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EVALUATION OF WATERMELON GERMPLASM AND ADVANCE BREEDING LINES AGAINST POWDERY MILDEW RACE '2F'

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Podosparea xanthii is the most frequently reported powdery mildew pathogen of watermelon (*Citrullus lanatus* L.). The utilization of genetic resources for disease resistance is economically and environmentally safer than the use of fungicides. This study was aimed to screen 107 available germplasm and advance breeding lines of watermelon against race 2F of powdery mildew. The melon differential lines were utilized to confirm the powdery mildew race in spring and autumn growing seasons. Here, we have reported some highly resistant cultigens including M16, M11 and M49 with lowest mean severity ratings of 0.58, 1.0 and 1.13, respectively. The mean severity ratings for sensitive cultigens were recorded the highest in ZXG1996 (6.24) and M01 (5.7) on the basis of polyhouse screening and controlled condition screening retest. Moreover, our study suggested that powdery mildew screening for watermelon in the polyhouse at seedling stage could be a good screening method to identify the response against race 2F of powdery mildew. The screening methods and disease assessments were verified in retest of cultigens in controlled environment condition. A significant positive correlation was recorded for mean disease severity rating and percentage disease index between polyhouse screening and controlled condition screening. The identified sources can be utilized for resistance mechanism studies and resistance breeding.
Keywords: Powdery mildew, race 2F, resistance screening, watermelon germplasm.

INTRODUCTION

Worldwide, nearly 10,000 species of angiosperms found to be infected with powdery mildew pathogenic fungi (Glawe, 2008). Two major causal organisms of the order Erysiphales were found to be responsible for powdery mildew in cucurbits, namely *Podosphaera xanthii* and *Golovinomyces cichoracearum* (McGrath *et al.*, 1996; Mandal *et al.*, 2020). *P. xanthii* is reported as the most prevalent and devastating fungi in Asia (Cohen *et al.*, 2004; Kasiamdari *et al.*, 2016). Although watermelon was considered to be free from powdery mildew with some exceptions (Tetteh, 2008), from last few decades, powdery mildew became an important limiting factor for watermelon production around the world. The watermelon powdery mildew disease is caused by *P. xanthii*, and it also has an important impact on low productivity in many other Cucurbits (Keinath and DuBose, 2004; Keinath, 2015). Isolates of powdery mildew from one species can infect other species of cucurbits (Cohen *et al.*, 2000; Lebeda *et al.*, 2011). A case of powdery mildew was first reported in 1925 in California. Since then, a number of physiological races have been discovered on the basis of the reactions after infection of different melon lines (Hong *et al.*, 2018). The cultivar, weather and geographical locations are the major factors which influence the powdery mildew race in the area (Cohen *et al.*, 2004). Races like 0, 1, 2 US, 2 France (2F), 3, 4, 5, N1 (race 6), N2 (race 7), N3, and N4 are commonly reported in many growing regions. Cases of incidence of races 1, 2, and 3 were reported mostly in America, whereas the incidence of races 0, 4, and 5 were identified in France. Race 1 and 2F are dominant and most prevalent in China region. According to McCreight (2006), it is possible to have as many as 28 races on melon based identification system with many variants across the present powdery mildew races (McCreight, 2006). A large development has been seen in breeding for resistant cultivars, but recurrent outbreak of the disease is reported regularly in different parts of the world due to breakdown of existing resistance by the appearance of new powdery mildew races (Hong *et al.*, 2018). The major example is emergence of race S in US after 2003 (Davis *et al.*, 2001; Davis *et al.*, 2007; Tetteh *et al.*, 2010). Similarly, four new races including 0, 2 US, 4 and 5 were observed first time during 2001-2002.

The powdery mildew disease is a constant threat to almost all the growing areas around the world and it can be observed throughout the year in many growing regions (Braun, 1995; McGrath, 2017). Powdery mildew mainly affects foliar parts and it can easily infect stems, petioles, cotyledons, hypocotyl and fruits (Garcia *et al.*, 2009). Infection in initial stages can cause poor growth and reduced vigour (McGrath *et al.*, 1996; Keinath and DuBose, 2004).

Powdery mildew pathogen on watermelon is relatively less apparent than melon and cucumber and the major symptoms are like occurrence of chlorotic spots during initial infection or fully developed lesions on the leaf surface. Powdery mildew is among the major constrains observed during all growing stages. Failure in proper control of powdery mildew at the initial stage of infection often causes loss in yield and it can be up to 50% in many cucurbits (Dhillon *et al.*, 2018). The reduction in leaf canopy can cause sunburn of fruits, which is not acceptable for marketing and export. The important economic impact of multiple cucurbits have driven research efforts to use genetic resistance (Prasanth *et al.*, 2019). Although, there is no standard definition for resistance to powdery mildew in cucurbits and the term 'resistance' is used to denote slow fungal growth and pathogen sporulation, considering that most resistant genotype may exhibit some initial disease symptoms (Cohen *et al*., 2004).

The use of fungicide is not advisable from the economic and environmental point of view and the development of resistant cultivars is more effective, eco-friendly over fungicide application (Kousik *et al.*, 2019). Moreover, it would be interesting and useful to obtain a source of resistance to powdery mildew for incorporation into commercial cultivars. The current study was aimed to 1) develop an efficient and reliable method for screening; 2) introduce a modified scoring scale for efficient results and inclusion of more variant symptoms and 3) screen the watermelon germplasms that have been developed by cucurbit germplasm innovation and genetic improvement research center, college of Horticulture, of Northwest A & F University, Yangling, Shaanxi, China.

MATERIALS AND METHODS

Plant Materials: A powdery mildew screening experiment was conducted in the polyhouse of cucurbit research Laboratory, College of Horticulture, Northwest A & F University during the autumn growing season. A total of 107 cultigens of *Citrullus* spp. were obtained from the watermelon repository of Northwest A & F University, Yangling, Shaanxi, China. All the cultivars and breeding lines were collectively referred as cultigens.

Inoculation of conidia on leaf segments: The initial collection was done from naturally infected plants available in greenhouse. One day before inoculation, highly infected leaves were shaken to remove old conidia and to produce inoculants consisting of vigorous young spores (Davis *et al.*, 2006). The spore suspension was prepared by detaching young leaves with heavy sporulation. The leaves were washed in beaker and filtered through a muslin cloth and diluted to spore concentration of 4×10^4 conidia $ml^{-1}$and it was checked by hemocytometer. 0.02% Tween-20 was added to suspension solution for better sticking. A fresh suspension was prepared for each day (Guo *et al.*, 2018).

Experiment-1

Race identification: A powdery mildew race identification was done in controlled condition of growth chamber at College of Horticulture, Northwest A & F University. To confirm the race of *P. xanthii* present, melon differentials were inoculated in the experiment. The race identification experiment was carried out with the ten melon indicator accessions including Iran H, Topmark, PMR45, PMR5, Vedrantis, Edisto 47, PI414723, PI124111, PMR6 and PI124112, and their responses were evaluated according to standard protocols (McCreight, 2006; Wang *et al.*, 2013). Seeds of the melon accessions were also obtained from watermelon repository of Northwest A & F University, Yangling, Shaanxi, China.

Experiment-2

Screening under polyhouse conditions: The experiment was carried out with 107 cultigens during autumn in 2019 when the climatic conditions were favourable for several disease development (Prasanth *et al.*, 2019). The seed of test cultigens of *Citrullus* spp. were sown in pro-trays (32 cells) and randomly placed on bench. The experiment was conducted with a minimum of three replications and 8 plants per replication. The cultigens were not considered for the experiment if the germination was not sufficient or insufficient number of plants per replication was found. Five random plants per replication were selected for disease scoring. Powdery mildew was mass propagated on the susceptible melon and cucumber plants and spores were collected for inoculation. Seedlings were inoculated at cotyledon and true leaf stage for a proper infection in all plants (Tetteh *et al.*, 2010; Prasanth *et al.*, 2019). Seedlings were maintained at normal greenhouse temperature and relative humidity. Spreader plants were placed between rows as an additional source of powdery mildew infection (Tetteh, 2008).

Environmental Conditions: Inoculated plants were kept in normal polyhouse condition. The experiment was conducted during the autumn season, which is favourable for powdery mildew growth.

Disease Assessment: Powdery mildew on watermelon seedlings were rated as disease incidence and severity at every week after inoculation. The scoring was done on minimum 5 plants per replication. Total plant disease severity was calculated by averaging leaf scorings.

Disease scoring was done based on percent leaf area infected and on 0–9 rating scale (Tetteh *et al.*, 2013) with little modification.

After calculating disease severity rating, the genotypes in the population were categorized into four categories namely (Tetteh *et al.*, 2013)

Table 1. Disease scoring scale (0-9) based on the leaf infection.

Score	% Disease	Description
0	0	No disease
1	0-3	Faint yellow specks on leaves or small leaf lesions
2	3-6	Chlorotic lesions covering 20% of leaves or few leaves with few lesions
3	6-12	Yellow chlorotic lesions on leaves turned to brown necrotic areas
4	12-25	Two to three healthy colonies of mycelium on leaves
5	25-50	Large leaf lesions with abundant sporulation
6	50-75	Less than 50% mycelium coverage on leaves
7	75-87	Represents 50 to 70%mycelium coverage on leaves and
8	87-100	50 to 70% mycelium coverage with large necrotic areas
9	100	All leaves fully covered with powdery mycelium or plant dead

Table 2. Genotype classification in different categories based on mean severity rating.

Response	Average Rating
Resistant	0-2
Moderately resistant	2.0-3.5
Moderately susceptible	3.6-5.0
Susceptible	>5.1

The additional response of cotyledon leaves and stems were included to draw a precise conclusion. Leaf and cotyledons gradings were used because of a weak significant correlation in previous studies (Davis *et al.*, 2007). The observation was recorded 5WAI in all the germplasm and advanced lines. The following ranking was followed S-heavily infected with spores/dead, MS-chlorotic lesions, MR-few visible spores and R-no sporulation (Ben-Naim and Cohen, 2015).

Experiment -3

Disease response of selected germplasms under controlled environmental condition: A retest was performed in growth camber (controlled condition) to verify and confirm the reaction of the selected germplasms against race 2F of powdery mildew. A set of representative cultigens from different response level were used in this experiment. Three random germplasms were selected from sensitive, resistance and intermediates (moderate sensitive and moderate resistance) groups. The experimental design was a randomized block with four replications. The germplasms were seeded in 50 cell trays. Plants with 2-3 true leaves and similar growth were selected and transplanted in plastic pots for further observations.

First rating (1WAI) was recorded after clear infection visibility of susceptible differentials. The second observation (5WAI) was recorded after sensitive plants started showing dry leaf symptoms. Ratings were not performed later than 2 months after transplanting. Stem and upper leaf ratings were recorded using nonlinear disease severity scale.

Disease assessment: Disease severity was examined on the leaves and stem of individual plants by using the previously described nonlinear scale suggested by (Tetteh, 2008). Where 0 = no symptom; 1 = faint yellow speck on leaves and first appearance of necrotic spots on the stem; 2 = chlorotic lesions on leaves with 2 to 3 necrotic spots on the stem; 3 = chlorotic lesions covering 20% of leaves and necrotic spots covering less than 10% of stem; 4 = yellow chlorotic lesions on leaves turned to brown necrotic areas and first sign of active mycelium sporulation on leaves or stem; 5 = 2 to 3 healthy colonies of mycelium on leaves or stem; 6 = approaching 20% mycelium coverage; 7 = 20 to 50% mycelium coverage; 8 = 50 to 70% mycelium coverage with large necrotic areas; 9 = plant fully covered with powdery mycelium or plant death. The final consideration was made on the basis of total plant rating. The cultigens were considered resistant if the total rating was ≤ 3; intermediate if the plant rating ranged from 3.1 to 4; or susceptible if the rating was > 4.0.

The percentage disease index (PDI) was calculated in retest experiment after 1 and 5 weeks after inoculation. The following formula was used to calculate the PDI (Prasanth *et al.*, 2019).

$$\text{PDI} = \frac{\text{Sum of numerical values}}{\text{Number of plants graded} \times \text{maximum rating}} \times 100$$

Growing condition: Seeds were transplanted to pot filled with a mixture of peat and vermiculite (2:1 v/v) and placed in a growth chamber at a temperature of 29/19°C (day/night), and a 16h light/8h dark cycle was maintained. A higher relative humidity (70-90%) was maintained to provide a favourable condition for infection.

Data analysis: All the presented data were summarized as a mean for each plant material used in Table 4 and 5. The correlation was performed with IBM SPSS 22.0 and plotted with the help of XLSTATv18. Software (Addinsoft, Paris, France).

RESULTS

Powdery mildew race confirmation: To confirm the powdery mildew fungus race present in the locality, spores were collected and tested on a set of melon differential cultivars (Table 3; Figure 1). From the set of cultivars used in race identification experiment, the differential cultivars like Vedrantais, Edisto47, PI414723, PI124111, PMR6 and PI124112 were found resistant, while Iran-H, Topmark, PMR45 and PMR5 responded to inoculation and visible symptoms were recorded on cotyledons and true leaves. The differential responses of the cultigens against the *P. xanthii* spores confirm the '2F' race. The same race of powdery

mildew was observed in both spring and autumn growing seasons.

Table 3. Differential host reactions to powdery mildew pathogens in different seasons.

S. No.	Melon lines	Response (Spring)	Response (Autumn)
1	Iran H	S	S
2	Topmark	S	S
3	PMR45	S	S
4	Vedrantais	S	S
5	PMR5	R	R
6	Edisto 47	R	R
7	PI 414723	R	R
8	PI 124111	R	R
9	PMR 6	R	R
10	PI 124112	R	R

S- sensitive R- resistant

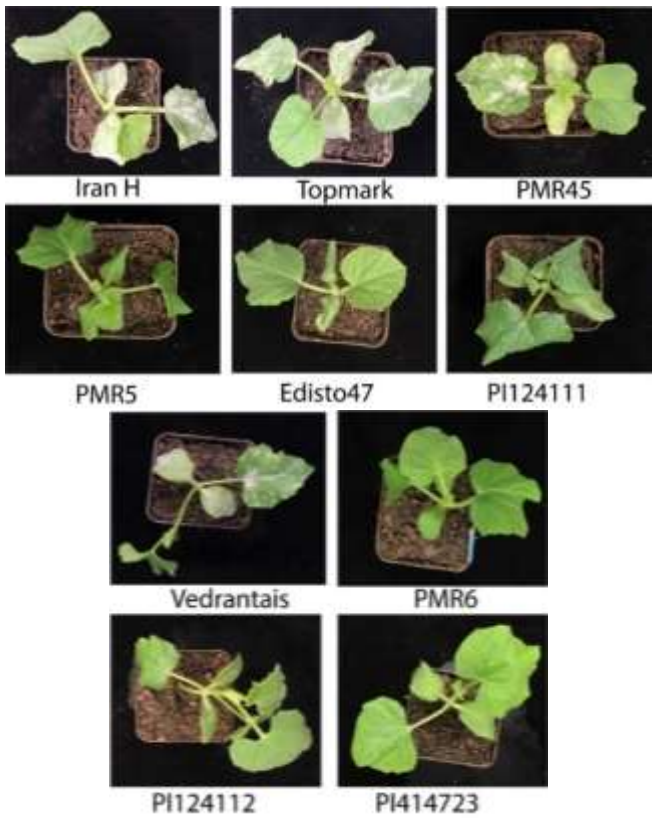

Figure 1. Differential host reactions to powdery mildew pathogens.

Evaluation of watermelon lines against race 2F: Powdery mildew development was rapid and severe in many watermelon cultivars used in the study. The appearance of the powdery mildew on different germplasm and advance breeding lines was confirmed by the presence of *P. xanthii* conidia on the abaxial surface on the leaves and stem. Significant development of powdery mildew was recorded in the most sensitive cultivars during all the performed experiment, including polyhouse and fully controlled condition. The similar results were recorded for many germplasm and advance breeding lines. During the polyhouse trial, wide range of reaction was observed. The symptoms ranged from little chlorotic appearance on resistant plants to full leaf coverage on sensitive plants. The overall response was concluded on the basis of mean severity rating recorded from 2WAI to 5WAI along with reaction on cotyledons and stem. A large number of germplasm and breeding lines were found in the intermediate group according to response. There were total of 107 materials for screening in polyhouse condition and only 5.8% cultigens were found with a higher level of resistance response, with mean severity rating between 0-2. A large number of cultigens had a moderate sensitive response and the share was 50.9%. 7.8% of plants were categorized under sensitive category with an average rating of ≥ 5.1. The cultigens like M16, M49 and M11 showed a higher level of resistance, whereas ZXG-1996, 04-1-2 and M01were recorded with the highest disease severity rating. A large number of cultigens were found to have an intermediate reaction against race 2F of powdery mildew. Apart from the leaves, the severity rating of the cotyledons of almost all the species was observed with small conidial growth.

Retest under controlled condition: A retest experiment was performed with randomly selected cultigens from different response group to validate the polyhouse screening results. Moreover, some additional information, such as stem rating was added to check the correlation between leaf and stem disease severity ratings. The retest was performed under controlled relative humidity and temperature. The response of the majority of plants was similar to the polyhouse screening and in some cultivars, the severity was relatively higher than polyhouse screening. The cultivar ZXG-1996 was recorded with the highest rating in both the observations. Sensitive cultivars such as 04-1-2, ZXG-1996 and M01 were also found sensitive in retest of cultigens. Similarly, the observation for the resistant cultigens were recorded similar in both the experiments. Some mean leaf ratings were slightly different because of the difference in growing conditions. Mean disease severity rating for resistance cultivar was recorded ≥ 5 in both the screening methods while it was observed between 0-2 for resistant cultigens.

Correlation between polyhouse screening and artificial screening: A significant positive correlation was recorded in Mean severity rating of the controlled condition and polyhouse condition. A similar positive correlation was observed in PDI of watermelons seedling screening under controlled condition and polyhouse condition. MSR (CC)

with MSR (PC) and PDI (CC) with PDI (PC) showed values 0.815, 0.962 respectively at significance level of alpha = 0.01. The plot (Figure 3) indicates a good agreement between the methods used for disease assessment and screening in different condition. The observation complements each other and provides significant evidence of disease severity among

Table 4. Ranking of the final total powdery mildew severity rating for the watermelon germplasm under greenhouse condition (Autumn 2019).

							Response***		
S. NO.	**CULTIGENS**	**2 WAI**	**3WAI**	**4WAI**	**5WAI**	**MSR**	**Cotyledon**	**Stem**	**Response**
1	M01	3.27	4.67	6.83	8.07	5.71	S	S	S
2	M04	2.33	2.67	4.33	5.80	3.78	S	MS	MS
3	M05								**
4	M07	3.50	4.80	4.97	5.33	4.65	MR	S	MS
5	M08	2.10	3.20	3.63	4.27	3.30	MS	MS	MR
6	M09	2.20	3.57	4.40	4.13	3.58	S	MS	MS
7	M11	0.00	0.00	1.43	2.57	1.00	S	MR	R
8	M12	2.23	3.43	4.27	6.43	4.09	S	MS	MS
9	M13	2.40	3.50	5.67	5.17	4.18	S	MS	MS
10	M16	0.00	0.33	1.00	1.00	0.58	MR	R	R
11	M17	1.77	2.37	4.30	5.67	3.53	MR	MS	MR
12	M20								**
13	M21								**
14	M22	1.47	2.67	3.67	4.87	3.17	S	S	MR
15	M24	2.37	3.43	3.80	5.30	3.73	MS		MR
16	M25	2.50	2.87	4.67	6.20	4.06	MR	S	MS
17	M29	0.00	3.57	5.63	5.80	3.75	MS	S	MS
18	M30 A-1	1.63	2.67	3.43	4.47	3.05	MR	MS	MR
19	M34	2.27	4.50	5.30	5.53	4.40	S	MS	MS
20	M35	2.13	3.67	5.37	6.00	4.29	S	MR	MS
21	M36	2.63	4.20	6.30	6.60	4.93	MS	MR	MS
22	M38	0.33	3.50	5.27	5.87	3.74	S	MR	MS
23	M39	2.27	4.27	5.37	5.53	4.36	MS	S	MS
24	M42	0.83	3.00	3.33	3.67	2.71	MS	MS	MR
25	M44	2.07	2.40	4.33	4.47	3.32	MR	MS	MR
26	M47	0.00	1.67	2.00	3.67	1.83	R	MS	R
27	M49	0.00	0.00	1.50	3.00	1.13	MS	MR	R
28	M51	2.00	2.50	3.67	5.40	3.39	S	S	MR
29	M52	2.57	4.33	4.33	3.30	3.63	MR	MR	MS
30	M53	2.00	2.50	3.30	3.57	2.84	MS	S	MR
31	M55	2.40	2.87	3.57	4.30	3.28	S	MS	MR
32	M56	2.63	3.27	3.57	4.63	3.53	MR	S	MR
33	M58	1.93	2.40	2.67	3.30	2.58	MS	S	MR
34	M61	2.37	3.10	3.10	3.30	2.97	MS	MS	MR
35	M62	2.50	4.20	6.30	7.20	5.05	S	S	S
36	M63	1.47	2.33	2.47	4.67	2.73	S	MR	MR
37	M64	1.37	2.67	3.27	4.00	2.83	MS	MR	MR
38	M65	2.43	3.60	5.20	5.83	4.27	S	MR	MS
39	M61-1	2.20	2.67	3.67	4.30	3.21	MR	R	MR
40	M67	2.57	3.47	4.23	7.33	4.40	MS	MS	MS
41	M68	3.10	4.63	5.30	6.73	4.94	MS	MS	MS
42	M69	2.20	4.63	5.20	6.03	4.52	S	S	MS
43	M70	2.20	2.87	3.20	5.20	3.37	MR	MS	MR
44	M86	2.20	3.63	3.63	5.20	3.67	MS	MR	MS
45	GS11	2.33	3.17	4.20	6.30	4.00	MS	S	MS
46	GS16	2.20	2.60	3.20	5.33	3.33	MS	S	MR
47	GS17	2.63	3.33	3.60	4.83	3.60	S	S	MS
48	GS20	2.13	3.20	5.53	7.20	4.52	S	MS	MS
49	GS25	3.10	3.33	4.27	5.33	4.01	S	S	MS
50	GS27-1	2.17	2.23	3.27	4.33	3.00	MS	MS	MR

							Response***		
S. NO.	**CULTIGENS**	**2 WAI**	**3WAI**	**4WAI**	**5WAI**	**MSR**	**Cotyledon**	**Stem**	**Response**
51	GS28	2.40	2.63	4.27	4.67	3.49	MR	MR	MR
52	GS30	3.20	4.30	5.80	6.53	4.96	S	MS	MS
53	GS36	2.20	3.50	4.20	5.67	3.89	S	MS	MS
54	GS37	2.17	2.20	4.30	6.20	3.72	MS	MS	MS
55	GS38	3.20	4.70	5.53	7.50	5.23	S	S	S
56	GS40	2.87	3.67	4.83	5.67	4.26	MS	MS	MS
57	F02	2.63	3.67	3.30	4.33	3.48	S	S	MR
58	F06	2.63	2.80	3.17	5.77	3.59	MS	S	MS
59	F09	2.17	4.40	4.30	6.20	4.27	S	S	MS
60	F10-1	1.83	3.57	4.70	4.67	3.69	MS	MR	MS
61	F12	2.20	4.70	5.37	6.33	4.65	S	S	MS
62	F19-3	2.17	2.43	2.63	3.77	2.75	MS	MR	MR
63	134-2C	3.10	3.20	3.77	4.10	3.54	MR	MS	MR
64	ZXG01338	2.17	2.57	3.40	6.27	3.60	MS	MS	MS
65	ZXG00437	3.07	3.90	5.80	7.63	5.10	S	MS	S
66	ZXG00884	0.60	2.57	3.27	3.77	2.55	MS	MR	MR
67	ZXG00133	2.70	3.20	4.30	7.37	4.39	S	S	MS
68	ZX00233	2.10	3.20	3.47	4.60	3.34	MS	MS	MR
69	ZXG IN-2	2.60	3.63	4.30	6.60	4.28	S	S	MS
70	35X2	2.60	3.63	3.63	5.30	3.79	MS	MR	MS
71	72	2.10	4.37	4.23	5.23	3.98	S	S	MS
72	13	1.20	1.70	2.10	4.53	2.38	MS	S	MR
73	44	1.67	2.27	3.27	6.37	3.39	S	S	MR
74	61	2.70	4.27	4.63	6.13	4.43	MS	MS	MS
75	99	2.30	3.63	4.20	6.67	4.20	MS	S	MS
76	10	3.10	3.20	4.53	5.77	4.15	S	S	MS
77	72X44	2.60	3.93	3.67	6.80	4.25	MS	S	MS
78	148	2.90	4.27	5.30	7.17	4.91	S	MS	MS
79	ZY15	2.37	3.70	4.27	6.17	4.13	S	S	MS
80	2006-5 F3	2.10	3.13	3.67	5.37	3.57	MS	S	MR
81	Y1F	2.20	3.63	4.60	6.80	4.31	S	MS	MS
82	YIM	0.00	1.67	1.87	2.00	1.38	MR	MR	R
83	Y59	2.60	3.20	5.20	6.63	4.41	MS	MS	MS
84	Y61	2.13	2.67	4.90	5.13	3.71	S	MR	MS
85	Y118	1.30	2.00	4.23	4.97	3.13	MR	MR	MR
86	S01	2.93	4.73	5.90	6.80	5.09	S	S	S
87	S02	2.93	4.30	6.80	7.97	5.50	S	MS	S
88	04-1-2	3.20	4.33	6.77	8.63	5.73	S	S	S
89	1207	2.13	3.67	4.13	4.10	3.51	MS	MS	MR
90	14R-4 F3	2.20	3.87	4.67	5.90	4.16	S	S	MS
91	90	2.00	3.20	5.33	6.27	4.20	MR	S	MS
92	P02	2.13	2.50	3.77	4.20	3.15	S	S	MR
93	T2-3-2-1-3	0.00	2.33	2.43	3.60	2.09	MS	MR	MR
94	YL	0.00	1.23	2.67	3.47	1.84	MR	R	R
95	SGBB								**
96	ZY-15	0.73	3.17	3.77	4.47	3.03	S	MR	MR
97	HONG XIAO YU	0.80	2.50	3.77	4.20	2.82	S	MS	MR
98	BLACK DIAMOND	2.13	3.30	5.33	6.33	4.28	MS	S	MS
99	KE LUAN SHENG	2.10	2.50	4.27	5.33	3.55	S	S	MS
100	ZHONG 10 YOU DAN	2.20	4.30	5.37	5.60	4.37	MS	S	MS
101	JOING MU YOU DAN	2.37	3.57	6.27	6.30	4.63	S	MS	MS
102	HEI MU DAN	2.50	3.57	3.87	4.47	3.60	MS	MR	MR
103	BEI GUA	0.67	2.33	3.40	4.60	2.75	MR	MR	MR
104	CHARLESON ELITE								**
105	XIAO ZI TANG LI	2.20	4.53	5.47	6.43	4.66	S	MS	MS
106	JING 5 FU	2.20	4.23	4.47	5.37	4.07	S	S	MS
107	ZXG-1996	3.77	4.90	7.50	8.80	6.24	S	S	S

No germination in seeds/not enough plant in replication. * data were collected 5weeks after inoculation (WAI)

Table 5. Disease reaction exhibited by selected cultigens in retest experiment. The mean disease severity was recorded on leaf and stem. PDI was calculated after 5 weeks of inoculation.

	Mean disease severity rating (0-9) scale									
		1 WAI				5 WAI				
Material	**Replication**	**Leaf**	**SD leaf**	**Stem**	**SD stem**	**Leaf**	**SD leaf**	**Stem**	**SD stem**	**PDI***
04-1-2	4	2.53	0.23	1.6	0.2	7.87	0.12	5.3	0.3	84.44
ZXG-1996	4	3.87	0.12	1.0	0.0	8.60	0.20	7.9	0.5	95.55
M01	4	3.33	0.12	1.8	0.5	7.13	0.12	5.5	0.2	94.16
M49	4	1.53	0.61	2.1	0.6	3.27	0.50	2.1	0.6	81.11
M11	4	0.67	0.31	1.6	0.2	2.67	0.20	1.5	0.5	90.00
M16	4	0.13	0.20	0.2	0.0	1.53	0.31	0.6	0.3	81.11
BEI GUA	4	2.07	0.42	1.0	0.2	4.20	0.20	1.7	0.5	84.00
Y118	4	2.27	0.31	0.4	0.5	4.27	0.53	2.3	0.2	80.00
GS28	4	2.40	0.40	0.8	0.2	4.07	0.35	1.5	0.1	76.00

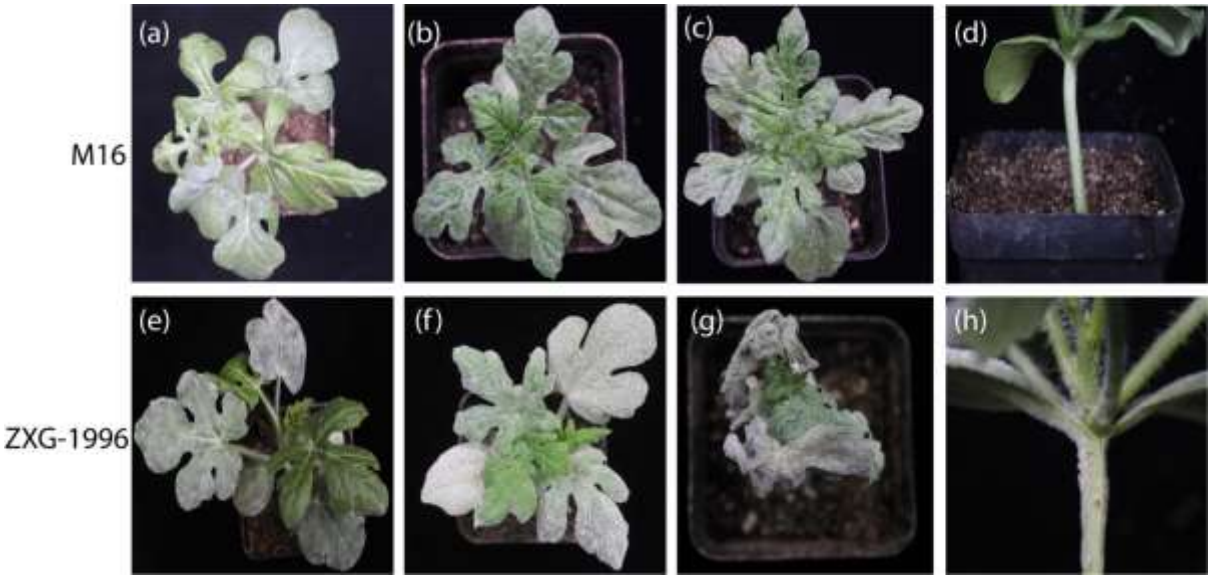

Figure 2. Watermelon cultigens response against powdery mildew. (a-d) leaf and stem response of M16-resistance cultigens. (e-h) leaf and stem response of ZXG-1996-sensitive cultivar against powdery mildew.

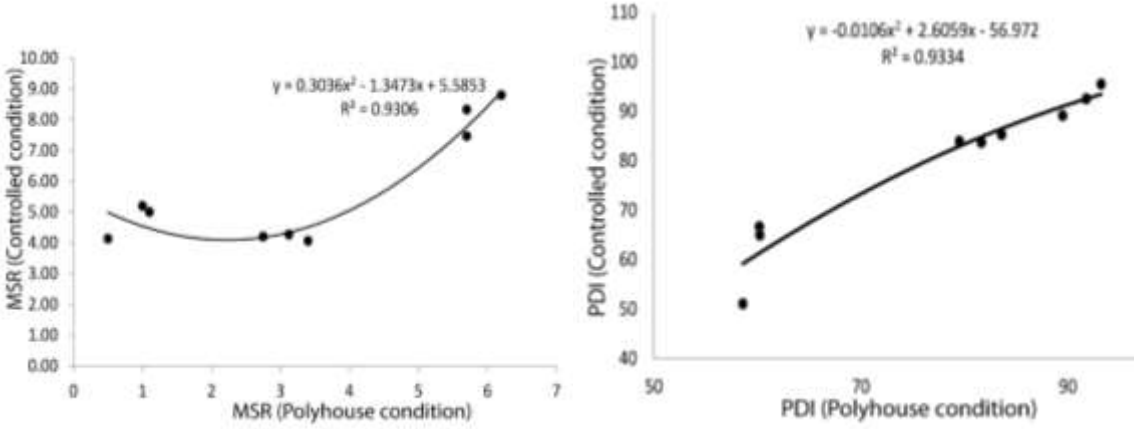

Figure 3. Correlation between qualitative and quantitative parameters under the artificial and controlled screening of watermelon genotypes against 2F powdery mildew race. (5 weeks after inoculation), MSR- Mean disease severity rating, PDI- percent disease index.

the watermelon seedlings. These results also illustrate that the screening system allows the disease screening trials of powdery mildew at a particular season, regardless of the weather condition.

Correlation of Leaf and Stem ratings: The leaf and stem disease severity rating of randomly selected plants from different response groups were used for correlation analysis. There was a significant and positive correlation was observed in later stage of disease infection. No positive correlation was recorded in leaf and stem ratings 1week after inoculation. Most of the cultigens were recorded with a higher rating in controlled condition than polyhouse condition. For race 2F resistance, a significant positive correlation (0.954, significance level 0.01) was observed between leaf and stem disease severity rating after 5 weeks of inoculation. Likewise, a strong positive correlation was observed in leaf ratings of 1WAI and 5WAI; the high correlation suggests that the disease progress was uniform in controlled environment condition.

Table 6. Correlation matrix of the mean disease severity ratings of leaf and stem under controlled condition screening of selected cultivars from different disease response group. WAI- Weeks after inoculation.

Correlation matrix (Pearson):

Variables	Leaf (1WAI)	Stem (1WAI)	Leaf (5WAI)	Stem (5WAI)
Leaf (1WAI)	1.0000	0.1946	0.9050**	0.8400**
Stem (1WAI)		1.0000	0.2955	0.3050
Leaf (5WAI)			1.0000	0.9540**
Stem (5WAI)				1.0000

**significance level alpha=0.01

DISCUSSION

The occurrence and breakdown of existing resistance shows the possibility of different races and appearance of new powdery mildew race in any particular area, so it is very crucial to keep eyes on the powdery mildew races for immediate assistance to watermelon growers (Cohen *et al.*, 2004; Huang *et al.*, 2018). A race monitoring system should be carried out to avoid losses from emerging threat. The race identification has its importance because the pathogenicity against the standard differential melon cultigens is known for identification of powdery mildew race (Wang *et al.*, 2013). In the current study, the cultigens were tested against the 2F race, which is a major devastating race in China and in local regions. The race identification was done in spring growing season and later retested for confirmation in the autumn growing season. The race confirmation was done according to race identification host and reactions were suggested by Wang *et al.* (2013). The differential responses of the cultigens against 2F race of *P. xanthii* may be due to the inherent genetic makeup or due to unique interaction between virulent genes of pathogen and genes of the host plant. In our study, the reactions of melon differentials against race 2F of powdery mildew were similar with Wang *et al.* (2013). Race 2F of powdery mildew was defined on the basis of the susceptibility of the melon differentials IranH, Topmark, PMR45 and Vedrantais and resistance of PMR5, Edisto, PI414723, PI124111, PMR6 and PI124112. (Wang 2013) Moreover, it may be possible that some particular race is more active in particular season; so, in our study, the reactions were observed in two different growing seasons and the consistency in results shows that race 2F of powdery mildew might be the dominant race for powdery mildew in this region. The cultigens of one country may exhibit different levels of resistance in another country due to variation in many environmental factors. So race identification experiment should be performed in controlled environment with prescribed melon host lines.

The screening method is a crucial factor to determine the authentic results. Our experiment was designed according to the methods used by Davis *et al.* (2006), Kousik *et al.* (2018), Kousik *et al.* (2019) and Prasanth *et al.* (2019). There are so many scoring systems that are available in different literature but the diseases scoring scale adopted in this study was selected on the basis of pre-experiments performed with 50 cultigens. Every day the reactions of the seedlings were observed and scoring of Tetteh *et al.* (2013) was found most suitable for our cultigens screening experiment. Similarly, few pre-experiments were performed for method of inoculation including dust and spray of inoculation. In our pre–experiments, it was found that inoculation suspension is the best method for mass screening as it provides the equal distribution on plant parts as well as provides an opportunity to know the concentration of inoculation. The retest experiment was performed to support the screening results obtained from polyhouse screening. The retest seems to be an effective method to validate the screening procedure and a similar method for screening was followed by Tetteh *et al.* (2010).

Different cultigens and advance breeding lines of watermelon studied in this research and M16, M11 and M49 were recorded as the most resistant against race 2F of powdery mildew based on the polyhouse scoring of seedlings, stem reaction and cotyledon leaf reaction. The data generated in polyhouse screening and retest in controlled condition were used for final conclusion. Powdery mildew lesions and conidia development were

clearly observed on sensitive cultivars (ZXG-1996, M01, 04-1-2) and moderate sensitive cultivars after one week of inoculation, whereas no conidial development was observed on any resistant cultigens after one week of inoculation. The resistant lines were observed with few small yellow spots in a controlled condition and this might be due to hyper-sensitive type reaction because of high inoculum levels in controlled condition (Hong *et al.*, 2018b). The response of the cultigens used in our study against race 2F of powdery mildew may be due to inherent genetic makeup or due to unique interaction. The resistant cultigens were recorded with the slow growth of powdery mildew on leaves and stem. Slow disease development is a unique reaction of disease resistant cultivars observed in various screening systems (Douglas *et al.*, 1984; Hautea *et al.*, 1987; Raju *et al.*, 1991).

Furthermore, the correlation study was performed and it indicates that both the methods were consistent and responses were similar (Prasanth *et al.*, 2019). The leaf and stem correlation are important to predict the resistance mechanism and the high correlation is directly linked with similar genes controlling the resistance (Davis 2007, Davis 2010). Similar observation was observed by Davis *et al.*(2007) for race 1W resistance. In our study, high positive correlation was recorded in leaf and stem rating after 5 weeks of inoculation.

Conclusion: Response of total 107 cultigens against powdery mildew race 2F was examined in the experiment. Some cultigens were proposed as resistant and sensitive resources for further genetic and molecular validations. High level of resistance was observed in few cultivars and most of the cultigens were recorded with intermediate reactions. The cultivars with high level of resistance can be utilized in future for breeding purpose. Further information about the parents and species could be useful for future strategies. We also conclude the conformity of powdery mildew race 2F in the locality. We propose a comprehensive study in selected cultivars to examine the effects of powdery mildew on production and productivity. In addition, we propose the utilization of resistant cultigens for resistant breeding approaches.

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