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PRELIMINARY SELECTION AND PHENOTYPIC CHARACTERIZATION OF MELON LANDRACES EXHIBITING RESISTANCE TO POWDERY MILDEW

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ABSTRACT

Powdery mildew is a devastating disease of melon worldwide. Safe guarding the melon production requires to tackle this disease, either by chemical control or by developing resistant cultivars with the latter being the most sustainable, inexpensive and environmentally friendly approach. The use of genetic resistance is a safe alternative to overcome the hazardous chemical contaminants. Thus, in the present study 56 melon landraces were evaluated for their response to powdery mildew and for a set of agro-morphological and quality traits. The results showed that 4 landraces were highly resistant to powdery mildew, with low symptoms and disease incidence not exceeding 10%, and 11 landraces were moderately resistant. The agro-morphological assessment of the selected resistant landraces showed that the fruit weight ranged between 433 and 1300 g with a total soluble solids ranging between 7.93 and 13.57 °Brix. This local germplasm is of great potential and the highly resistant landraces should be exploited as potential sources of resistance to powdery mildew in future breeding programs.

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INTRODUCTION

Melon (*Cucumis melo* L., 2n= 2x= 24), is a cross-pollinating crop with a small diploid genome size of 450Mb (Garcia-Mas *et al.*, 2012). It is a commercially important horticultural crop worldwide, belonging to the Cucurbitaceae family, and exhibits high levels of diversity in their morphological, physiological and biochemical properties (Pitrat, 2016). In Tunisia, the area allocated to melon production is around 17,900 ha with a production of 149,000 tons (FAO, 2016). Several fungal diseases affect seriously melon crops (Ayed *et al.*, 2007; Daami-Remadi *et al.*, 2007; Camele *et al.*, 2009) decreasing yield, affecting fruit quality, and forcing intensive use of chemical sprays.

Powdery mildew is a common fungal disease and a serious foliar disease of melon crops worldwide grown both under greenhouse and open field conditions. This disease is mainly caused by two biotrophic fungal species: namely *Podosphaera xanthii* (ex. *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (ex. *Erysiphe cichoracearum*) (Robinson and Decker-Walters, 1997). Two races of *G. cichoracearum* and 22 races of *P. xanthii* have been reported on melon (McCreight, 2006). These are fungi whose mycelial filaments colonize the surface of their host's epidermis and attach themselves to it using suckers that allow them to feed (Stadnik *et al.*, 2001). Powdery mildew symptoms can be present at all stages of melon development: from the cotyledon stage to the harvest stage. On leaves, whitish powdery spots appear on the upper and the lower side of the leaf blade and these become stunted and eventually dried. Damage can also occur on stems but rarely on fruits (Stadnik *et al.*, 2001). Fungus spores are transmitted through the air as soon as favorable conditions have set in (temperatures between 26-32 °C and high humidity). Consequently, it decreases the photosynthetic potential, and concomitantly decreases the fruit quality and yield (Stadnik *et al.*, 2001).

Fungicide resistance of powdery mildew is a serious problem worldwide. Therefore, searching for suitable sources of resistance and breeding for resistance are considered as very important targets for improving melon crops. Development of resistant varieties is a sustainable approach for the success of melon production. Resistant lines and varieties that belong to different melon types (cantaloupes, charentais, galia, etc.) have been developed in the USA, France, Israel and other countries (Karchi, 2000; McCreight, 2006). The genetic control of powdery mildew resistance is not fully understood. A monogenic dominant control was reported by some researchers (McCreight, 2006; Dogimont, 2011) while others reported a monogenic recessive control (McCreight, 2003).

Landraces are important resources of resistance (Ricciardi *et al.*, 2003; Chikh-Rouhou and Garcés-Claver, 2021) and the identification of novel sources of resistance is the first step in breeding program for disease resistance. Therefore, the aim of the present study was the evaluation of melon landraces for their response to Powdery mildew under natural infection. The resistant landraces were also characterized for their main ago-morphological traits to assess their suitability as potential genitors for future melon breeding programs.

MATERIAL AND METHODS

Plant material

The plant material used consisted of 56 local melon landraces collected from local farmers of different melon-growing regions in Tunisia (**Table 1**). For each melon landrace, seeds were sown in cell trays containing peat:perlite (1:1 v:v) potting mix and maintained under growth chamber conditions (25°C in 16h day, 16°C in 8h night). At the two-true-leaf growth stage, seedlings were transplanted to a sandy loam soil in an unheated greenhouse under daylight conditions, located at the Experiment Station of Sahline (N35° 45' 02'', E 10° 42' 44''). Water was applied to plants with drip irrigation 2 to 3 days a week whenever the soil at ~2 cm below the surface felt dry to the touch.

Table 1. Melon landraces used in the study.

Codes	Local name	Codes	Local name	Codes	Local name
1	Arbi-Gal	20	Beji	39	Wardanine 2
2	Arbi-Tra	21	Arbi-Lbn	40	Wardanine 3
3	Dziri kbir	22	Ananas	41	Local
4	Dziri Sidi BZ	23	Arbi 3	42	Sahrawi
5	Mazoul Pig	24	M.Mehdia-14	43	Arbi-Fallah1
6	Dziri-1	25	FL-38	44	Arbi-Fallah2
7	Arbi kairouan	26	M-4	45	Fallah Dziri
8	Arbi 14	27	M-12	46	Dziri local
9	Arbi 15	28	Horchay 35	47	Bayoudh
10	Arbi-Rp1	29	Nabil-a	48	Chemam
11	Arbi-Rp2	30	Fallah	49	Kahlawi
12	RD	31	M. Inc 1	50	Jbeniana
13	Arbi-Stmb	32	M. Inc 2	51	Mallagui
14	Asli kbir-1	33	Fakous Inc3	52	Arbi-Inc1
15	Asli kbir-2	34	M. Inc 4	53	Arbi-Inc2
16	HTM 20	35	Wardanine	54	Arbi-Inc3
17	Nefza 28	36	Mo3tmar	55	Arbi-TRK
18	Nefza 29	37	Bembla	56	Dziri Manz Kamel
19	Maatmer	38	Wardanine 1		

Experimental design

The experimental design was a randomized complete block design with two blocks. Each replication consisted of 56 landraces and six seedlings per landrace. They were subjected to agricultural practices commonly adopted by farmers in the region and irrigated and fertilized as needed. Plant to plant distance was 40 cm between seedlings within the same row and 80 cm between rows.

Screening for powdery mildew resistance under natural infection

To evaluate the resistance of melon landraces to powdery mildew under natural infection, disease symptoms on leaves were evaluated based on a 0 to 4 rating scale from Camele *et al.* (2009) where: (0): no symptoms of powdery mildew, (1): less than 5% of the leaf surface with powdery mildew symptoms, (2): 5 to 20% of the leaves with symptoms, (3): 21 to 50% of the leaves showing symptoms, and (4): more than 50% of leaves attacked. Disease incidence (DI) and disease severity (DS) were calculated as follow (Camele *et al.*, 2009):

DI (%) = n*100/N, where n: the number of symptomatic plants, and N: total number of plants. DS (%) = $\sum$ (ni Xi) * 100 / (the highest value from the scale * N), where Xi: the level of attack, ni: the number of plants with the same attack level, and N: total number of plants.

Each 5 days, developing symptoms were evaluated according to the rating scale detailed above and the area under the disease progress curve (AUDPC) was as well estimated from those scores. The AUDPC integrates both the intensity of symptoms and the time taken for their expression. The AUDPC was calculated using the following formula of Perchepied and Pitrat (2004): $AUDPC = \sum_i [(x_i + x_{i+1})/2]\ (t_{i+1} - t_i)$, where i = 1 to 3 scorings, x_i = mean disease score of each plant at date i, x_{i+1} = mean disease score of each plant at date i+1, and $t_{i+1} - ti$ = number of days between scoring date i and scoring date i+1.

The Spanish line Bola de Oro 'BO' was used as susceptible control to powdery mildew and the Indian line 'PI414723' was used as resistant control.

Agro-morphological evaluation of the resistant/tolerant landraces

At harvest, the average weight of fruits produced per plant was determined. The average yield per plant was noted at the last harvest. Three fully ripe fruit per landrace were randomly harvested from each block (6 fruits per landrace) and fruit quality was determined by assessing the Total soluble solids content (TSS) using a digital refractometer (Atago PR-100, Tokyo, Japan). Some qualitative traits were also evaluated such as separation of peduncle from fruit, fruit grooves and netting, fruit skin and flesh color.

Statistical analyses

Data collected were subjected to ANOVA analysis using the statistical program SPSS version 20 (SPSS for windows, IBM, Armonk, NY). Means were compared based on the least significant difference (LSD) test at p = 0.05. Agglomerative Hierarchical Cluster analysis was used to determine differences and similarities among landraces, and the distance measure used was Euclidean distance computed between each population by the Ward method using SPSS.

RESULTS

Phenotypic evaluation of melon landraces response to Powdery mildew

Presence of a visual white powdery mass (typical symptoms of powdery mildew) was developed on leaf surfaces and petioles of the susceptible control 'BO' and the susceptible landraces. Low symptoms were observed on leaf surfaces of the resistant control 'PI4147123' and the resistant landraces. Large variability was observed among landraces for the disease severity (Figure 1) and the AUDPC values (Supplementary material).

The assessment of the disease severity showed differences between melon landraces. In fact, it was possible to classify them into different groups according to the symptoms' severity; landraces with disease severity not exceeding 10%, those not exceeding 25% and those greater than 30%. Among the 56 landraces evaluated, four landraces (codes: 15, 20, 22, and 55) were highly resistant as they showed disease severity not exceeding 10% like the resistant control 'PI414723' (Figure 1). Eleven landraces (codes: 21, 23, 25, 28, 29, 45, 47, 52, 53, 54, and 56) were moderately resistant as they exhibited a moderate level of attack not exceeding 25%. The remaining landraces were found to be moderately to highly susceptible to powdery mildew disease as the susceptible control 'BO' (Figure 1).

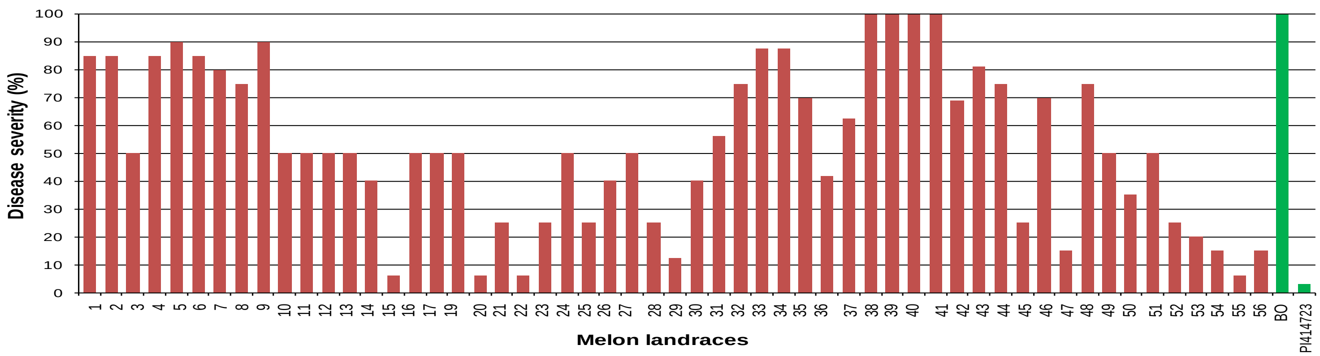

Figure 1. Disease severity of melon landraces evaluated for their response to powdery mildew. Bola de Oro 'BO' and PI414723 were the susceptible and resistant controls, respectively.

The AUDPC values varied among landraces; thus, it was possible to detect genotypic differences in the response to powdery mildew. The AUDPC values for the susceptible control 'BO' was significantly higher than those for most of the tested landraces, while the AUDPC for the resistant control 'PI414723' was lower (Supplementary material).

Agro-morphological evaluation of the selected resistant landraces

The 14 landraces ranked as being resistant to powdery mildew, under our experimental conditions, showed variability in their qualitative and quantitative parameters as demonstrated in Table 2. The greatest diversity was observed for fruit characteristics (Figure 2).

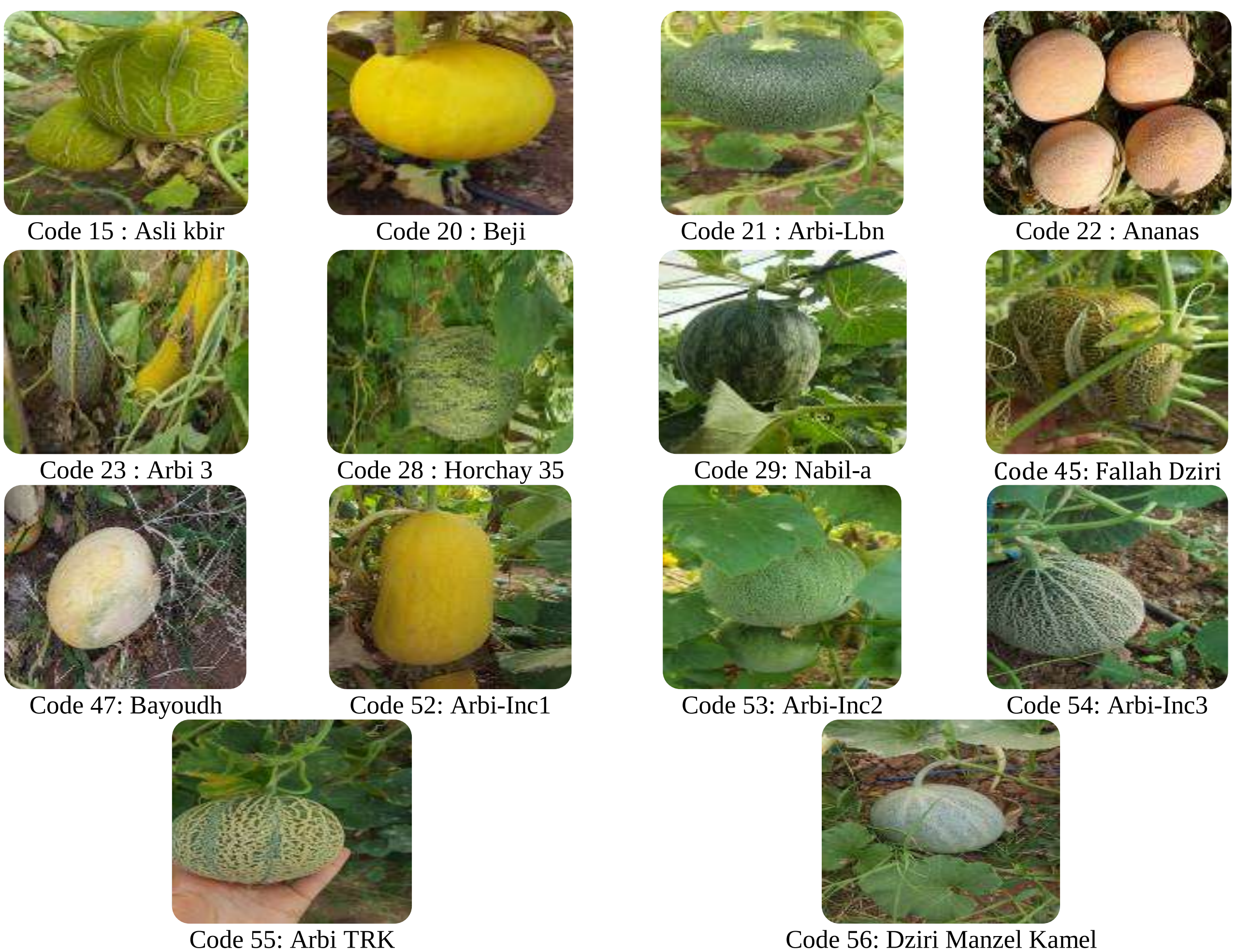

Figure 2. Fruit trait diversity of the 14 melon landraces selected as being resistant to powdery mildew.

Table 2. Evaluation of some agro-morphological traits of the selected resistant melon landraces.

Code	Weight (g)	Predominant Skin color	Flesh color	Fruit Cork formation	Peduncle dehiscence	TSS (°Brix)	Reaction classification to PM*
15	1266.67± 251.66 a	Green	Orange	Present	-	11.08±1.65 b	HR
20	1266.67± 461.88 a	Yellow	Green	Absent	-	10.17±0.46 b	HR
22	500.00± 0.00 c	Green	Greenish white	Present	-	11.23± 0.50 b	HR
55	666.67± 288.67 abc	Green	Orange	Present	+	11.32±0.88 b	HR
21	866.67± 321.45 abc	Green	Orange	Absent	-	10.83±0.22 b	MR
23	766.67± 251.66 abc	Green	Orange	Present	+	11.03±0.66 b	MR
28	1000.00± 0.00 abc	Green	Orange	Absent	-	10.2± 0.12 c	MR
29	1066.67± 57.73 abc	Green	Orange	Absent	-	13.57± 1.57 a	MR
45	433.33± 57.73 c	Yellow	Green	Present	+	10.0± 0.57 c	MR
47	633.33± 750.55 abc	Yellow	Yellowish white	Absent	+	9.93±0.33 c	MR
52	700.00± 435.89 abc	Yellow	Yellowish white	Absent	-	8.81±0.45 d	MR
53	1233.33± 665.83 ab	Green	Green	Present	+	7.93±0.65 d	MR
54	600.00± 264.57abc	Green	Orange	Present	+	10.13±0.26 c	MR
56	1300.00± 519.61a	Green	Orange	Present	+	10.5±0.19 c	MR

For each column, means followed by different letters are significantly different based on LSD test at $p < 0.05$).

* Reaction classification to powdery mildew (PM) as Highly resistant (HR; disease incidence ≤10%) and Moderately resistant (MR; disease incidence not exceeding 25%). The landrace code 25, *flexuosus* botanical group, moderately resistant was not evaluated in this trial.

In fact, the external fruit color varied from yellow to green while the flesh color was either white, greenish white, green, or orange. The cork formation was noted as well in all selected resistant melon landraces.

At maturity, for 7 out of the 14 melon landraces selected, separation of peduncle was easy due to the formation of an abscission zone (Table 2), a crack was then visible at the peduncle basis thus leaving a clear scar when removed (Figure 3). These fruits were characterized generally by low storability. The 7 remaining landraces were characterized by a non-dehiscent peduncle (Table 2, Figure 3) and their maturity was indicated by a change in fruit color (change from green to yellow or orange). The Total soluble sugars (TSS) influence the sweetness of melon. For our selected landraces, this parameter varied between 7.93 and 13.57 °Brix and was highest in landrace '29' (Table 2).

Figure 3. Peduncle dehiscence in melon fruits. (A) a crack is visible at the base of the peduncle which leaves a clear scar when removed. (B) Non-dehiscent peduncle.

A hierarchical classification of the 14 selected resistant melon landraces (Figure 4), carried out from the mean values of the quantitative traits evaluated, clustered the phenotypic diversity of the selected landraces into two distinct groups, each group was characterized by specific parameters: fruit weight and total soluble sugars (Table 3).

The first group consisted of 6 landraces (Codes 15, 20, 56, 53, 28, and 29) characterized by the greatest fruit weights ranging between 1000 and 1300g, with an average of 1188.89 g and a TSS exceeding 10.2 °Brix except for landrace '53' which was a non-sweet melon (7.93 °Brix) (Table 2). The second group consisted of 8 landraces (Codes 22, 45, 47, 54, 55, 52, 21, and 23) characterized by an average fruit weight varying between 433.33 and 866.67 g and a TSS exceeding 10 °Brix except for landraces '47' and '52' (9.93 and 8.81 °Brix, respectively).

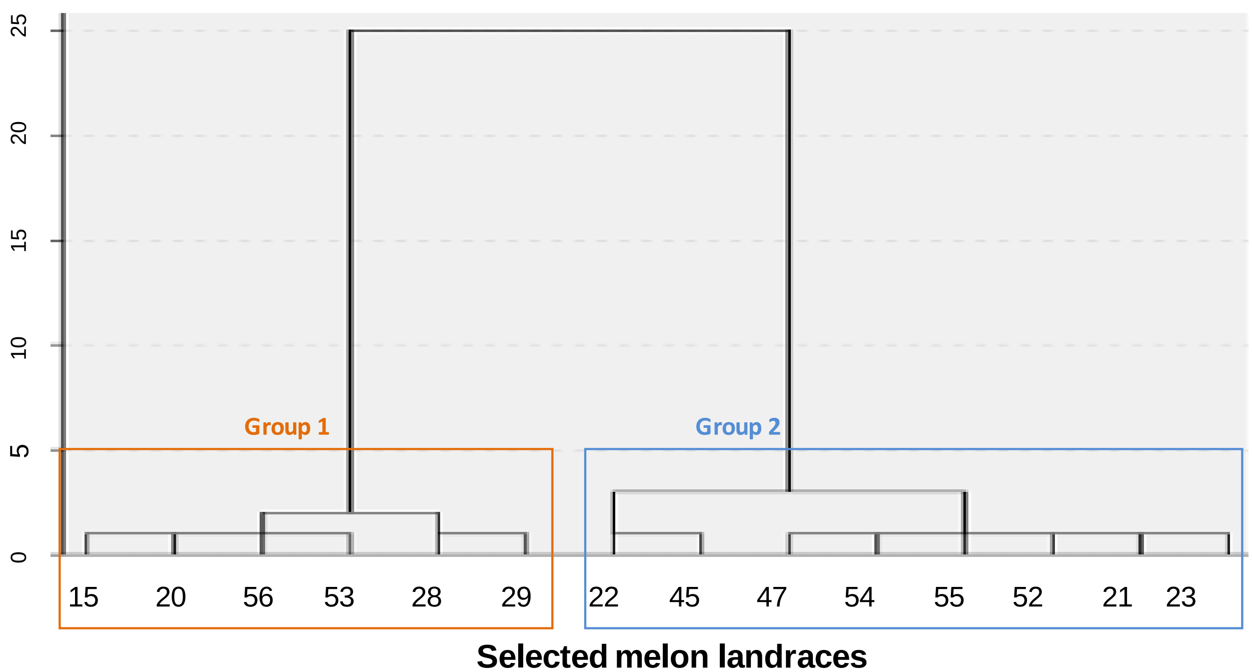

Figure 4. Dendrogram of hierarchical classification of the selected melon landraces resistant to powdery mildew. Numbers across the left refer to the level of truncation.

Table 3. Characteristics of each melon group formed by Hierarchical clustering.

Trait	Group 1 (N=6)	Group 2 (N=8)
Weight (g)	Min 1000 Max 1300	Min 433.33 Max 866.67
TSS (°Brix)	Min 7.93 Max 13.57	Min 8.93 Max 11.32

DISCUSSION

Tunisia has not been considered as a diversification center for *Cucumis melo*, however, a large diversity in seed, flower and fruit traits was recently reported in a collection of landraces by Chikh-Rouhou *et al.* (2021c). Besides, the importance of the Tunisian melon genetic resources as a valuable genetic reservoir was demonstrated (Chikh-Rouhou *et al.*, 2021a). The landraces are a very important source of genetic diversity, constituting an important genetic resource for plant breeders (Chikh-Rouhou and Garcés-Claver, 2021). In the present study, diversity within melon landraces as for their response to powdery mildew, affecting melon crops in Tunisia, has been found.

Powdery mildew, mainly induced by *Podosphaera xanthii*, is a devastating disease associated to melon crops worldwide. This disease is managed using fungicide sprays leading consequently to heavy environmental contamination (Sales Júnior *et al.*, 2011). The use of genetic resistance is a safe alternative to control this pathogen. Landraces are important sources of resistance and the identification of novel sources of resistance is the first step in breeding program for disease resistance. In this research a phenotypic evaluation of several melon landraces for their response to powdery mildew was carried out, under natural infection.

Disease incidence surveys of melon landraces tested indicated that 7.40% of them were highly resistant and 20.37% were moderately resistant. Screening of melon collection is an initial step for development of a new breeding program to increase resistance to powdery mildew. The basic methods used for the powdery mildew screening are whole plant infection and observation, and leaf disks assay (Cohen *et al.*, 2000; Velkov and Petkova, 2014; Ivanova *et al.*, 2019). Disease ratings in these reports have been based on incidence (resistant versus susceptible) or severity (numerical scale reflecting leaf area infected by powdery mildew). The highly resistant landraces selected in the present study, could be explored as potential sources of resistance to powdery mildew in future breeding programs. Thus, a complete characterization of this resistance is necessary, by performing crosses between susceptible and resistant accessions. Strategies such as association mapping and QTL mapping represent an opportunity to elucidate the genes or regions of the genome involved in such resistance.

The need to develop resistant varieties is a challenge for every breeding program. However, the 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. Researches are ongoing in order to identify the most predominant races of the pathogen in the Tunisian greenhouse conditions (unpublished data). Previous work on local melon germplasm indicated they could be a potential source of resistance to biotic stress (Chikh-Rouhou *et al.*, 2020) especially to Fusarium wilt (Daami-Remadi *et al.*, 2007; Ayed *et al.*, 2007; Chikh-Rouhou *et al.*, 2018; Chikh-Rouhou *et al.*, 2021b) and to aphid attack (Chikh-Rouhou *et al.*, 2019). It is important to select melon genotypes resistant to fungal diseases and at the same time having good fruit quality. Fruit quality and productivity of resistant genotypes need to be further studied to select the promising ones.

The evaluation and characterization of resistant genotypes is an important objective in melon breeding programs. Melon varieties are characterized by specific traits concerning appearance of fruits, taste, aroma, etc. (Velkov and Petkova, 2014; Pitrat, 2016; Chikh-Rouhou *et al.*, 2021b). Combining resistance and fruit quality in one genotype is a challenge. In this study, four melon landraces were selected as being highly resistant to powdery mildew and 11 as moderately resistant. Their agro-morphological characterization showed that the fruit weight ranged between 433 and 1300 g with a total soluble solids ranging between 7.93 and 13.57 °Brix. The TSS, is a primary determinant of fruit quality, it influences the flavor of melons. Indeed, improvement of fruit sweetness is an important breeding goal as well. Landraces ‘15’ and ‘29’ were found to be very interesting with fruit weight exceeding 1 kg and TSS exceeding 11°Brix. Most of the resistant landraces exhibited characteristics of the botanical variety *inodorus* and *reticulatus* which are highly appreciated in Tunisia. Besides, the peduncle dehiscence was evaluated; this trait is correlated with melon shelf life (Chikh-Rouhou *et al.*, 2021c). Fruits with a dehiscent peduncle are characterized generally by low storability because of their quick softening upon ripening whereas, fruits with a non-dehiscent peduncle, like *inodorus* types, are considered as non-climacteric types and had a longer shelf life exceeding 45 days (Chikh-Rouhou *et al.*, 2021b; Chikh-Rouhou *et al.*, 2021c). Climacteric and non-climacteric varieties exist in the same species of *Cucumis melo*, based on their ripening related respiration rate and ethylene evolution profiles (Giovannoni, 2007; Pitrat, 2016). Indeed, these authors showed that the climacteric melons have orange flesh, high aroma and short shelf-life and that non-climacteric melons usually display pale-green flesh, low level of aroma and slow softening resulting in typically longer shelf-life than climacteric varieties, and reported that these differences are more likely the result of genetic differences in ethylene synthesis or response. All these aspects need to be further taken into account once designing a melon breeding program.

CONCLUSION

The identification of melon landraces exhibiting resistance to powdery mildew disease, the most devastating disease of melon causing decreased crop yield and fruit quality decline, is essential to promote melon landraces growing in Tunisia. The selected resistant landraces with high-yield or high soluble solid content could be explored for genetic research, like gene mapping, and could also be used as parent lines in breeding programs to transfer the resistance gene(s) into the susceptible lines exhibiting good agronomic characters.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.