horticulturae

MDPI

Article

Resistance of Tunisian Melon Landraces to *Podosphaera xanthii*

Hela Chikh-Rouhou [1,*], Ana Garcés-Claver [2,3], Lydia Kienbaum [4,5], Abdelmonem Ben Belgacem [1] and Maria Luisa Gómez-Guillamón [6]

1 Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB) BP 57, Chott-Mariem LR21AGR03-Production and Protection for a Sustainable Horticulture, University of Sousse, Sousse 4042, Tunisia
2 Department of Plant Science, Agrifood Research and Technology Centre of Aragon (CITA) Avda. Montañana 930, 50059 Zaragoza, Spain
3 AgriFood Institute of Aragon—IA2 (CITA-University of Zaragoza), 50059 Zaragoza, Spain
4 Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, 70599 Stuttgart, Germany
5 Selecta One (Klemm & Sohn GmbH & Co. KG), Hanfäcker 10, 70378 Stuttgart, Germany
6 Instituto de Hortofruticultura Subtropical y Mediterranea-La Mayora, CSIC-UMA, Avda, Dr. Wienberg s/n., Algarrobo, 29750 Malaga, Spain
* Correspondence: hela.chikh.rouhou@gmail.com

Abstract: Powdery mildew caused by *Podosphaera xanthii* is among the most threatening fungal diseases affecting melons on the Mediterranean coast. Although the use of genetic resistance is a highly recommended alternative to control this pathogen, many races of this fungus have been described and, therefore, resistance is usually overcome; thus, breeding for resistance to this pathogen is a challenge. Several melon genotypes carrying resistance to powdery mildew have been described but their agronomical and fruit characters are usually far away from the required melon types in many commercial markets. Taking this into consideration, looking for novel sources of resistance in Tunisian landraces is a very convenient step to obtain new resistant melon varieties/hybrids suitable for Mediterranean markets. Several Tunisian melon landraces have been tested against three common races in Mediterranean regions (Race 2, Race 3.5, and Race 5), using phenotypic approaches in two independent experiments (artificial inoculations in a growth chamber and natural conditions of infection in a greenhouse). The results of the artificial inoculations showed that all the tested landraces were susceptible to Race 3.5 and Race 5 and several landraces were resistant to Race 2. Under natural conditions of infection, Race 2 of *P. xanthii* was the race prevalent in the plot and the resistance of TUN-16, TUN-19, and TUN-25 was confirmed. The found resistances were race-specific and underlie a high genetic influence reflected in the high value of the estimated heritability of 0.86. These resistant landraces should be considered as a potential source of resistance in breeding programs of melons belonging to inodorus and reticulatus groups, but further research is necessary to elucidate the genetic control of the found resistances and to provide useful molecular markers linked to *P. xanthii* Race 2 resistance.

Keywords: powdery mildew; phenotypic evaluation; heritability

Citation: Chikh-Rouhou, H.; Garcés-Claver, A.; Kienbaum, L.; Ben Belgacem, A.; Gómez-Guillamón, M.L. Resistance of Tunisian Melon Landraces to *Podosphaera xanthii*. *Horticulturae* **2022**, *8*, 1172. https://doi.org/10.3390/horticulturae8121172

Academic Editors: Hillary Righini, Roberta Roberti and Stefania Galletti

Received: 7 October 2022
Accepted: 1 December 2022
Published: 9 December 2022

1. Introduction

Powdery mildew is a major problem in melon production worldwide since it occurs all year round regardless of the growing system in cultivated fields or greenhouses [1]. The disease is caused by two pathogens: *Podosphaera xanthii* (Castagne) U. Braun and Shishkoff (synonym *Sphaerotheca xanthii* (Castagne) L. Junell) and *Golovinomyces orontii* (DC) V.P. Heluta (synonyms *Erysiphe cichoracearum* DC, *G. cichoracearum* DC). The most prevalent species is *P. xanthii* but in some countries, *G. orontii* can also be found [2,3]. *P. xanthii* occurs more frequently in temperate subtropical and tropical areas, while *G. orontii* occurs more frequently in temperate areas under field conditions [2]. The infection is evident by the

development of white mycelia on leaves and stems and, in severe cases, also affects fruits and floral structures. Severely infected leaves may become chlorotic, or even necrotic and brittle. As a consequence, the fungus causes a reduction in plant growth, premature desiccation of the leaves, and consequent reduction in the quality and marketability of the fruits [3]. No previous research has been published on the incidence and pathogenic variability of powdery mildew in melon crops in Tunisia. However, it has been observed during surveys that the severity of the attacks is devasting if not treated.

Spraying chemicals is the most extended method used to control this disease but the fungus is able to develop resistance, leading to a lack of efficiency in chemical control in many cases [4,5]. So, the genetic control of resistance against powdery mildew fungi represents a serious challenge to researchers and breeders. Breeding of melons for resistance to powdery mildew is hindered partially due to the diverse genetic pool from which many sources of genetic resistance have been identified [1,6].

Many physiological races of *P. xanthii* have been identified according to their reactions on different melon lines [1,3]. The predominant race in powdery mildew populations changes depending on the melon cultivar, the growing season, and the geographical area [7]. It has been suggested that a large fraction of the reported races are not relevant to the majority of *P. xanthii* resistance breeding, which may have to be performed on a regional basis for subsets of races. Only a limited number of races highly harmful to cucurbit crops are distributed over a large area [1]. Races 1, 2, 3.5, and 5 are reported to be the most extended in Southern European and Mediterranean regions [8–10]. Resistance to Race 1 could be controlled by one recessive gene in the Indian line 'PI313970′ [6], while resistance to Race 2 is more complex, and both partial and total resistances have been described [10]. Resistance to Race 5 has been described under monogenic and dominant control in the Indian melon line 'PI124112′ [11], and Yuste-Lisbona et al. [10] described a dominant–recessive epitasis controlling resistance to Race 5 as well as to Races 1 and 2 in the Zimbabwean melon line 'TGR-1551′. So far, no resistance against Race 3.5 has been published/studied, although in current experiments carried out by the International Seed Federation Disease Resistance Terminology Working Group (ISF DRT WG), melon lines PI313970, PI124112, Arum, and SV1105, among others, have shown complete or intermediate resistance to several isolates of this race [12]. Most of the sources of resistance to *P. xanthii* originate from India (e.g., PI124112, PI414723, PI134198, PI313970) and several genes have been described controlling mainly Races 1, 2, and 5 [10,13]. Although most of them are race-specific genes, there are melon genotypes carrying genes controlling resistance to more than one race (e.g., PI124112, PI414723, PI313970, TGR-1551). To date, 19 powdery mildew resistance genes and 12 major quantitative trait loci (QTLs) associated with this trait have been reported [6,11,14,15].

Breeding programs require sources of resistance and a few have been reported in melon, mostly in *momordica* and *acidulus* horticultural groups [10,16]. Vegetable seed companies are using these sources of resistance to develop commercial melon varieties with resistance to *P. xanthii* [12]. However, the existence of many races reducing the durability of the resistance [1,3] makes it necessary to find new resistant genotypes with different genetic backgrounds. Local landraces represent a valuable genetic resource for breeding in a changing environment. They exhibit fine adaptation to the specific environment in which they have evolved under years of domestication. The agronomic traits and fruit characteristics of Tunisian landraces are similar to the commercial types demanded by Mediterranean markets, and they also carry adaptation to the environment and cultivation methods [17,18]. Thus, Tunisian melon germplasm, unexplored to date for powdery mildew resistance, could be of great potential and should be exploited. This study is aimed at identifying novel sources of resistance in Tunisian local germplasm for powdery mildew resistance, in order to obtain new resistant melon varieties/hybrids adapted to their cultivation in Mediterranean areas.

2. Materials and Methods

2.1. Plant Material and Fungal Isolates

Tunisian melon landraces belonging to different botanical groups, collected from local farmers of the Centre-East region of the country and maintained at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB, Tunisia), were used in the experiments (Table 1). Most of these landraces have been characterized for their morpho-agronomic traits [18], fruit quality [19], and also evaluated for their resistance to Fusarium wilt and *A. gossypii* [17,20].

Table 1. Tunisian melon landraces used and their main characteristics regarding some biotic stress resistances.

Codes	Accessions	Horticultural Group	Resistance to Fusarium wilt [17]	Resistance to Aphid [20]
TUN-2	Maazoun Menzel Chaker	*inodorus*	+	−
TUN-3	Maazoun Mehdia (MM2009)	*inodorus*	+	−
TUN-4	Maazoun Fethi	*inodorus*	−	−
TUN-5	Fakous (FL)	*flexuosus*	+	−
TUN-7	Trabelsi	*inodorus*	−	−
TUN-8	Galaoui	*reticulatus*	−	−
TUN-9	Dziri (DZ P5 2011)	*inodorus*	+	−
TUN-13	Arbi1	*inodorus*	−	−
TUN-16	Sarachika	*inodorus*	+	−
TUN-18	Rupa	*cantalupensis*	+	−
TUN-19	Chamem (Ananas type)	*reticulatus*	+	+
TUN-24	Maazoun (Kairouan)	*inodorus*	−	−
TUN-25	Asli	*inodorus*	−	−
TUN-26	Stambouli	*inodorus*	+	−

(+) and (−) indicate the presence and absence of resistance, respectively.

A set of differential melon lines were used to confirm and identify the race in both assays (artificial and natural conditions of infection): 'PMR-45′, resistant to Races 0 and 1, and susceptible to Races 2, 3, 3.5, 4, and 5 of *P. xanthii;* 'Edisto-47′, resistant to Races 0, 1, 2, 3, and 4 and susceptible to Races 3.5 and 5; 'PMR-5′, resistant to Races 0, 1, 2, 3, 4, and 5 and susceptible to Race 3.5; and 'PI414723′, with resistance to all races. The Spanish cultivar 'Bola de Oro', susceptible to all *P. xanthii* races, was used as a susceptible control. The identification of the species causing powdery mildew in the greenhouse was conducted by examination of conidia for the presence of fibrosin bodies and the production of forked germ tubes [3,21].

Three races of *P. xanthii* were used in the artificial inoculations: Race 2 (isolate SF30), Race 3.5 (isolate M23), and Race 5 (isolate C8), which are the most frequent and widespread races in melon production in Mediterranean areas. These isolates came from melon crops from different areas of Spain (Almeria, Murcia, and Malaga, respectively) and were maintained in axenic conditions on cotyledons of the zucchini cultivar 'Black Beauty' and the Spanish susceptible melon cultivar 'Amarillo' as described by Yuste-Lisbona et al. [10].

2.2. Phenotypic Evaluation by Artificial Inoculations

Seeds of the melon accessions were sown into sterilized sand and grown in a growth chamber under 24 °C day/16 °C night conditions with a 16:8 h (light:dark) photoperiod. Fourteen accessions and twelve plants per accession were inoculated. For artificial inoculations of Races 2 and 5, inoculation was carried out by depositing a small amount of conidia, taken from two–three infected cotyledons maintained in axenic conditions, at two spots (at each side of, and equidistant from, the main leaf vein) on the second true leaf of each plant [10] (Figure 1A). Once inoculated, plants were maintained in a controlled growth chamber under 32 °C day/22 °C night conditions with a 16:8 h (light:dark) photoperiod.

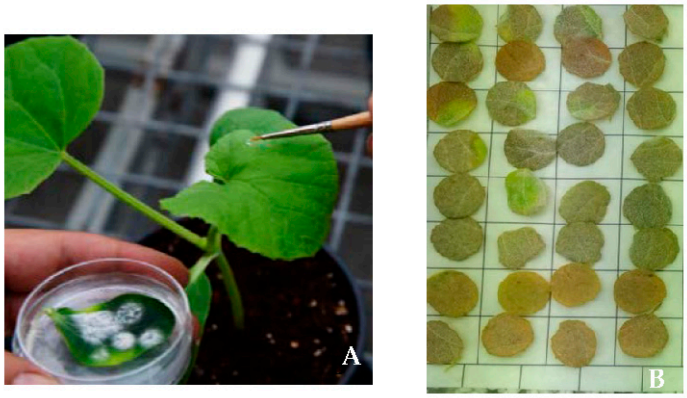

Figure 1. (**A**) Plants inoculated artificially by depositing a small amount of conidia at two spots on the second true leaf of melon plants. (**B**) Leaf disk inoculation method.

Inoculations with Race 3.5 were carried out on leaf discs (Figure 1B). For the leaf disc inoculation method, one leaf disc of 18 mm in diameter was taken from the second true leaf of each plant at the 3-4 leaf stage; discs were placed adaxially upwards on moistened filter paper in clear polystyrene boxes. Each box was placed on the base of a settling tower of plastic (124 × 580 × 580 mm) and a cover with a centered hole of 150 mm of diameter. Inoculation was performed by blowing, through the hole, the conidia present on the surface of two–three infected cotyledons, obtained in axenic conditions. Inoculated discs were incubated in growth chambers under 25 °C/18 °C day/night conditions and a 12 h photoperiod as suggested by [22,23].

Inoculations were repeated two times for each race. For each accession and *P. xanthii* race, symptoms were evaluated 12–15 days post-inoculation. Plants were scored according to the level of fungus sporulation, using a scale of 0 to 3 [10] as follows: 0 = no visible sporulation; 1 = low level of sporulation; 2 = moderate level of sporulation; and 3 = profuse sporulation. Melon accessions with a mean disease score between 0 and 1 were considered resistant (R) (the infection did not progress), whereas those >1, where the infection progressed, were considered susceptible (S) [10].

2.3. Phenotypic Evaluation under Natural Infection

Accessions were evaluated under normal daylight conditions in a greenhouse at the Experimental Station of Sahline, Tunisia (N 35°45′02″, E 10°42′44″). Tun-9 and Tun-18 were not included in the assay due to the poor number of available seeds. The experiment was carried out, in a randomized complete block design with three replications of 6 plants each, and was repeated two times. The evaluation was performed when all plants of the *P. xanthii*-susceptible control showed severe symptoms of infection. The percentage of affected plants was scored for each melon accession.

Powdery mildew on each infected leaf was evaluated based on the following rating scale: 0 = no disease; 1 = less than 5% of the leaf area infected; 2 = 5% to 20% of the leaf area with symptoms; 3 = 21% to 50% of the leaf area infected; and 4 = more than 50% of the leaf area infected. For each melon accession, the mean and standard error of the disease score were calculated. The disease incidence was also calculated for both methods (artificial inoculation and natural infection) as follows:

$$D(\%) = \frac{\sum(n_i\, X_i)}{(4 * N)} \times 100$$

where:

X_i: the rating scale;

n_i: the number of plants with the same rating scale;

N: total number of plants.

2.4. Statistical Analyses

To analyze the responses of the accessions and the influence of the methodology used (artificial inoculation in the growth chamber or natural conditions of infection) on the response to the disease caused by *P. xanthii* Race 2, an ANOVA using Type III error was performed. All analyses were carried out using R 4.1.2 [24] with the statistical packages emmeans [25], lme4, and car. For plotting the results, the R library ggpplot2 [26] was used. Model selection by Akaike's Information Criterion (AIC) resulted in the set-up of a final model:

$$y_{ijk} = \mu + m_i + mb_{ij} + a_k + ma_{ik} + e_{ijk}$$

where:

μ = general effect;
m_i = effect of the i-th method;
mb_{ij} = nested effect of the i-th method within the j-th block;
a_k = effect of the k-th accession;
ma_{ik} = interaction effect between the i-th method and the k-th accession;
e_{ijk} = residual error of observation y_{ijk}.

Cullis broad-sense heritability [27] was calculated from the model estimations to analyze the ratio of genetic variance compared to the total phenotypic variance observed. The least squares means were obtained for the significant interaction effect between accessions and methods and were compared in the form of a letter display using the Tukey test ($p < 0.05$). BLUP (best linear unbiased prediction) estimates were calculated across both methods for the estimation of the pure genetic effects of the accessions regarding their response to *P. xanthii* Race 2. Additionally, the estimated genetic values were calculated and correlation analyzed to estimate the relationship between the performance of accessions under the two methodologies (artificial inoculation and natural conditions of infection).

3. Results

3.1. Phenotypic Evaluation by Artificial Inoculations

All plants of the susceptible control 'Bola de Oro' showed symptoms when inoculated with Races 2, 5, and 3.5; five Tunisian accessions, TUN-9, TUN-16, TUN-18, TUN-19, and TUN-25, were found to be resistant to Race 2 since they did not show any powdery mildew symptoms or the disease did not progress ($\leq$1) (Figure 2; Table 2); however, no resistance to Race 5 nor to Race 3.5 was found as all of the landraces showed highly susceptible responses (> to 2.5).

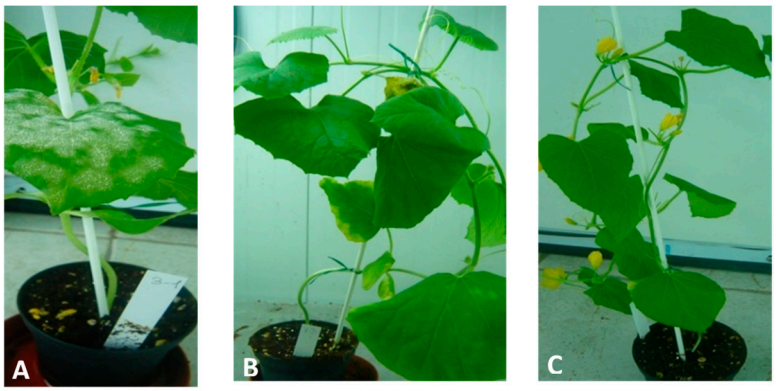

Figure 2. (**A**) Symptoms in susceptible control 'Bola de Oro' in comparison to the resistant accessions, (**B**) Tun-19 (Chamem) and (**C**) Tun-25 (Asli). All of them were inoculated with Race 2 of *P. xanthii*, with subsequent symptom evaluation 15 days post-inoculation in a growth chamber. Plants were scored according to the level of fungus sporulation using a scale of 0 to 3.

Table 2. Phenotypic evaluation of the resistance to *P. xanthii* Races 2, 5, and 3.5, by artificial inoculation in growth chamber conditions. Values are mean (n = 12) ± SE.

	Disease Score			**Reaction Classification** *		
	Race 2	**Race 5**	**Race 3.5**	**Race 2**	**Race 5**	**Race 3.5**
TUN-2	3.00 ± 0.00	2.85 ± 0.12	2.79 ± 0.39	S	S	S
TUN-3	1.30 ± 0.30	2.65 ± 0.18	3.00 ± 0.00	S	S	S
TUN-4	1.50 ± 0.25	3.00 ± 0.00	2.75 ± 0.39	S	S	S
TUN-5	1.10 ± 0.20	3.00 ± 0.00	-	S	S	-°
TUN-7	3.00 ± 0.00	2.85 ± 0.14	2.95 ± 0.14	S	S	S
TUN-8	2.70 ± 0.20	3.00 ± 0.00	2.83 ± 0.40	S	S	S
TUN-9	1.00 ± 0.50	3.00 ± 0.00	-	R	S	-
TUN-13	1.40 ± 0.40	2.60 ± 0.20	3.00 ± 0.00	S	S	S
TUN-16	0.00 ± 0.00	2.50 ± 0.24	3.00 ± 0.00	R	S	S
TUN-18	0.00 ± 0.00	2.65 ± 0.20	2.93 ± 0.17	R	S	S
TUN-19	0.40 ± 0.25	2.00 ± 0.35	-	R	S	-
TUN-24	2.00 ± 0.54	3.00 ± 0.00	3.00 ± 0.00	S	S	S
TUN-25	0.20 ± 0.20	2.50 ± 0.20	2.95 ± 0.15	R	S	S
TUN-26	3.00 ± 0.00	2.85 ± 0.14	2.75 ± 0.39	S	S	S
Differential set						
Bola Oro	2.00 ± 0.10	2.85 ± 0.14	3.00 ± 0.00	S	S	S
PMR45	2.00 ± 0.00	2.50 ± 0.20	3.00 ± 0.00	S	S	S
Edisto47	0.00 ± 0.00	2.85 ± 0.20	3.00 ± 0.00	R	S	S
PMR5	0.00 ± 0.00	0.50 ± 0.10	2.00 ± 0.20	R	R	S
PI414723	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.25	R	R	R

* Reaction classification as resistant (R; disease score ≤ 1) and susceptible (S; disease score > 1), ° Due to their poor germination ability, TUN-5, TUN-9, and TUN-19 were not tested against Race 3.5.

3.2. Phenotypic Evaluation under Natural Infection and Identification of The Prevalent Race

The disease of powdery mildew was recognized by the presence of a visual white powdery mass, mainly composed of mycelia and conidia, on leaf surfaces (Figure 3A,B), petioles, and young stems. The microscopic observation allowed for the identification of *P. xanthii* as the causal agent and the race of *P. xanthii* prevalent in the greenhouse plot was determined as Race 2 based on the resistance or susceptibility of the differential standard host cultivars used. Thus, 'Bola de Oro' and 'PMR-45′ were rated as susceptible (disease score = 4) while 'Edisto-47′, 'PMR-5′, and 'PI414723′ were rated as resistant with score means of 0.33, 0.16, and 0.33, respectively (Table S1).

Figure 3. Assessment of Powdery mildew symptoms under natural infection. (**A**) 'PMR-45′ and (**B**) 'Bola de Oro' (both accessions were susceptible to Race 2), (**C**) Resistant accession Tun-25 (Asli). Symptom evaluation was made when all plants of the susceptible accession showed severe symptoms of infection.

Most of the powdery mildew-infected plants exhibited white colonies that covered the leaves, except plants of TUN-16, TUN-19, and TUN-25 (Figure 3C), which showed mild symptoms and the disease incidence was not higher than 10% (Figure 4), confirming the results of the artificial inoculations. All remaining landraces were susceptible, showing symptoms exceeding 30% in the susceptible lines 'Bola de Oro' and 'PMR-45' (Figures 3 and 4).

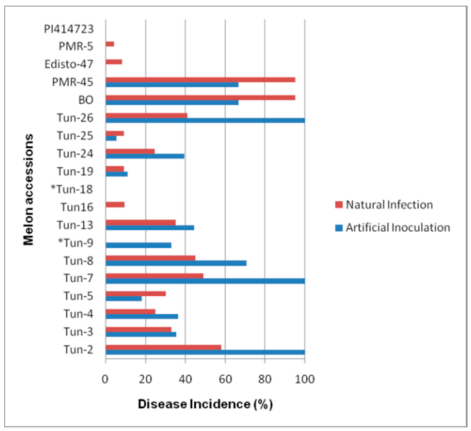

Figure 4. Disease incidence (%) of melon landraces and control genotypes evaluated to *P. xanthii* Race 2, under natural infection and under artificial inoculation. 'Bola de Oro' (BO) and 'PMR-45' were the susceptible controls; 'Edisto-47', 'PMR-5', and 'PI414723' were the resistant controls. *Tun-9 and *Tun-18 were not evaluated under natural infection due to poor numbers of available seeds.

The behavior of the different accessions against Race 2 was evaluated under artificial inoculation in the growth chamber and under natural conditions of infection. The disease score data were analyzed using a two-factorial analysis of variance. For both evaluations, the effects of the accession (G) and method of evaluation (M) were highly significant, and the interaction GxM was also observed (Table 3). However, replication effects were not significant (Table 3), indicating that there were no measurable systematic environmental effects between the blocks.

Table 3. Analysis of variance for effects of the melon accession (G), method of evaluation (M), and their interaction (GxM) on the *P. xanthii* Race 2 disease score.

Sources	df	Sum of Square	F Value	P (>F)
Block:Method	2	0.831	0.796	0.395
Accession (G)	15	222.89	28.673	$<2.2 \times 10^{-16}$ ***
Method (M)	1	11.26	21.730	3.276×10^{-7} ***
Interaction G × M	15	32.76	4.214	1.175×10^{-5} ***
Residuals	158	81.88		

df: Degrees of freedom, *** highly significant at $p < 0.001$.

The regression line of the *P. xanthii* Race 2 responses between the methods of evaluation and the confidence interval was determined, showing that accessions outside the grey area are deviating, highlighting differences in their reaction among the methods (Figures 5 and S1). These differences were specially observed in the susceptible accessions, where their susceptible responses were more or less pronounced depending on the method of evaluation. For example, the accession Tun-26 showed profuse sporulation by artificial inoculation, but it showed only 5% to 20% infection of the total leaf area under natural infection conditions.

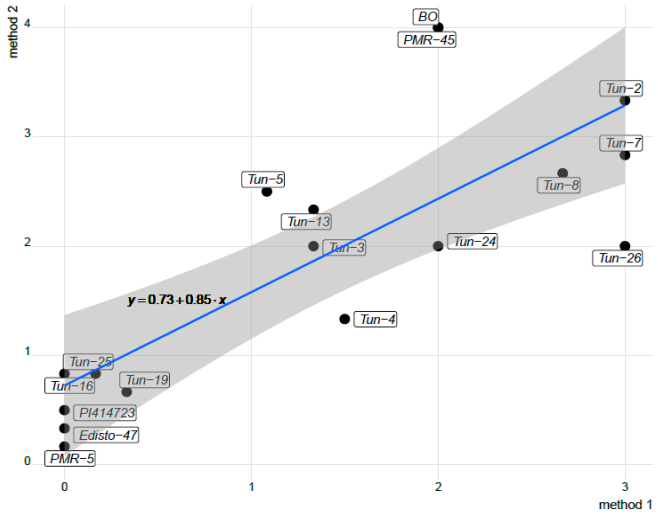

Figure 5. Display of the differences in the *P. xanthii* Race 2 responses between the methods of evaluation (Method 1: artificial inoculation in growth chamber; Method 2: natural conditions of infection under greenhouse). Accessions lying near the regression line show the same reaction whatever the method used. The confidence interval is marked with a grey area. All accessions outside the grey area are deviating, showing differences in their reaction among the methods. The straight regression line and the highly significant correlation (0.79***) of genetic estimations between Methods 1 and 2 generally indicate a high correspondence of landrace responses between the two methods. Tun-9 and Tun-18 were evaluated in Method 1 but not in Method 2, so they are not presented in this figure.

The extent of variance components influencing the response to *P. xanthii* Race 2 showed that 57% of the total variance could be explained by the accession, i.e., genetic variance, with a high value of 0.86 for the estimated heritability (Figure 6). The interaction between accession and method of evaluation explained 11% of the total variance and the method used contributed only 6% to the total phenotypic variance, suggesting a minor influence. Residual variance captured a quarter of the total variance, highlighting that only a small part of the total variance could not be explained by any of the applied factors.

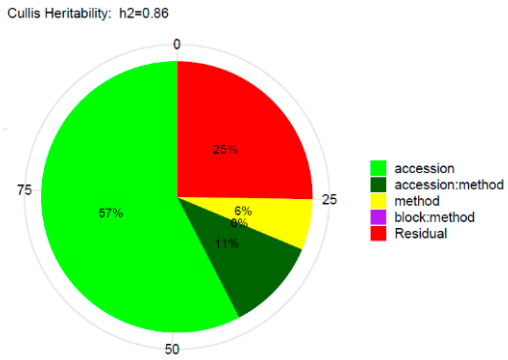

Figure 6. Display the extent of variance components influencing the responses to *P. xanthii* Race 2 analyzed over both methods. The accession covers with 57% most of the variance, followed by the residual variance with 25%. The interaction between accession and method explains 11% and the method effect 6%. 0% of the total variance is captured by the design effect block:method.

When analyzing variance components separately for each method (Figure S2), it becomes clear that artificial inoculation in the growth chamber had a larger genetic variance (81%) than natural infection in the greenhouse (67%). The residual component, which might refer to other environmental effects, was higher under natural infection (33% vs. 19%).

Estimated BLUPs (the pure genetic estimation values) of the accessions regarding the response of the accessions to Race 2 of *P. xanthii* were calculated (Table 4), where the genetic variance was separated from other influencing factors (method of evaluation, accession x method, and residual effects). The breeding values that the breeder would use span a range of 0.267 (resistant) to 2.987 (susceptible) and showed a consistent phenotyping ranking. In addition, the identified resistances in TUN-16, TUN-19, and TUN-25 were validated by low BLUPs values which ranged between 0.561 and 0.634.

Table 4. Estimated BLUPs of the accessions regarding their response to *P. xanthii* Race 2. All other investigated effects (block, method, method:accession, residuals) are separated from these effects, leading to pure genetic estimations for resistance to Race 2.

Accession	BLUP for Disease Scoring
PMR-5	0.267
Edisto-47	0.340
PI414723	0.414
TUN-16	0.561
TUN-19	0.634
TUN-25	0.634
TUN-4	1.443
TUN-3	1.663
TUN-5	1.774
TUN-13	1.811
TUN-24	1.958
TUN-26	2.399
TUN-8	2.546
TUN-7	2.766
BO	2.840
PMR-45	2.840
TUN-2	2.987

4. Discussion

The existence of many races of *P. xanthii* reduces the durability of the resistance, which is usually overcome; this makes it necessary to find new resistant genotypes with different genetic backgrounds. Although several genotypes carrying resistance to powdery mildew have been described and used to develop commercial melon varieties with resistance to *P. xanthii*, their agronomical and fruit characteristics are usually far away from the required melon types in certain markets. Thus, looking for novel sources of resistance in unexplored landrace collections, such as those held in the CRRHAB (Tunisia), is a very convenient step to test the possibility of obtaining new resistant melon varieties/hybrids.

The predominant pathogens of powdery mildew populations change depending on the melon cultivar, the growing season, and the geographical area [7]. In Tunisia, no previous research has been carried out leading to the identification of powdery mildew species and races. Under the greenhouse conditions observed during this study, *P. xanthii* Race 2 was identified as the pathogen responsible for powdery mildew. Determination of the predominant races of *P. xanthii* in Tunisian melon-growing regions is of substantial significance both for combating the disease and for breeding purposes. Race composition can vary over time and screening tests are required every year.

The ANOVA analysis showed that the response to *P. xanthii* Race 2 infection was significantly different among accessions. Additionally, significant differences were observed between the employed methods: both methodologies were useful and accurate in identifying resistance, but accessions were more susceptible when artificial inoculation in the growth chamber was used. Significant effects of the interaction accession x method have been also observed. This interaction was observed only in some susceptible accessions; thus, the susceptible response shown by accessions TUN-4 and TUN-26 was higher when artificial inoculation in the growth chamber was used; however, TUN-8, TUN-13, BO, and

PMR-45, showed higher susceptibility in natural conditions of infection. Genetic effects explained 57% of the total phenotypic variation observed, followed by the interaction effect between the method of evaluation and the accession (11%) and, thereafter, the method effect (6%).

Correlation analysis revealed a highly significant relationship ($r^2 = 0.791^{***}$, $p < 0.05$) in the response of the accessions between the artificial inoculation in the growth chamber and the natural conditions of infection, which confirms that the use of a controlled growth chamber and artificial inoculations could speed up the evaluation of the response to *P. xanthii* Race 2 in breeding programs and also confirms that the evaluation is not dependent on the crop cycle and can be carried out at any time. Obviously, greenhouse or field trials are needed to complement and confirm the results of artificial inoculation in growth chambers.

In our study, a very strong genetic influence on the response to *P. xanthii* Race 2 was found. This was reflected in the high value of the estimated heritability (0.86), showing that selection regarding resistance in this broad gene pool and the successive development of resistant varieties are very promising. A complete genetic characterization of the found resistances is necessary to identify the number of loci/alleles controlling this resistance that seems to be race-specific.

Most of the resistances found in *P. xanthii* are race-specific. Many commercial varieties with specific resistances have been developed but they become susceptible to infection shortly after they are exploited, because of frequent changes in pathogen populations [1]. Therefore, the pyramiding of resistance genes to specific races in a single genotype, or the use of resistance that is not race-specific, should give more comprehensive and durable protection [1]. Consequently, the search for and utilization of new genes that confer resistance to powdery mildew are still primary objectives in melon breeding. The results obtained after artificial inoculation showed that all the melon landraces were susceptible to Races 3.5 and 5 and five landraces were resistant to Race 2. Under natural conditions of infection, TUN-16, TUN-19, and TUN-25 showed low symptoms and the disease incidence was not higher than 10%, confirming the results of the artificial inoculations. Only a few sources of resistance to powdery mildew have been reported in melon, mostly belonging to *flexuosus*, *conomon*, *momordica*, and *acidulus* horticultural groups [10,16]. Resistance to different races of *P. xanthii* has been generally found in accessions from India [28]. Most of these sources are quite different from Tunisian commercial types, which makes it difficult to eliminate undesirable melon characteristics introgressed into the commercial melon lines together with the resistance gene/s. The resistant landraces identified in this study belong to *inodorus* (Dziri: TUN-9, Sarachika: TUN-16, and Asli: TUN-25), *cantalupensis* (Rupa: TUN-18), and *reticulatus* (Chamem: TUN-19) horticultural groups, which are similar to commercial types of melons regarding their agronomic traits and fruit characteristics. Special attention should be given to the resistant landrace TUN-19 Chamem, of Ananas type, since it also carries the *Vat* gene of resistance to *Aphis gossypii* [20] and the *Fom*-1 gene of resistance to Races 0 and 2 of *Fusarium oxysporum* f.sp *melonis* [17]. TUN-19 is a potential landrace with high value as a donor of different diseases and pest resistances for melon breeding programs to develop commercial melons of the Ananas type, which are highly appreciated not only in Tunisia but also in other Mediterranean countries. Furthermore, these Tunisian landraces are productive [18], are adapted to local cultivation conditions, and do not have non-desirable traits [29]. Thus, the use of these new resistance sources will promote breeding programs to obtain melon varieties/hybrids.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8121172/s1, Table S1: Phenotypic evaluation of the resistance to *P. xanthii* Race 2, under natural infection, Figure S1: Least squares mean for the significant interaction effect between method and accession. Figure S2. Display of the extent of variance influencing the *P. xanthii* Race 2 response in each method used.

Author Contributions: Conceptualization and methodology, H.C.-R. and M.L.G.-G.; formal analysis, L.K. and H.C.-R.; writing—original draft preparation, H.C-R. and A.G.-C.; writing—review and editing, H.C.-R., A.G.-C., L.K. and M.L.G.-G. Regarding A.B.B., he collaborated in the artificial inoculation with Races 2 and 5. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded, in part, by the Spanish Ministry Economy and Competitivity co-funded with FEDER (AGL2017-85563-C2-2-R), the PID2020-116055RB-C22 I+D+I project funded by MCIN/AEI/10.13039/501100011003, and the A11-20R project funded by the Aragon Government.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The Ministry of Higher Education and Scientific Research of Tunisia (MESRST) through the funding allocated to the Research Laboratory LR21AGR03. The authors thank Rafika Sta-Baba for providing seeds of some landraces and Mohamed Ferjani (Sahline Station) for his help in the greenhouse experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mccreight, J. Melon-Powdery Mildew Interactions Reveal Variation in Melon Cultigens and *Podosphaera xanthii* Races 1 and 2. *J. Am. Soc. Hort. Sci.* **2006**, *131*, 59–65. [CrossRef]
2. Křístková, E.; Lebeda, A.; Sedláková, B. Species Spectra, Distribution and Host Range of Cucurbit Powdery Mildews in the Czech Republic, and in Some Other European and Middle Eastern Countries. *Phytoparasitica* **2009**, *37*, 337–350. [CrossRef]
3. Lebeda, A.; Křístková, E.; Sedláková, B.; McCreight, J.D.; Coffey, M.D. Cucurbit Powdery Mildews: Methodology for Objective Determination and Denomination of Races. *Eur. J. Plant Pathol.* **2016**, *144*, 399–410. [CrossRef]
4. Hollomon, D.W.; Wheeler, I.E. Controlling Powdery Mildews with Chemistry. In *The Powdery Mildews: A Comprehensive Treatise*; American Phytopathological Society (APS Press): St. Paul, MN, USA, 2002; pp. 249–255.
5. Pérez-García, A.; Romero, D.; Fernández-Ortuño, D.; López-Ruiz, F.; De Vicente, A.; Torés, J.A. The Powdery Mildew Fungus *Podosphaera fusca* (Synonym *Podosphaera xanthii*), a Constant Threat to Cucurbits. *Mol. Plant Pathol.* **2009**, *10*, 153–160. [CrossRef]
6. McCreight, J.D. Genes for Resistance to Powdery Mildew Races 1 and 2U.S. in Melon PI 313970. *HortScience* **2003**, *38*, 591–594. [CrossRef]
7. Hosoya, K.; Kuzuya, M.; Murakami, T.; Kato, K.; Narisawa, K.; Ezura, H. Impact of Resistant Melon Cultivars on *Sphaerotheca fuliginea*. *Plant Breed.* **2000**, *119*, 286–288. [CrossRef]
8. Bardin, M.; Nicot, P.C.; Normand, P.; Lemaire, J.M. Virulence Variation and DNA Polymorphism in *Sphaerotheca fuliginea*, Causal Agent of Powdery Mildew of Cucurbits. *Eur. J. Plant Pathol.* **1997**, *103*, 545–554. [CrossRef]
9. Del Pino, D.; Olalla, L.; Pérez-García, A.; Rivera, M.; García, S.; Moreno, R.; Vicente, A.; Tores, J. Occurrence of Races and Pathotypes of Cucurbit Powdery Mildew in Southeastern Spain. *Phytoparasitica* **2002**, *30*, 459–466. [CrossRef]
10. Yuste-Lisbona, F.J.; López-Sesé, A.I.; Gómez-Guillamón, M.L. Inheritance of Resistance to Races 1, 2 and 5 of Powdery Mildew in the Melon TGR-1551. *Plant Breed.* **2010**, *129*, 72–75. [CrossRef]
11. Perchepied, L.; Bardin, M.; Dogimont, C.; Pitrat, M. Relationship between Loci Conferring Downy Mildew and Powdery Mildew Resistance in Melon Assessed by Quantitative Trait Loci Mapping. *Phytopathology* **2005**, *95*, 556–565. [CrossRef]
12. Grimault, V.; Houdault, S.; Himmel, P. Development of Differential Hosts to Identify Commercially Relevant Races of Melon *Podosphaera xanthii* against Which Vegetable Seed Companies Make Claims of Resistance. *Cucurbit Genet. Coop. Rpt.* **2020**, *43*, 1–3.
13. Pitrat, M. Melon Genetic Resources: Phenotypic Diversity and Horticultural Taxonomy. In *Genetics and Genomics of Cucurbitaceae*; Grumet, R., Katzir, N., Garcia-Mas, J., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 25–60. [CrossRef]
14. Yuste-Lisbona, F.J.; Capel, C.; Sarria, E.; Torreblanca, R.; Gómez-Guillamón, M.L.; Capel, J.; Lozano, R.; López-Sesé, A. Genetic Linkage Map of Melon (*Cucumis melo* L.) and Localization of a Major QTL for Powdery Mildew Resistance. *Mol. Breed.* **2011**, *27*, 181–192. [CrossRef]
15. Howlader, J.; Hong, Y.; Natarajan, S.; Sumi, K.R.; Kim, H.-T.; Park, J.-I.; Nou, I.-S. Development of Powdery Mildew Race 5-Specific SNP Markers in *Cucumis melo* L. Using Whole-Genome Resequencing. *Hortic. Environ. Biotechnol.* **2020**, *61*, 347–357. [CrossRef]
16. Nunes, E.W.; Esteras, C.; Ricarte, A.O.; Martínez-Perez, E.; Gómez-Guillamón, M.L.; Nunes, G.H.; Picó, M.B. Brazilian Melon Landraces Resistant to *Podosphaera xanthii* Are Unique Germplasm Resources. *Ann. Appl. Biol.* **2017**, *171*, 214–228. [CrossRef]
17. Chikh-Rouhou, H.; Gómez-Guillamón, M.L.; González, V.; Sta-Baba, R.; Garcés-Claver, A. *Cucumis melo* L. Germplasm in Tunisia: Unexploited Sources of Resistance to Fusarium Wilt. *Horticulturae* **2021**, *7*, 208. [CrossRef]

18. Chikh-Rouhou, H.; Mezghani, N.; Mnasri, S.; Mezghani, N.; Garcés-Claver, A. Assessing the Genetic Diversity and Population Structure of a Tunisian Melon (*Cucumis melo* L.) Collection Using Phenotypic Traits and SSR Molecular Markers. *Agronomy* **2021**, *11*, 1121. [CrossRef]
19. Chikh-Rouhou, H.; Tlili, I.; Ilahy, R.; R'Him, T.; Sta-Baba, R. Fruit Quality Assessment and Characterization of Melon Genotypes. *Int. J. Veg. Sci.* **2021**, *27*, 3–19. [CrossRef]
20. Chikh-Rouhou, H.; Ben Belgacem, A.M.; Sta-Baba, R.; Tarchoun, N.; Gómez-Guillamón, M.L. New Source of Resistance to Aphis Gossypii in Tunisian Melon Accessions Using Phenotypic and Molecular Marker Approaches. *Phytoparasitica* **2019**, *47*, 405–413. [CrossRef]
21. Ballantyne, B. Powdery Mildew on Cucurbitaceae: Identity, Distribution, Host Range and Sources of Resistance. *Proc. Linn. Soc. New South Wales* **1975**, *99*, 100–120.
22. Lebeda, A.; Sedláková, B. Screening for Resistance to Cucurbit Powdery Mildew (*Golovinomyces cichoracearum*, *Podosphaera xanthii*). In *Mass Screening Techniques for Selecting Crops Resistant to Diseases*; IAEA: Vienna, Austria, 2010; Volume 19, pp. 295–307. ISBN 978-92-0-105110-3. Available online: https://www.iaea.org/publications/7758/mass-screening-techniques-for-selecting-crops-resistant-to-disease (accessed on 1 August 2021).
23. McGrath, M.T. Heterothallism in *Sphaerotheca fuliginea*: Mycologia: Volume 86, No 4. Available online: https://www.tandfonline.com/doi/abs/10.1080/00275514.1994.12026445 (accessed on 1 August 2021).
24. R Core Team. *R: A Language and Environment for Statistical Computing*; R Core Team: Vienna, Austria, 2013.
25. Lenth, R.V. R Package Emmeans: Estimated Marginal Means. R. 3 August 2022. Available online: https://github.com/rvlenth/emmeans (accessed on 1 August 2022).
26. Wickham, H. Ggplot2 Book. Perl. 11 August 2022. Available online: https://github.com/hadley/ggplot2-book (accessed on 1 August 2022).
27. Cullis, B.R.; Smith, A.B.; Coombes, N.E. On the Design of Early Generation Variety Trials with Correlated Data. *J. Agric. Biol. Environ. Stat.* **2006**, *11*, 381. [CrossRef]
28. Dhillon, N.; Monforte, A.; Pitrat, M.; Pandey, S.; Singh, P.; Reitsma, K.; Garcia-Mas, J.; Sharma, A.; Mccreight, J. Melon Landraces of India: Contributions and Importance. *Plant Breed Rev.* **2011**, *35*, 85–150. [CrossRef]
29. Chikh-Rouhou, H.; Gómez-Guillamón, M.L.; Garcés-Claver, A. Melon Germplasm from Tunisia with Immense Breeding Value. *CGC Rep.* **2021**, *44*, 7–11.