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Effect of Powdery Mildew on the Photosynthetic Parameters and Leaf Microstructure of Melon

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Abstract: Powdery mildew is a fungal disease devastating to crops, causing significant quality and yield loss. As one of the most important fruits in the world, melon also is damaged by powdery mildew. The present study investigated the effect of powdery mildew on the photosynthetic parameters and leaf microstructure of melons, the ultrastructure of the leaf surface, photosynthetic index, chlorophyll content, yield, and quality index of five thick-skinned and differently shaped melon varieties. The net photosynthetic rate, transpiration rate, leaf water use efficiency, and chlorophyll levels were significantly ($p < 0.05$) higher in Kangbing F3800 plants compared to the other four varieties. In the case of powdery mildew infection, the total number of stomata in the upper and lower epidermis was particularly high in the Zhongtian No. 8 and Zhongtianxueqiong varieties, respectively. The stomatal length and width were highest in the upper epidermis of Zhongtian No. 12 leaves and in the lower epidermis of Zhongtian No. 8 leaves compared to the other varieties. The total yield and meat thickness were significantly ($p < 0.05$) higher in the Zhongtianxueqiong variety than the others, along with the low edge sugar content. Overall, powdery mildew impacted differently the photosynthetic and leaf surface characteristics of the five melon varieties. Kangbing F3800 emerged as the most resistant variety, making it the preferred choice for introducing and promoting thick-skinned melon varieties in the Ningxia Hui Autonomous Region of China.

Keywords: disease index; chlorophyll; stomata; yield; meat thickness

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1. Introduction

Melon belongs to the *Cucumis* genus of the Cucurbitaceae family. It is an annual vine and an important cash crop, which widespread total area of melon cultivation in the world was 1.07 million hm^2, and the output was 28 million tons, in 2020 (https://faostat.fao.org/, 1 May 2024). There are 130 genera and 800 species of melons [1], with China, Turkey, the United States of America, Korea, and Iran accounting for most of the output [2]. Crucially, approximately half of all melons are cultivated in China [3]. The Ningxia Hui Autonomous Region is next to the Helan Mountain. It benefits from abundant sunshine, large daily temperature differences, and fertile soil, which give cultivated melons a high sugar content and crisp meat, as well as tender and juicy consistency. Watermelons and melons have a long history of cultivation in Ningxia, where they have become an important source of income [4].

Powdery mildew is a serious fungal disease affecting greenhouse and cucurbita-ceous crops worldwide [5,6]. Powdery mildew damages and affects the growth of the leaf blade, the sheath presenting as white spots, and results in a ~50% yield reduction of crops [7–10]. This is the same in melons, where it mainly compromises the vegetative structures (leaves, stalks, and stems), which become covered with white powder-like spots. Symptoms are rarely observed in fruits, although weight-based productivity and fruit quality (loss of aroma and sugar content) may be affected. Elevated temperature and humidity are the leading causes of this disease [11]. Currently, powdery mildew is kept under control by chemical means, even though this approach may lead to resistance, environmental pollution caused by the overuse of fungicides, and food safety concerns [12]. Future environmentally friendly attempts to control the spread of powdery mildew should mainly focus on improving the excessively hot and humid environment in which melons are cultivated and breeding new varieties resistant to this fungus [13–15].

The hyphae of powdery mildew develop on plant surfaces, including leaves, stems, and cotyledons, where they form white, powdery spots. They lower the chlorophyll content and ability of melon leaves to perform photosynthesis. This leads to a drop in the photosynthetic quantum yield, blocks the electron transport chain, and lowers the photosynthetic rate, ultimately negatively affecting melon yields and quality [16,17]. Stomata could play an important role in restricting fungal invasion, with stomatal size playing a crucial role [18]. In fact, stomatal number and size can serve as reliable indicators of resistance to powdery mildew in melons [19].

In view of the complex interactions between powdery mildew and melon plants, this study sought to determine the effect of this fungus on different varieties of melons grown in the same greenhouse. The selected varieties were Zhongtianxueqiong, Zhongtian No. 9, Zhongtian No. 8, Zhongtian No. 12, and Kangbing F3800. Analysis of the photosynthetic indices, chlorophyll content, yield, leaf quality, and leaf surface revealed the effect of powdery mildew on the photosynthetic performance and leaf structure of melons. This study investigated and compared many key melon varieties under the stress of powdery mildew, which increases our understanding and knowledge of melon planting from the theoretical level in Zhongwei City, China, and lays a theoretical basis for further melon variety breeding.

2. Materials and Methods

2.1. Experimental Material and Conditions

The five varieties of thick-skinned melons tested in this study (Zhongtianxueqiong, Zhongtian No. 9, Zhongtian No. 8, Zhongtian No. 12, and Kangbing F3800), along with their seed supplies, are detailed in Table 1.

Table 1. Test varieties and seed supply units of the melons involved in this study.

Treatment	Variety	Seed Source
T1	Zhongtianxueqiong	Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, China
T2	Zhongtian No. 9	Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, China
T3	Zhongtian No. 8	Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, China
T4	Zhongtian No. 12	Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, China
T5	Kangbing F3800	KNOWN-YOU SEED CO., LTD., Taiwan, China

The experiment was conducted in Fengwan Village, Guanqiao Township, Haiyuan County, Zhongwei City, Ningxia Hui Autonomous Region, China (105°69′26.86″ E, 98 36°36′74.65″ N, altitude 1547 m). The region has a continental monsoon climate, with an average annual precipitation of 352.9 mm mostly distributed from July to September, an annual evaporation of 16,444.7 mm, an average altitude of 1780 m, and an average annual temperature of 7.7 °C.

Melons were grown in a large arch shed with a steel frame, using a unified method for seedling rearing and climbing. Each treatment in a plot was completely different when using plastic greenhouses with concrete walls to ensure that these plots were unaffected by external factors and powdery mildew. Each treatment had the same greenhouse environment with temperature and humidity, water and fertilizer conditions, and management measures.

Seedlings were sown on 5 March 2023 and settled on 1 April 2023. Plants were grown in double rows, with a ridge width of 80 cm, height of 20 cm, furrow width of 60 cm, and row spacing of 40 cm × 60 cm. Three repeats per variety, 50 plants per replicate, and random block arrangement were applied. Three-vine pruning and artificially assisted pollination of female flowers were performed. The soil in the test field was uniformly fertile loam. During the preparation of the field prior to planting, 4000 kg of organic earthworm manure; 20 kg of nitrogen, phosphorus, and potassium compound fertilizer; and 20 kg of diammonium phosphate were applied every 667 m^2. The irrigation method was drip irrigation under film.

2.2. Index Measurement

2.2.1. Determination of Melon Appearance

Fruit shape, skin base color, skin cover color and pattern, flesh color, and adhesion of melons were measured by observing ten randomly selected specimens from each variety.

2.2.2. Determining Photosynthetic Indices and Chlorophyll Content

On sunny days (10 days before the first harvest), the photosynthetic index of the melon leaves was determined between 9 a.m. and 11 a.m. using a portable photosynthesizer (infrared gas analyzer LCpro T Advanced Portable Photosynthesis System, ADC Co., Hoddesdon, UK). Light intensity was set to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature was set to 25 ± 2 °C, and the instrument was calibrated every hour. Four points on the melon leaves were randomly selected to determine the net photosynthetic rate, transpiration rate, intercellular carbon dioxide concentration, and stomatal conductance. Water use efficiency (net photosynthetic rate/transpiration rate) was calculated.

The chlorophyll content was measured using a handheld chlorophyll meter (SPAD-502Plus; Konica Minolta Sensing Company, Tokyo, Japan). Leaves with the same photosynthetic index were selected, and measurements were performed four times. The average value of the four readings represented the relative chlorophyll content (SPAD value), which was then used to calculate the specific chlorophyll content according to the following formula [20]:

$$Y = 0.0996X - 0.152$$

where X is the SPAD value of the seedlings, and Y is the specific chlorophyll content of the seedlings (mg/dm^2).

2.2.3. Powdery Mildew Disease Index Investigation

Based on the occurrence and development of powdery mildew in melon fields, the disease is classified according to the proportion of powdery mildew area to the total leaf area [21,22]. When the white powder was light and the diseased leaf area accounted for less than 5%, 5–10%, 10–50%, and over 50% of the total leaf area, the disease was marked, respectively, as grade 1, 2, 3, or 4, in accordance with Table S1 [23].

$$\text{Disease index (DI)} = (\Sigma(\text{disease leaf number} \times \text{grade}))/((\text{investigation leaf number} \times \text{highest disease grade})) \times 100\%$$

2.2.4. Observation and Determination of the Leaf Epidermis Ultrastructure

Briefly, fresh melon leaf tissues were preserved on dry ice and sent to Wuhan Servicebio Biotechnology Corporation, Wuhan, China, for analysis.

2.2.5. Determining Melon Yield and Fruit Quality Index

Ten melons were randomly selected from each variety. The melons were assayed using a ruler, Vernier caliper, electronic scale, and handheld sugar meter (ATAGO PAL-1; Atago Co., Ltd., Tokyo, Japan). The following fruit characteristics were quantified: longitudinal diameter, transverse diameter, meat thickness, central soluble solid content, and average yield. The texture, fiber, juice, fragrance, and flavor of the melon were measured using the taste method. The shape of the fruit, skin base color, skin cover color and pattern, flesh color, and adhesion were measured by observing the fruits.

2.3. Data Analysis

Excel 2019 (Microsoft Corp., Redmond, WA, USA) was used to collect and record the data. Significant differences among the five different melon varieties were examined by one-way analysis of variance (one-way ANOVA) in SPSS 22.0 (SPSS, Chicago, IL, USA). Tukey's test was used to determine whether the differences among the means were statistically significant. In all tests, a p-value < 0.05 was considered statistically significant. Data were visualized using SigmaPlot 12.5 (Grafiti LLC., Palo Alto, CA, USA) and R 4.0.3 (https://www.r-project.org/, 1 May 2024.)

3. Results

3.1. Appearance of the Five Tested Varieties of Melon

Among the five tested melon varieties, their appearances, including fruit shape, skin base color, skin covering color and pattern, flesh color, texture, and adhesion, were different (Figure 1 and Table S2). According to tasting, the five melon varieties had different smells and tastes (Table S2).

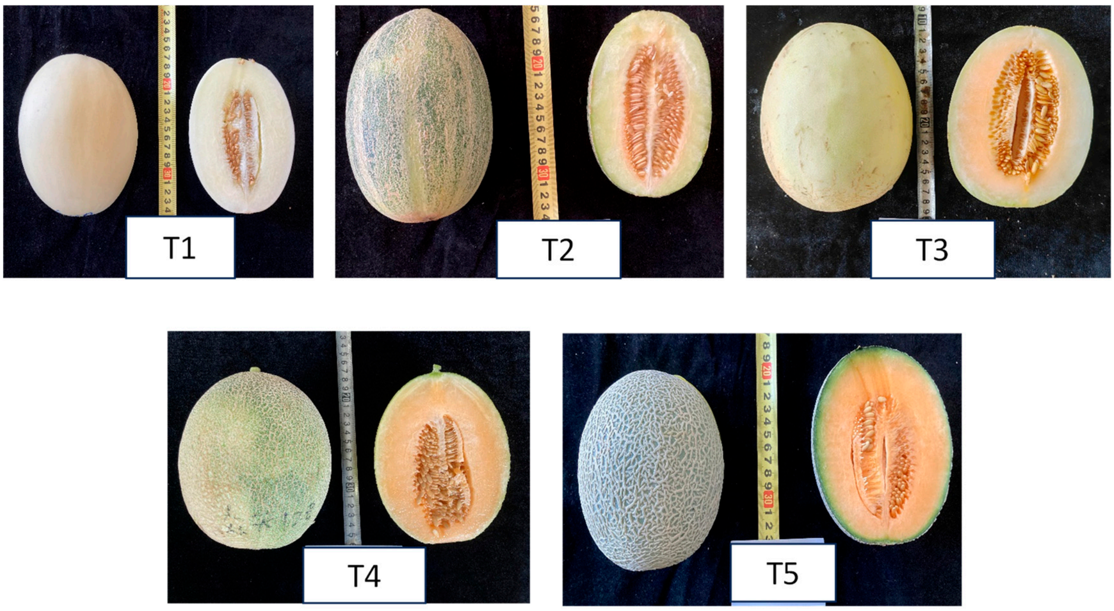

Figure 1. Profile photos of the five melon varieties (T1 was Zhongtianxueqiong; T2 was Zhongtian No. 9; T3 was Zhongtian No. 8; T4 was Zhongtian No. 12; T5 was Kangbing F3800).

3.2. Effect of Powdery Mildew on the Photosynthetic Parameters of Melon Leaves

The photosynthetic rate was significantly higher in sample T5 than in samples T3 and T4, which, in turn, were higher than those of samples T1 and T2 (Figure 2A). Powdery mildew had no significant effect on the stomatal conductance of melon leaves (Figure 2B). The transpiration rate was significantly higher in sample T5 and significantly lower in sample T2 (Figure 2C). The intercellular carbon dioxide concentration was significantly higher in samples T1 and T3 than in sample T5 (Figure 2D). Leaf water use efficiency was significantly higher in sample T5 and significantly lower in sample T2 (Figure 2E). T5 had the highest chlorophyll content than the other treatments, and T2 had the lowest chlorophyll content than the other treatments—especially, chlorophyll content of T2 accounted for 37.8% of T5 (Figure 2F).

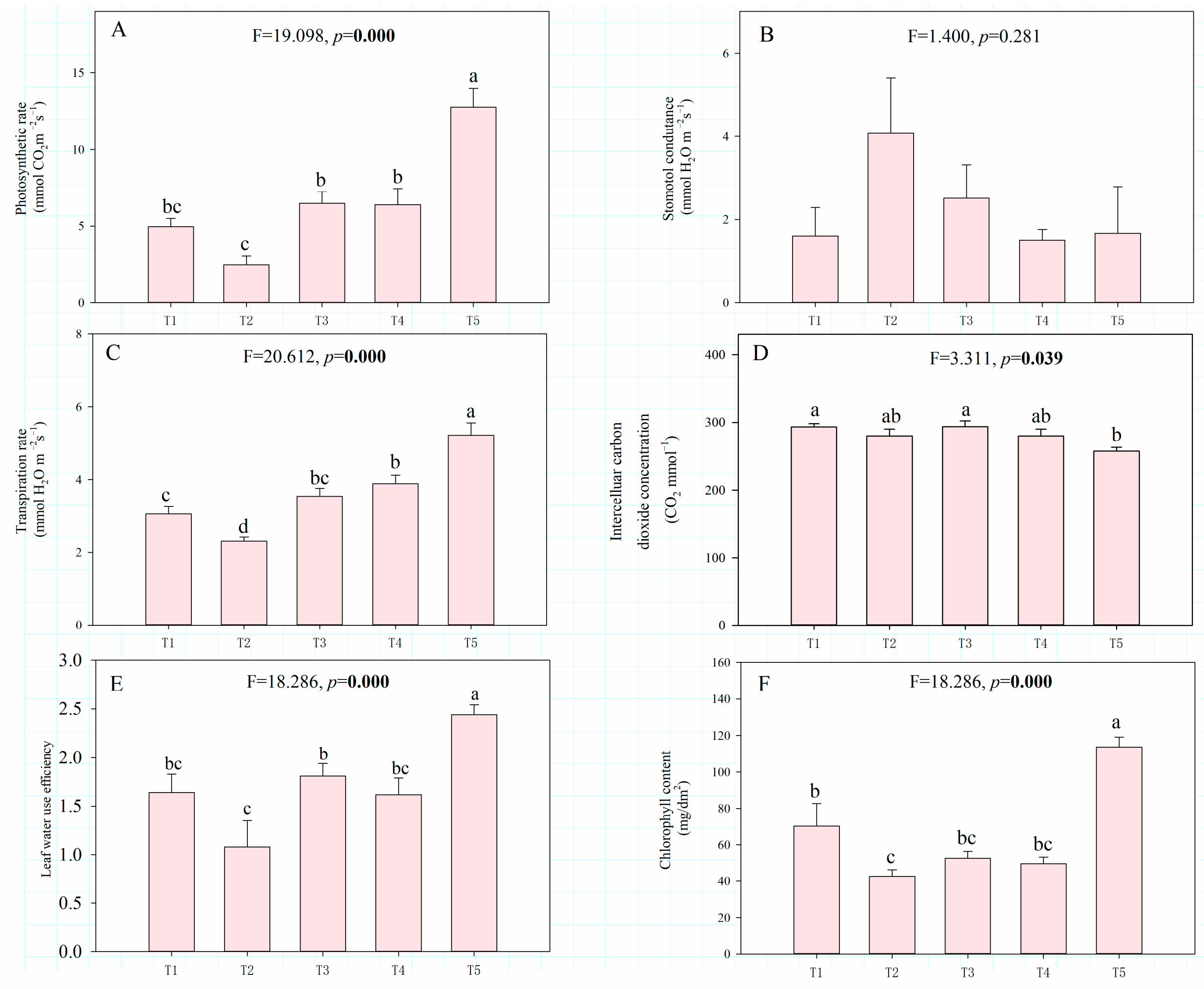

Figure 2. Effects of powdery mildew on the photosynthetic characteristics of melon leaves. (**A**) Photosynthetic rate of melon leaves; (**B**) stomatal conductance of melon leaves; (**C**) transpiration rate of melon leaves; (**D**) intercellular carbon dioxide concentration of melon leaves; (**E**) leaf water use efficiency of melon leaves; (**F**) chlorophyll content of melon leaves. Different lowercase letters represented significant differences among different treatments.

3.3. Effect of Powdery Mildew on the Disease Index of Melon Leaves

The powdery mildew disease index varied across melon samples. The leaves of sample T5 had no powdery mildew and were immune, with a disease index of 0 (Figure 3). The disease index of sample T3 was significantly lower than that of sample T1, a moderately resistant variety. Conversely, the disease index of sample T4 was significantly higher than that of T1. Finally, the highest disease index was observed for sample T2, which was a susceptible variety (Figure 3).

3.4. Effect of Powdery Mildew on the Ultrastructure of Melon Leaves

The number of open stomata in the upper epidermis of the leaves was the highest (79) in sample T3 and the lowest (38) in T4 (Figure S1 and Table 2). T1 and T4 presented no closed pores in the upper epidermis. In contrast, the resistant T5 variety displayed 22 closed pores in the upper epidermis. The maximum number of stomata on the leaves of T3 was 87, whereas the minimum number on the leaves of T4 was 38 (Figure S1 and Table 2). T1 and T4 had the highest stomatal opening and closing rates (1.00). T4 exhibited the highest upper epidermal stomatal length (17.43) and width (4.32). Instead, T2 showed the lowest upper epidermal stomatal length (10.36) and T5 the lowest upper epidermal stomatal width (2.77) (Figure 4 and Table 2).

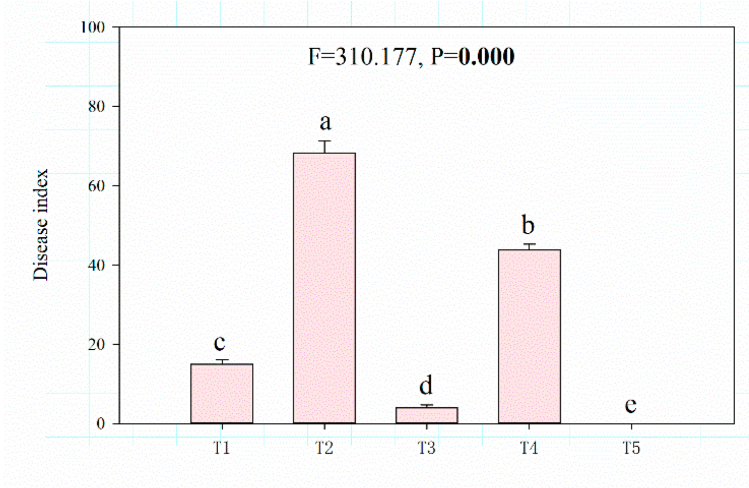

Figure 3. Effects of powdery mildew on the disease index of melon leaves. Different lowercase letters represented significant differences among different treatments.

Table 2. Summary of the scanning electron microscope indices of the melon upper epidermis.

Scanning Electron Microscope Index		T1	T2	T3	T4	T5
The number of gas holes in the table		54	58	79	38	54
Number of closed stomata in the upper epidermis		0	16	8	0	22
Stomatal opening and closing rate		1.00	0.78	0.91	1.00	0.71
Surface porosity	Total number of pores in the upper epidermis	54	74	87	38	76
	Unit area	300,394.10	300,394.10	300,394.10	300,394.10	300,394.10
	Density	0.00	0.00	0.00	0.00	0.00
Upper epidermal stomatal length (μm)		15.77	10.36	11.21	17.43	12.47
Upper epidermal stomatal width (μm)		4.10	3.04	3.62	4.32	2.77

The number of open stomata in the lower epidermis was the highest (125) in T4 and the lowest (60) in T5 (Figure S2 and Table 3). The number of closed stomata in the lower epidermis was at least four in T5, but it reached up to eight in T1. The highest total number of stomata in the lower epidermal leaves was 131 in sample T4, and the lowest was 64 in T5 (Figure S2 and Table 3). The stomatal opening rate was up to 0.96 in sample T3 (Figure 5 and Table 3). The lower epidermis stomatal length and width were the highest in T3, with values of 20.04 and 6.48, respectively. In contrast, the lowest values were recorded in T1 and T4 (9.48 and 3.40, respectively) (Figure 5 and Table 3).

Table 3. Summary of the scanning electron microscope indices of the lower epidermis of the melons.

Scanning Electron Microscope Index		T1	T2	T3	T4	T5
Number of open stomata in the lower epidermis		83	113	119	125	60
Number of closed stomata in the lower epidermis		5	8	5	6	4
Stomatal opening and closing rate		0.94	0.93	0.96	0.95	0.94
Stomatal density of lower epidermis	Total number of stomata in the lower epidermis	88	121	124	131	64
	Unit area	300,394.10	300,394.10	300,394.10	300,394.10	300,394.10
	Density	0.00	0.00	0.00	0.00	0.00
Stomatal length of lower epidermis (μm)		20.04	9.48	13.28	12.33	13.45
Lower epidermal stomatal width (μm)		6.48	3.59	4.99	3.40	4.10

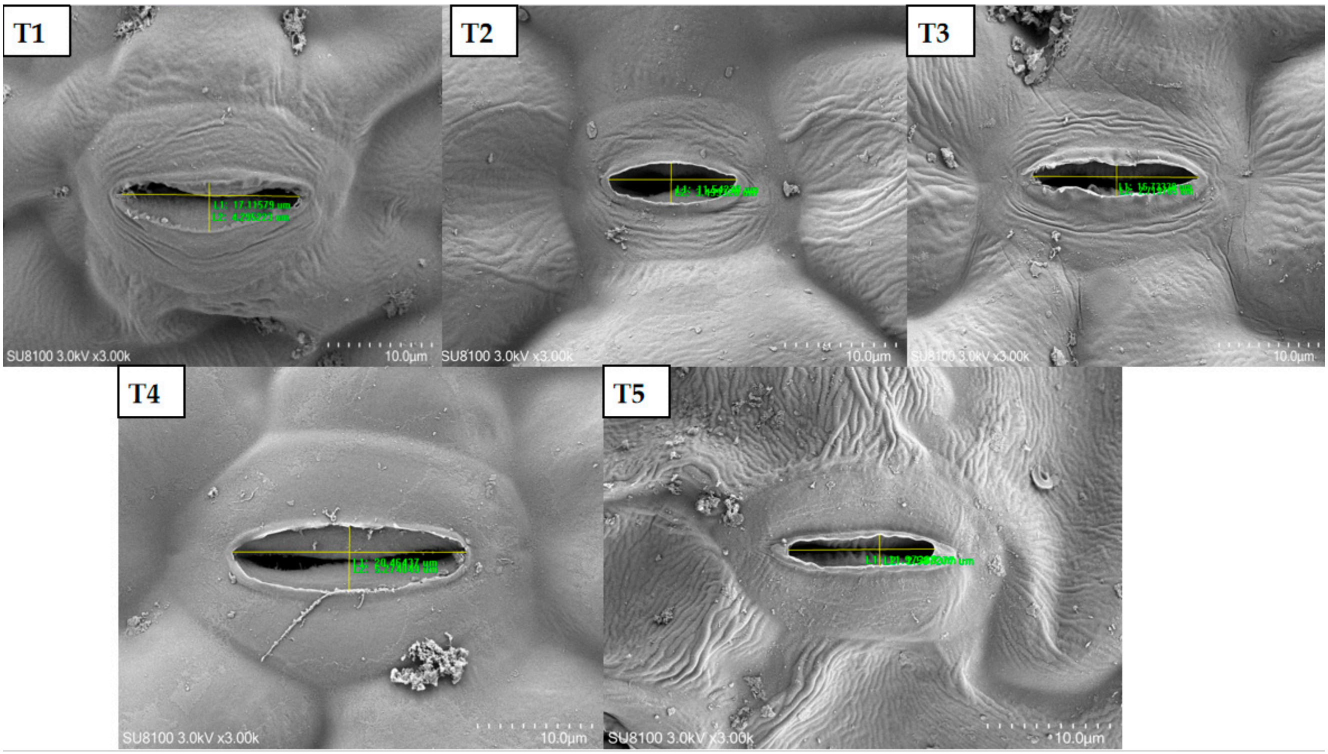

Figure 4. Scanning electron microscope image of the upper epidermal stomata of a melon leaf with powdery mildew (μm).

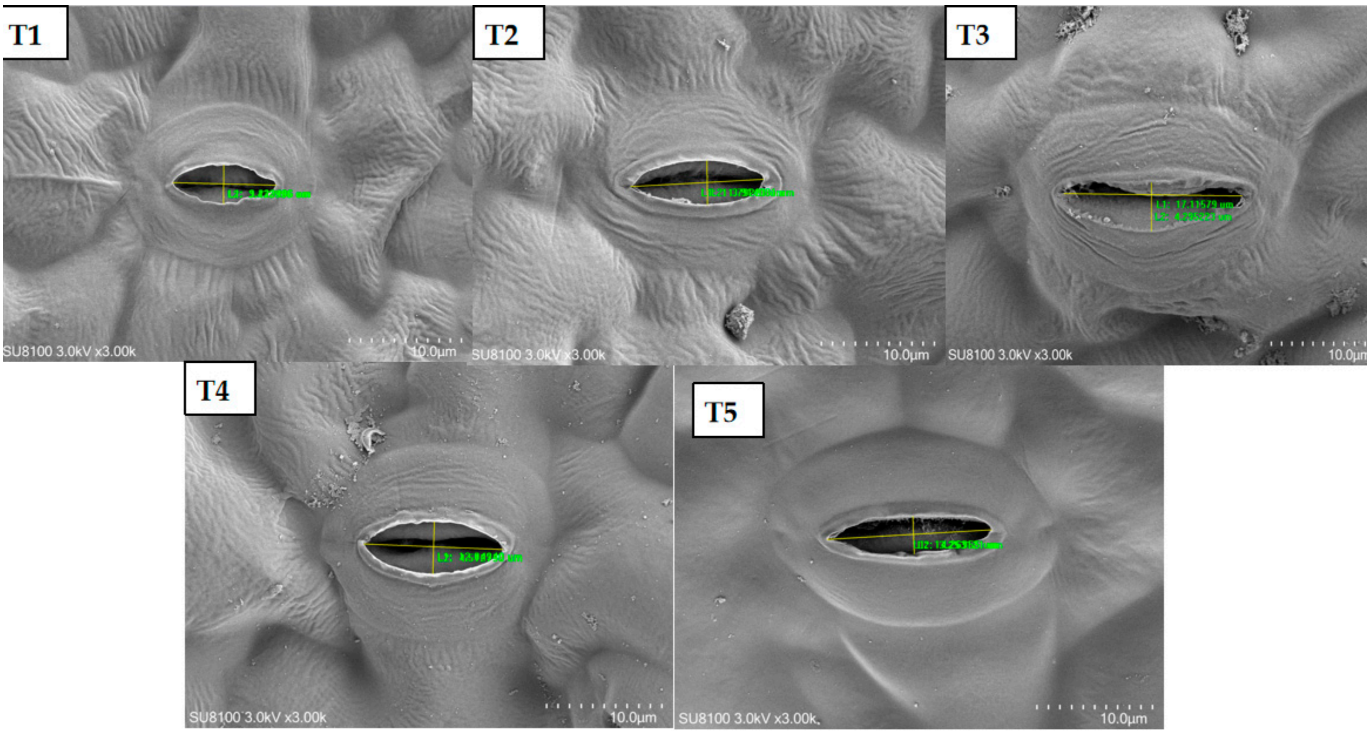

Figure 5. Scanning electron microscope image of stomata in the lower epidermis of a melon leaf with powdery mildew (μm).

3.5. Effect of Powdery Mildew on the Yield and Quality of Melon Varieties

The total output and meat thickness were significantly higher in sample T1. There was no significant difference among the five varieties in terms of the central soluble solid content, although that of sample T1 was significantly lower than that of samples T2, T3, and T4 but did not differ substantially from sample T5 (Table 4).

Table 4. Effects of powdery mildew on the fruit variety and yield of melon. (Different lowercase letters mean significant difference at $p < 0.05$ among different treatments at 0.05 level).

	Total Output (Kg/hm^2)	Meat Thickness (cm)	Thick-Skinned (cm)	Central Soluble Solids Content (%)	Central Soluble Solids Content (%)
T1	3822.48 ± 146.88 a	4.63 ± 0.07 a	0.37 ± 0.07	16.83 ± 0.38	7.4 ± 1.1 c
T2	2972.95 ± 130.96 b	3.3 ± 0.2 c	0.47 ± 0.03	16.9 ± 0.4	12.9 ± 1.36 a
T3	3246.56 ± 107.04 b	3.7 ± 0.06 ab	0.6 ± 0.06	17.37 ± 0.24	12.37 ± 0.13 a
T4	3152.63 ± 34.92 b	3.77 ± 0.07 ab	0.47 ± 0.03	17 ± 0.36	11.8 ± 0.23 ab
T5	2893.5 ± 242.41 b	4.07 ± 0.23 b	0.67 ± 0.2	15.83 ± 1.33	9.57 ± 0.64 bc

4. Discussion

Five melon varieties had different performances under the same stress of powdery mildew. In particular, Kangbing F3800 emerged as the most resistant variety with high photosynthesis indices, making it the preferred choice for introducing and promoting thick-skinned melon varieties in the Ningxia Hui Autonomous Region of China.

Photosynthesis is one of the most important processes for plants, since it converts light energy into chemical energy [24,25]. Powdery mildew is an air-borne fungal disease with a strong negative impact on photosynthesis [26]. In severe cases, powdery mildew conidia cover the affected parts of the plant, such as the leaves and stalks, resulting in a significant reduction in the areas of the host plant available for photosynthesis [27,28]. In recent years, improvements in agriculture and industrial practices in China have resulted in a dramatic increase in the planting area devoted to melons. However, elevated temperature and humidity have aggravated the occurrence and harm inflicted by powdery mildew [7]. One solution is the breeding of melon varieties resistant to this fungus. Kang et al. [29] analyzed the disease index of melons after controlled inoculation with powdery mildew, resulting in a quick and efficient method for breeding resistant varieties of muskmelon. Wang et al. [30] screened 23 melon germplasm resources immune to powdery mildew, including Kangbing F3800 and 1489, which disease index was 0. In another study, Wang et al. [31] used an in vitro method to identify a resistance to powdery mildew in 23 germplasm resources in Ningxia; the authors screened one immune specimen and three highly resistant ones, which laid the foundation for the effective control of powdery mildew and the breeding of resistant varieties of Ningxia melons. In this study, five thick-skinned melon varieties were selected, and Kangbing F3800 presented resistance to powdery mildew. Kangbing F3800 could be used as a key resource in the development of further varieties resistant to this fungus.

The results of this study illustrated that Kangbing F3800 was a good melon variety for resisting powdery mildew. Therefore, this melon variety should be utilized for variety promotion to local farmers and the mining of powdery mildew resistance genes. Up to now, the research on the key genes of resistance to powdery mildew has been more in-depth in wheat breeding, with researchers identifying a number of key genes that confer resistance to powdery mildew in wheat plants [32–34]. The photosynthetic indices and powdery mildew infection among the five melon varieties were significantly different under the same growth conditions. Melon resistance breeding using Kangbing F3800 should be conducted to pay more attention to the key genes of resistance to powdery mildew.

The leaf surface is the first plant structure contacting pathogens, and it is the first barrier preventing germs from penetrating into plant cells. Therefore, understanding leaf surface properties, such as stomatal size and density, can help identify the factors responsible for disease resistance. The number of setae and stomata on the surface of melon leaves does not correlate with resistance to powdery mildew [35]. However, the upper and lower epidermis of leaves from resistant varieties tend to be much thicker, and the papillae are denser and larger [36]. Exogenous hormones promote leaf transpiration and stomatal opening in bluegrass, enhance the ability to capture and utilize light energy, maintain normal growth and development during fungal infection, and improve resistance to disease [37]. The number of stomata per unit area of the leaves from muskmelon varieties resistant to bacterial fruit spoilage is higher than that from susceptible varieties, although the morphology and size of stomata have no bearing on disease resistance [38]. Instead, resistant specimens display longer and denser villi, as well as more frequent pores on the skin surface, but no difference in the morphological structure of the fruit epidermis [39]. The photosynthetic parameters are related to the above leaf surface characteristic, with wheat flag leaves having higher stomatal density on the front plane to absorb the energy from sunlight and lower stomatal density on the back plane [40]. However, the stomatal length of the epidermis and epidermal stomatal width of the lower leaves were higher than the upper leaves of Kangbing F3800. This melon variety could complete photosynthesis with resistance to powdery mildew infection.

This study compared five different melon varieties and illustrated that Kangbing F3800 had good resistance to powdery mildew. This increased our understanding and knowledge of melon planting in Zhongwei, China, and laid a theoretical basis for further melon variety breeding. This variety has key genes for resisting powdery mildew. Further research should pay attention to this melon variety; it could produce more economic value for local farmers under powdery mildew stress.

5. Conclusions

This study revealed the impact of powdery mildew on the photosynthetic parameters and leaf microstructure of melons. Accordingly, the net photosynthetic rate, transpiration rate, leaf water use efficiency, and chlorophyll level were higher in the disease-resistant Kangbing F3800 variety. Varieties susceptible to powdery mildew infection were characterized by an elevated total number of stomata in the upper (Zhongtian No. 8) and lower (Zhongtianxueqiong) epidermis, as well as elevated stomatal length and width in the upper (Zhongtian No. 12) and lower (Zhongtian No. 8) epidermis. The five varieties of melon displayed diverse shapes. Finally, the total yield and meat thickness were significantly higher in the Zhongtianxueqiong variety; however, they were offset by the low edge sugar content.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14060886/s1, Table S1: Evaluation criteria of resistance level of melon powdery mildew; Table S2: Appearance characteristics of five melon varieties; Figure S1: Scanning electron microscope image of upper epidermis of melon leaves; Figure S2: Scanning electron microscope image of mildew on lower epidermis of melon leaves.

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

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