* collected in In-PsPiñSp in Valencia (Table 1, Table S1). The second assay was carried out in 2015 in Mossoró, Rio Grande do Norte State Brazil; this assay included ten Brazilian accessions and one Indian accession, scored as resistant (5 accessions) and moderately resistant (6 accessions) in Valencia 2013 (Table 2, Table S1). The accession PI 313970, reported

previously as resistant to several American races (McCreight and Coffey, 2011) was also included in the Brazilian assay. Six accessions that were resistant in Brazil 2015 were included in the third assay (Table 2, Table S1) performed in Valencia to test their resistance to race 3.5 (Valencia 2016).

The three experiments were performed under greenhouse conditions. The Valencia 2013 assay was conducted from June to July in the following conditions: average air temperature of 27.8ºC and average humidity 61.5 %. Seeds were placed in a pre-germination chamber for 24 h at a temperature 37.5ºC. Afterwards the seedlings were transferred into plastic pots (one seedling/pot) with commercial substrate (Huminsubstrat N3®) at the cotyledon stage. Seedlings were watered daily and no pesticides were applied during the experiment. A completely randomized design with three replications and five plants per accession and replication was used. Artificial inoculations were performed on plants at the three true leaf stage (20 days after sowing), taking conidia and mycelium from Piel de Sapo plants used as inoculum source and picking up with a brush (no.2) on the three leaves. The symptoms assessment was carried out 25 days after inoculation, according to the methodology employed by McCreight (2003). Using this method, leaf symptoms were evaluated based on mycelial growth and sporulation using a 1-9 scale, where: 1= no evidence of disease, 2= a trace of hyphae, 3= hyphae restriction (slightly larger hyphaes and in larger quantities than in score 2, but also with no detectable sporulation), 4= a few colonies are present (sporulation), 5= scattered colonies, 6= numerous colonies (more sporulation), 7= 50% of adaxial leaf surface is covered with hyphae and spores and the abaxial surface present few colonies (abundant sporulation), 8= greater than 50% of adaxial leaf surface is covered with hyphae and spores and the abaxial surface present scattered colonies (abundant sporulation), and 9= greater than 75% of adaxial leaf surface is covered with hyphae and spores and the abaxial surface present numerous or coalescent colonies (abundant sporulation). This evaluation was made by observing the signs of the fungus with a magnifying glass (x 10) to better notice differences in the amount and size of the hyphae. According to these scores, the genotypes were classified as resistant (R, scores 1-3.9), moderately resistant (MR, scores 4-5.9), susceptible (S, score 6-6.9) and highly susceptible (HS, scores 7-9) (scale modified from that of McCreight (2003)).

In Brazil 2015 the assay was carried out from May to June, with an average air temperature of 32.9ºC and humidity 61.5 %. The management of seedlings was done as previously described. A completely randomized design with three replications and twelve plants per accession and replication was applied. Since the objective of the experiment was the evaluation of each

accession against three races of *P. xanthii* (1, 3, and 5), we used the methodology developed by Yuste-Lisbona *et al*. (2010) that allows the assessment of several races at the same time and on the same leaf. For each of the three isolates provided by Sakata Seed Sudamerica Ltda, conidia were obtained from monospore culture and transferred to fresh melon cotyledons that were maintained *in vitro* under axenic conditions, as described by Álvarez and Torés (1997). Seedlings were inoculated with the three races using an eyelash brush in the third fully expanded leaf, by placing the inoculum of each race at two equidistant spots (in relation to the midrib) on each leaf (a total of six inoculation spots per leaf). Leaves were visually scored for the presence of fungal structures 19 days after inoculation, following the recommendation of Yuste-Lisbona *et al*. (2010). In this case, the symptoms were rated in a 1-4 scale, where: 1= no visible sporulation, 2= low level of sporulation without progress of the fungus growth, 3= moderate level of sporulation, and 4= abundant sporulation. According to Yuste-Lisbona *et al*. (2010), plants with scores 1-2 were considered resistant (R), whereas those with scores 3-4 were considered susceptible (S). This criterion was also used in the last experiment performed in Valencia in 2016, which was carried out from March to May. In this last assay, seedlings were inoculated in the third fully expanded leaf with race 3.5 of *P. xanthii,* collecting conidia and mycelium with a tiny spatula from the Piel de sapo plants used as source of inoculum, by placing the inoculum at 4 spots on the leaf. Plants were scored for resistance 14 days after inoculation.

Evaluation of the response to *P. xanthii* under field conditions

In addition to these greenhouse assays, four assessments under field conditions were carried out in different Brazilian locations in the State of Rio Grande do Norte (Brazil) (in areas where the occurrence of severe powdery mildew attacks is always present). In this assay the same set of 11 selected accessions assayed in Brazil 2015 and the 10 differential hosts were used (Table 3, Table S1). Experiments in Mossoró 2014 and Assú were carried out between June and August (2014), while the experiments in Mossoró 2015 and Baraúna were conducted between September and November (2015). All experiments were carried out in a randomized block design with three replications of 10 plants each (plant spacing of 2.0 x 0.3 m). The evaluations were performed 20 days after transplanting, when powdery mildew attack in the field was very intense and all susceptible plants showed severe symptoms of infection. Three leaves of each plant were sampled and evaluated according to the scale used in Valencia 2013.

Molecular characterization and statistical analysis

A molecular analysis of the accessions used in this study was performed. Total DNA was extracted from young leaves following the method described in Doyle and Doyle (1990) with minor modifications. DNA was treated with RNAse A in the first step, and the final wash was done with 70% ethanol, containing 15 mM ammonium acetate to improve its quality. DNA concentrations (in TE buffer) were measured with the Nanodrop ND-1000 Spectrophotometer v.3.5 and adjusted to 10-15 ng/µl for subsequent genotyping. A set of 123 SNP markers, evenly distributed throughout the genome, was selected. This SNP collection was identified *in silico* in several re-sequencing projects reported in Blanca *et al.* (2011 and 2012), where 8 pools representing all the cultivated and wild melon types were analysed. This SNP set was previously employed with success in other characterization studies (Esteras *et al.*, 2013; Leida *et al*., 2015; Sabato *et al.*, 2015), being used to genotype the whole core collection of melons maintained in the COMAV (see Table S1). In this work, we generated the genotyping data of all the Brazilian, Indian and reference genotypes that had not been genotyped before with the same SNP set. This allowed us to study the genetic relationships of the assayed accessions with the full core collection (in total 227 genotypes). Basic information about these SNPs is supplied in Table S2 (Supporting information), and more detailed information is available in the Melonomics database (https://melonomics.net/) and in the consensus melon map (Díaz *et al*., 2015).

SNP genotyping was performed using the iPLEX ® Gold MassARRAY® Sequenom system at the Epigenetic and Genotyping unit of the University of Valencia (Unitat Central d´Investigació en Medicina (UCIM), University of Valencia, Valencia, Spain). This genotyping technology relies on single base extension (SBE) using mass-modified dideoxynucleotide terminators of an oligonucleotide primer that anneals immediately upstream of the polymorphic site of interest to generate different allelic products and uses the MALDI-TOF mass spectrometry to distinguish the mass of the different alleles (Gabriel *et al.*, 2009).

A Principal Coordinate Analysis (PCoA) was performed with GenAlEx 6.501 in order to study the genetic relationships among the assayed accessions, mainly between the new accessions phenotyped against *P. xanthii* and the previously reported resistance sources, and between them and the accessions included in the core collection that represent the diversity of the species (Table S1).

Also, a selected set of accessions (78, see Table S1) were employed to carry out a Cluster Analysis using the PowerMarker software (Liu and Muse, 2005). This subset of accessions comprised all the genotypes tested for powdery mildew resistance in the three greenhouse assays, including the differential hosts and accessions representative of each melon horticultural group. Nei's genetic distance (Nei *et al.*, 1983) and NJ method were used, and the support values for the degree of confidence at the nodes of the dendrogram were analysed by bootstrap re-sampling 1,000 times. Phylip 3.69 software (Felsenstein, 1997) was employed to construct the consensus tree and TreeView32 (Page, 1996) to visualize it.

Results

Valencia 2013 assay

Twenty-five days after the inoculation, the differential cultivars Vedrantais and PMR 45 were highly susceptible, showing severe symptoms of the disease, and the breeding lines AR Hale's Best Jumbo and WMR 29 were susceptible (Table 1). The accessions, cultivars and breeding lines Edisto 47, PI 124112, PMR 5, PMR 6, and PI 414723 behaved as resistant. The susceptibility of Vedrantais, PMR 45 and WMR 29 and the resistance of Edisto 47 would suggest the presence of race 4. MR-1 behaved as moderately resistant, with some plants scored as resistant and other as susceptible (score range 3-7). .

A significant effect of the accessions was found (χ^2 = 595.99, p <0.001) for their reaction to *P. xanthii*. The accessions assayed were grouped into four classes as highly susceptible, susceptible, moderately resistant and resistant (Table 1). Most of the accessions (79%) were susceptible or highly susceptible, being many of them (57%) highly susceptible, with average scores ranging from 7.0 to 9.0 (abundant sporulation). Four accessions, from the core and Indian collections, (Ac-G22843Se, Con-GMJa, Con-Pat81Ko, and Mom-SM113Ind) and 13 Brazilian landraces were susceptible, with average scores ranging from 6 to 6.9. Some Indian wild types (Ag-WM19Ind, Ag-WM24Ind, and Ag-WM64Ind), the African Ac-TGR1843Zimb (=PI 482429), the Japanese Con-FreeCJa, and the Brazilian accessions AL-3, BA-2, CE-1, MA-6, PI-3, RN-9 were classified as moderately resistant, with average scores from 4 to 4.6. Certain variability in the response was detected in these accessions, with some plants scored as susceptible (scores of 6).

Six accessions were assessed as completely resistant (average scores 1-1.5): four Brazilian landraces of diverse origins (AL-1, BA-3, CE-3, and RN-2) and two Acidulus accessions, one from India (Ac-AM55Ind) and other from Zimbabwe (TGR1551).

Brazil 2015 assay (races 1, 3 and 5)

The accessions scored as resistant and the moderately resistant ones from the Brazilian collection in the Valencia 2013 assay, were also evaluated under greenhouse conditions against races 1, 3 and 5 in Brazil. All the accessions that were resistant in Valencia showed clear resistance against the three races (average score 1) (Table 2), whereas most of the Brazilian accessions moderately resistant in Valencia were susceptible against the three races in Brazil (average scores 3-4), also showing certain variability within accession. Only the RN-9 accession, moderately resistant in Valencia 2013, showed resistance to the three races employed in Brazil 2015. The Indian accession PI 313970, used as reference, was also resistant to all the races tested. In general, the reaction of the differential hosts was the expected for the three races, except for the susceptible reaction of Edisto 47 to race 3.

Valencia 2016 assay (race 3.5)

The accessions showing resistance in both, Valencia 2013 and Brazil 2015 assays, and the reference PI 313970, were also tested against the race 3.5 (Table 2). The Brazilian accessions AL-1, BA-3 and RN-2, as well as the Indian Ac-AM55Ind and PI 313970 were clearly resistant (average scores 1 to 1.3). The accession CE-3 showed some variability in its response to powdery mildew, with plants displaying scores from 1 to 3. Among the differential hosts, the Edisto 47 was scored as resistant, but showed both resistant and susceptible plants (scores ranging from 1 to 4), whereas the response of the other differential hosts was that expected for the race 3.5.

Field assays

Recent surveys carried out in 2015 seem to point races 1, 3, 5 and 3.5 of *P. xanthii* as the most frequent ones in Mossoró, Baraúna, Assú and other melon-producer areas in Rio Grande do Norte State in Brazil (unpublished). According to the race differentials assayed in this work, race 3 seems to be the predominant one at least in Mossoró (Table 3). However, similarly to Valencia

2013, MR-1 showed resistant and susceptible plants (scores from 3 to 7). Also PI 124112 showed this variable behaviour. The races present in Mossoro and Assú overcame the resistance of the accession PI 313970, but PI 414723 was resistant in all the localities.

The accessions with the best response to the powdery mildew Brazilian populations in the field were AL-1, BA-3, CE-3, RN-2, and Ac-AM55Ind, which were fully resistant in all locations (average score of 1) (Table 3). Nearly all the accessions selected as moderately resistant in the first assay ranged mainly from susceptible to highly susceptible (average scores from 4.3 to 9) in the field, showing in general much more variability in their response than in the greenhouse assays (Table 3).

Molecular analysis

The molecular analysis was done with the same set of SNPs employed previously to analyse the full core collection, representing all the melon horticultural groups, so the relationships among all accessions could be studied. Genotyping results are shown in Table S3 (Supporting information). The first three coordinates of the PCoA explained 45.3%, 4.5% and 3.7% of the total variation respectively (Fig. 1). Accessions belonging to subsp. *melo* and subsp. *agrestis* were clearly separated according to the first coordinate, while the second coordinate separated accessions according to their horticultural groups or origin within each subspecies.

Three groups of Brazilian accessions could be distinguished, one within subsp. *melo* accessions (Group I), another within subsp. *agrestis* (Group II) and the third one in an intermediate position between both subspecies (Group III). Most of the accessions in the Group I (MA-2, MA-5, PB-1, PB-2, PE-3 and RN-6) were susceptible or highly susceptible to the fungus, except the resistant/moderately resistant RN-9. They were molecularly close to Chate (Chate-CarIta, Chate-CarBIta) and Flexuosus types (Flex-AlficozSp), French Cantalupensis (Can-NCFran, Can-PresFran, Can-NYIsr, Can-CAFran, and Can-SucrFran), and some unclassified landraces with diverse origins. This group was located between most Cantalupensis types in the upper part of the PCoA and most Inodorus, Ameri and subsp. *melo* landraces in the bottom according to the second coordinate. The Brazilian accessions included in Group II were dispersed among subsp. *agrestis* accessions. In this group most of the Brazilian accessions displayed a resistant/moderately resistant response to *P. xanthii* (AL-1, AL-3, CE-3, MA-6, PI-3, and RN-2).

Accessions in this group were molecularly similar to the wild *agrestis* and to the group of Acidulus and Tibish, most African and Indian, that also included the resistant accessions Ac-AM55Ind, TGR1551 and PI 313970. A few accessions of this group (PE-1, PE-2, SE-2), were highly susceptible to *P. xanthii*, and similar to the accessions of the Conomon group from Far Eastern countries. The remaining Brazilian accessions (Group III) were interspersed with the Indian Momordica of the core collection and with the resistant Momordica used as differential hosts PI124112, PI414723 and MR-1). Most of the accessions in this group were susceptible to the fungus except BA-2 and BA-3.

To better observe the relationships of the new selected resistant sources with the previously reported accessions, we constructed a dendrogram using only the genotypes assessed for powdery mildew resistance (Fig. 2). This Cluster Analysis confirmed that most of the highly susceptible Brazilian accessions belong to subspecies *melo* or are similar to the Conomon group of the subsp. *agrestis,* whereas most of the resistant accessions are closer to wild *agrestis*, especially Indian wild forms, Acidulus and some Momordica types. All the new Brazilian resistance sources were molecularly different from all the resistant genotypes of reference and the differential hosts used in this study.

Discussion

Cucumis melo exhibits high levels of diversity in morphological, physiological and biochemical properties (Burger *et al*., 2010; Esteras *et al*., 2013; Leida *et al*., 2015; Pitrat 2016). In addition, diversity within the species has been reported in the response to different diseases (Fergany *et al*., 2011; Ambrósio *et al*., 2015, López *et al*., 2015). Although recent studies locate a close relative of wild melons in Australia (Sebastian et al., 2010), most studies agree with the fact that the highest levels of variation are in the Indian subcontinent, considered the main domestication center for the species (Roy et al., 2012, Dhillon et al., 2012; Pitrat 2016). Brazil has not been considered as a diversification center for melons, however a large diversity (in seed, flower and fruit traits) was recently reported in a collection of landraces from the northeast of the country by Dantas *et al*. (2015). In our work, diversity in the response to the most important races of *P. xanthii* affecting melon crops in Brazil and Spain has been found within this Brazilian collection.

In this study, we used a set of differential hosts to determine the races used in each assay. Some races had been previously characterized, and in most cases the response of the differential hosts confirmed the presence of the corresponding race. However, we found variation in the response of the breeding line MR-1, that was expected to be resistant to race 4, the most probable race present in the isolate used in Valencia 2013. MR-1 and PI 124112 gave also variable response in Brazilian fields, even though they were expected to be resistant to race 3. This variability could be due to the occurrence of genetic variation within the accessions. However, all the accessions used in this study were reproduced by several selfing cycles, so a large intra-accession variation is not expected. The occurrence of race mixtures in the *P. xanthii* population (for example the presence of race 3.5 or other uncharacterized races in Brazilian fields) and the differential aggressiveness of fungal isolates may also account for part of this variation. MR-1 and PI 124112 are accessions in which genes of resistance to other *P. xanthii* races have been reported. The occurrence of defeated race-specific resistance genes could also alter the response of these genotypes, as it has been recently proposed for lettuce response to powdery mildew (Simko *et al.,* 2014). We also found discrepancies in the response of Edisto 47. This was the melon cultivar used to define race 3 (Thomas, 1978). However, in Brazil 2015 this cultivar was scored as susceptible after the inoculation with the race 3 provided by Sakata. We have also found variable results of the response of Edisto 47 to race 3 in the literature. In fact, Pitrat et al. (1998) reported this cultivar as susceptible to race 3, but later studies describe it as resistant (Hosoya et al., 2000; Pitrat 2008). In Valencia 2016, Edisto 47 also showed variable results even though it was expected to be susceptible to race 3.5 (McCreight et al., 2012).

Despite the variable response of some differential host, several Brazilian landraces consistently showed high levels of resistance to the fungus, suggesting that Brazilian germplasm diversity could be a great source of variability for melon breeding. The whole Brazilian collection assayed here for *P. xanthii* resistance was previously molecularly characterized with a limited set of SSRs (Dantas *et al*., 2015). This analysis suggested a close genetic relatedness of the Brazilian germplasm to different melon accessions coming from the centers of origin and primary diversification of melons, mainly with accessions from the Flexuosus/Chate, Momordica, and Conomon groups. In the present study, much more reference accessions have been included to better represent the diversity of melons, and a 123-SNPs genotyping platform has been used. Our molecular results confirmed the previously observed diversity of these Brazilian accessions, and

allowed a more accurate classification of them. PCoA results for the whole melon collection are coherent with the classical clusters obtained in previous genetic diversity studies (Esteras *et al*., 2013; Leida *et al*., 2015; Nimmakayala *et al*., 2016). Brazilian accessions represented most of the species diversity, and three groups could be distinguished: one within subsp. *melo* (along with Chate, Flexuosus and Ameri types, representing old landraces from Central Asia and North Africa (Blanca *et al*., 2012), and some French Cantaloupe varieties), a second one within subsp. *agrestis* (along with African and Asian wild *agrestis*, Acidulus, and Tibish, and with Far Eastern Conomon), and a third group in the intermediate position (along with the Momordica accessions).

Most of the Brazilian accessions found to be resistant to *P. xanthii* in the present study are within the subsp. *agrestis* group. In fact, the only Brazilian accession molecularly grouped within the subsp. *melo* that displayed resistance against several races and isolates, in greenhouse and field assays, was RN-9. This accession is morphological and molecularly similar to the Chate/Flexuosus horticultural group (Dantas *et al*., 2015), which has been suggested to be one of the first cultivated forms of melons, especially in the Mediterranean region, displaying high genetic variation (Esteras *et al*., 2013; Leida *et al*., 2015). Resistance to *P. xanthii* is not usually found within subsp. *melo*, but some Asian snakemelons (Flexuosus group) were reported to be heterogeneous for the resistance to *P. xanthii* (Dhillon *et al.*, 2012). The other accessions included in this study that are resistant to *P. xanthii* and belong to the subsp. *melo* are some differential hosts, that are indeed cantaloupe cultivars and breeding lines with resistance genes introgressed from the subsp. *agrestis* (*Pm-1* in PMR 45, PMR 5, and PMR 6; *Pm-2* in PMR 5 and PMR 6; *Pm-w* in WMR 29; *Pm-Edisto47-1* and *Pm-Edisto47-2* in Edisto 47) (Jagger et al., 1938; Bohn and Whitaker, 1964; Pitrat, 1991; Ning *et al*., 2014). These differential hosts clustered separately from RN-9 and the Chate/Flexuosus landraces, thus suggesting that RN-9 has a different genetic background. RN-9 is molecularly closer than the resistant sources of the subspecies *agrestis* to the most economically important melon horticultural groups grown in western countries (Inodorus and Cantalupensis), which makes this melon accession interesting for powdery mildew resistance breeding programs.

Four Brazilian accessions were highly resistant in all the assays (AL-1, BA-3, CE-3 and RN-2). All of them were similar to the Indian Momordica and to the African and Asian wild *agrestis* and Acidulus group, within the subspecies *agrestis*, in which the resistance to *P. xanthii* had been reported previously. Molecular results indicate that all these accessions are different from each other, and different from any of the accessions previously reported as resistant within the horticultural groups used in this study (carriers of the resistance genes *pm-S* in PI 313970; *Pm-3* and *Pm-6* in PI 124111 (the accessions from which MR-1 was developed); *Pm-x* in PI 414723; and *Pm-4* and *Pm-5* in PI 124112) (Dogimont, 2010-11).

Only three genotypes assayed in this study displayed similar resistance level to *P. xanthii* in all assays as these four resistant Brazilian accessions. All of them are Indian accessions, one Momordica (PI 414723) and two Acidulus (PI 313970 and Ac-AM55Ind). The accession PI 414723 has been reported to be resistant to most races of *P. xanthii*, but susceptible to races 2US and S (McCreight, 2003, 2006; McCreight and Coffey, 2011). Since PI 414723 remained resistant in all the assays, we assume that these races were not present in the four locations assayed in Brazil. PI 313970 has been also reported as resistant to many races (McCreight, 2003, 2006; reviewed by Bojórquez-Ramos *et al*., 2012 and by Maia, 2012), but in our study some Brazilian races present in Mossoró and Assú fields overcome its resistance. The third Indian accession (Ac-AM55Ind) was fully resistant in all conditions. Our molecular results indicated that this accession is quite similar to the African Ac-TGR1843Zimb and TGR1551. TGR1551 displays multiple resistances to virus, fungi and pests. It has been reported to carry a dominant gene (*Pm-R*, in LGV*)* and another recessive (probably in LGVIII) for resistance to *P. xanthii* races 1, 2 and 5 (see Table S1; Gómez-Guillamón *et al*., 2006; Yuste-Lisbona *et al*., 2011). Despite their closeness, Ac-AM55Ind and TGR1551 have molecular and morphological differences, so it remains to be studied if their resistance to powdery mildew has a common genetic basis.

These results indicate that Brazilian germplasm could be an important source of new alleles of resistance to *P. xanthii*. The genetic background of these new sources of resistance seems to be different to other previously studied genetic backgrounds (Gómez-Guillamón *et al*., 2006; Fergany *et al*., 2011; Roy *et al*., 2012), suggesting that their resistance to powdery mildew could

be controlled by different *loci*/alleles. Further studies on the genetic control of the resistance in these materials will be necessary.

Conclusions

Melon production is seriously affected by powdery mildew infection. The most important species causing this disease is *P. xanthii*, a fungus with high levels of diversity, which reduces the durability of the resistance in melon crops and makes necessary the searching of new suitable genotypes donors of resistance. The new Brazilian sources resistant to the most important races of *P. xanthii* affecting melon crops in two of the main melon producer countries and molecularly different to previously reported sources could carry new interesting resistance genes. Despite their more acidic and low-sugar flesh, the Brazilian landraces are highly productive, adapted to Brazilian cultivation conditions, and they have less non-desirable traits than melon wild types, so the use of these new resistance sources will encourage breeding programs in Brazil and Spain.

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Supporting information

Table S1. Acessions analysed in this work for resistance to several races to *P. xhantii* and SNP-genotyped. Information about the taxonomy, origin and previously assayed accessions is given.

Table S2. Information about the collection of 123 SNPs employed in the genotyping assay.

Table S3. Genotyping results for the accessions analysed.

Tables

Table 1. Average scores of disease severity on leaves and reaction of melon accessions inoculated in a greenhouse in Valencia in 2013 with an isolate of *P. xanthii* collected from Piel de Sapo in a greenhouse and maintained on this cultivar.

Accession	Score[1]	Reaction[2]	Accession	Score	Reaction
Brazilian germplasm			Core collection		
AL-1	1.0	R	Ac-AM55Ind	1.5 (1-2)	R
AL-2	6.2 (5-7)	S	Ac-G22843Se	6.5 (6-7)	S
AL-3	4.1 (3-6)	MR	Ac-TGR1843Zimb	4.0	MR
BA-1	7.5 (7-8)	HS	Ac-5394Zamb	7.5 (7-9)	HS
BA-2	4.4 (4-5)	MR	Ag-AHKInd	8.0	HS
BA-3	1.0	R	Ag-C38Nig	7.5 (7-9)	HS
BA-4	6.9 (6-8)	S	Ag-TayCam	7.5 (7-8)	HS
BA-5	6.3 (6-8)	S	Ag-TendSud	8.0 (7-9)	HS
CE-1	4.1 (4-5)	MR	Ag-WM3Ind	8.5 (8-9)	HS
CE-2	7.7 (7-8)	HS	Ag-WM7Ind	9.0	HS
CE-3	1.0	R	Ag-WM9Ind	8.5 (8-9)	HS
CE-4	6.0 (5-7)	S	Ag-WM19Ind	4.5 (4-6)	MR
MA-1	6.1 (5-7)	S	Ag-WM22Ind	8.0 (7-9)	HS
MA-2	7.2 (6-8)	HS	Ag-WM24Ind	4.5 (4-6)	MR
MA-3	6.2 (6-7)	S	Ag-WM44Ind	7.5 (7-9)	HS
MA-4	7.5 (7-9)	HS	Ag-WM64Ind	4.0	MR
MA-5	8.0	HS	Ag-15591Gha	8.5 (8-9)	HS
MA-6	4.4 (3-5)	MR	Ag-WChInd	7.0	HS
PB-1	8.8 (8-9)	HS	Am-KokUzb	9.0	HS
PB-2	6.1 (5-7)	S	Am-NanaGeorg	8.0 (7-9)	HS
PB-3	6.4 (6-7)	S	Can-GuCUSA	7.0 (6-8)	HS
PB-4	8.1 (8-9)	HS	Chi-VellInd	7.5 (7-8)	HS
PE-1	8.2 (6-9)	HS	Con-FreeCJa	4.5 (4-5)	MR
PE-2	8.5 (8-9)	HS	Con-GMJa	6.5 (5-8)	S
PE-3	8.5 (8-9)	HS	Con-Pat81Ko	6.5 (4-8)	S
PE-4	8.9 (8-9)	HS	Con-PauPol	8.0 (7-9)	HS
PI-1	7.1 (6-8)	HS	Con-SCKo	7.0	HS
PI-2	6.4 (5-7)	S	Dud-QPMAfg	8.0 (7-9)	HS
PI-3	4.2 (4-5)	MR	Flex-AcukTur	9.0	HS
RN-1	6.1 (5-7)	S	Flex-SilkaSud	8.5 (8-9)	HS
RN-2	1.0	R	Flex-SnakeSA	9.0	HS
RN-3	6.0 (5-7)	S	In-PsPiñSp	7.5 (7-8)	HS
RN-4	6.2 (5-7)	S	La-TGR96Zimb	7.5 (7-8)	HS

RN-5	8.3 (8-9)	HS	Mom-AM7Ind	9.0	HS
RN-6	8.2 (7-9)	HS	Mom-AM78Ind	8.0	HS
RN-7	8.7 (7-9)	HS	Mom-SM81Ind	7.0 (6-8)	HS
RN-8	6.0	S	Mom-SM113Ind	6.0 (5-7)	S
RN-9	4.6 (4-6)	MR	Tibish-KSud	9.0	HS
SE-1	7.5 (7-9)	HS	TGR1551	1.5 (1-3)	R
SE-2	8.3 (8-9)	HS			
Race differential genotypes					
Vedrantais	9.0	HS	Edisto 47	2.5 (2-3)	R
PMR 45	7.0 (6-8)	HS	PI 414723	1.0	R
PMR 5	1.0	R	MR-1	5.5 (3-7)	MR
PMR 6	1.0	R	PI 124112	1.5 (1-2)	R
WMR 29	6.1 (5-7)	S	AR Hale's Best Jumbo	6.5 (5-8)	S
χ^2	595.99 ($p < 0.001$)[3]				

[1] Score mean for the accessions tested and score range in parenthesis when there was variation. Score scale according McCreight (2003) (see material and methods for description).

[2] Classification of reaction as resistant (R) [score mean: 1-3.9], moderately resistant (MR) [4-5.9], susceptible (S) [6-6.9] and highly susceptible (HS) [7-9].

[3] Chi-square (χ^2) by Kruskal-Wallis' test.

Table 2. Average scores of disease severity on leaves and reaction of melon accessions inoculated in a greenhouse with races 1, 3, 5 (Brazil, 2015) and 3.5 (Valencia, 2016) of *P. xanthii*.

	Race 1		Race 3		Race 5		Race 3.5	
Accession	Score[1]	Reaction[2]	Score	Reaction	Score	Reaction	Score	Reaction
AL-1	1.0	R	1.0	R	1.0	R	1.2 (1-2)	R
AL-3	3.0 (2-4)	S	3.0 (2-4)	S	3.0	S		
BA-2	3.0 (2-4)	S	3.0	S	3.0	S		
BA-3	1.0	R	1.0	R	1.0	R	1.1 (1-2)	R
CE-1	4.0	S	3.0	S	4.0	S		
CE-3	1.0	R	1.0	R	1.0	R	1.8 (1-3)	R
MA-6	3.0 (2-4)	S	3.0 (1-4)	S	4.0	S		
PI-3	4.0	S	3.0	S	3.0 (2-4)	S		
RN-2	1.0	R	1.0	R	1.0	R	1.3 (1-2)	R
RN-9	1.0	R	1.0	R	1.0	R		
Ac-AM55Ind	1.0	R	1.0	R	1.0	R	1.0	R
Differential genotypes								
Vedrantais	4.0 (1-4)	S	4.0	S	3.5 (3-4)	S	3.2 (3-4)	S
PMR 45	1.0	R	4.0	S	4.0	S	3.0 (3-4)	S
PMR 5	1.0	R	4.0	S	1.0	R	3.0 (3-4)	S
PMR 6	1.0	R	4.0	S	1.0	R		
WMR 29	1.0	R	4.0	S	3.0 (2-4)	S	3.0 (3-4)	S
Edisto 47	1.0	R	4.0	S	4.0	S	2.1 (1-4)	R
PI 414723	1.0	R	1.0	R	1.0	R	1.0	R
MR-1	1.0	R	1.0	R	1.0	R		
PI 124112	1.0	R	1.0	R	1.0	R	3.9 (3-4)	S
PI 313970	1.0	R	1.0	R	1.0	R	1.0	R

[1] Score mean for the accessions tested and score range in parenthesis when there was variation. Scores scale according to Yuste-Lisbona *et al*. (2010) (see material and methods for description).

[2] Classification of reaction in resistant (R: scores 1-2) and susceptible (S: scores 3-4) according Yuste-Lisbona *et al.* (2010).

Table 3. Average scores of disease severity on leaves and reaction of melon accessions to *P. xanthii* evaluated in field conditions in four sites of Brazil.

	Mossoró (2014)		Mossoró (2015)		Assú (2014)		Baraúna (2015)	
Accession	Score[1]	Reaction[2]	Score	Reaction	Score	Reaction	Score	Reaction
AL-1	1.0	R	1.0	R	1.0	R	1.0	R
AL-3	6.7 (6-8)	S	6.0 (5-7)	S	6.1 (6-7)	S	4.3 (4-5)	MR
BA-2	8.1 (8-9)	HS	9.0	HS	8.1 (7-9)	HS	6.1 (5-7)	S
BA-3	1.0	R	1.0	R	1.0	R	1.0	R
CE-1	7.2 (6-8)	HS	7.0 (6-8)	HS	7.7 (7-8)	HS	4.9 (4-7)	MR
CE-3	1.0	R	1.0	R	1.0	R	1.0	R
MA-6	6.6 (6-7)	S	6.0 (5-6)	S	7.0 (6-9)	HS	6.3 (6-7)	S
PI-3	6.7 (6-8)	S	6.3 (4-8)	S	6.5 (5-7)	S	6.1 (6-7)	S
RN-2	1.0	R	1.0	R	1.0	R	1.0	R
RN-9	1.0	R	2.1 (1-3)	R	4.9 (4-6)	MR	4.7 (4-6)	MR
Ac-AM55Ind	1.0	R	1.0	R	1.0	R	1.0	R
Race differentials								
Vedrantais	7.8 (6-9)	HS	8.1 (8-9)	HS	8.5 (7-9)	HS	9.0	HS
PMR 45	6.4 (5-8)	S	6.8 (4-9)	S	6.2 (4-9)	S	8.3 (7-9)	HS
PMR 5	6.9 (5-8)	S	6.2 (5-8)	S	7.2 (6-9)	HS	6.6 (5-8)	S
PMR 6	6.9 (5-9)	S	6.7 (4-8)	S	7.1 (5-9)	HS	7.3 (6-9)	HS
WMR 29	5.6 (4-6)	S	6.0 (4-7)	S	7.3 (6-8)	HS	4.7 (3-7)	MR
Edisto 47	6.0 (5-8)	S	6.1 (4-7)	S	4.0 (3-5)	MR	4.1 (3-5)	MR
PI 414723	1.0	R	1.0	R	1.0	R	1.0	R
MR-1	4.1 (3-6)	MR	4.2 (3-7)	MR	4.1 (3-6)	MR	2.4 (2-3)	R
PI 124112	4.4 (3-7)	MR	4.6 (3-6)	MR	6.3 (5-8)	S	4.0 (3-6)	MR
PI 313970	4.5 (3-7)	MR	1.0	R	4.6 (4-7)	MR	1.0	R

[1] Score mean for the accessions tested and score range in parenthesis when there was variation. Score scale according McCreight (2003).

[2] Classification of reaction as resistant (R) [score mean: 1-3.9], moderately resistant (MR) [4-5.9], susceptible (S) [6-6.9] and highly susceptible (HS) [7-9].

Figures

Fig. 1. Principal Coordinate Analysis (PCoA) performed using the 123 SNP platform to determine the genetic relationships of the Brazilian landraces (Br) and the whole melon collection. The symbols and colors in the legend indicate the respective horticultural group. The three groups detected within the Brazilian subset are indicated, as well as the accessions reported as resistant (in red) or moderately resistant (in orange) in the Valencia 2013 assay and other accessions bearing resistance genes according to previous studies (in red and underlined).

Fig. 2. NJ tree based on 123-SNP genotyping showing relationships among the resistant and moderately resistant accessions found in this study with representative types including previously reported resistant genotypes. Four groups are indicated in different color: Resistant (red), moderately resistant (orange), susceptible (black) and highly susceptible (purple) according to Valencia 2013 assay (including race differentials). Bootstrap values higher than 500 are shown. The five Brazilian and the Indian accessions found resistant in this work are highlighted with bold font.

* Tested in Brazil 2015 and Valencia 2016 assays (not tested in Valencia 2013).

**Representative accessions of a specific horticultural group.