

COMPARATIVE EVALUATION OF RESISTANCE TO FOUR MAJOR DISEASES IN FOUR WATERMELON VARIETIES

JUNJIE HU

School of Agriculture, Jilin Agricultural Science and Technology College, Jilin 132101, China

Corresponding email: hujunjie700116@126.com

Abstract

Disease outbreaks and continuous cropping challenges have increased the demand for resistant watermelon cultivars. This study evaluated four commercial varieties ('Jike 20', 'Chenlu 182', 'Zhemi 6', and 'Aihua 2') for resistance to powdery mildew, anthracnose, *Fusarium* wilt, and vine decline under controlled conditions. 'Jike 20' showed the strongest overall resistance (HR to three diseases, MR to vine decline), making it a promising candidate for breeding programs. In contrast, 'Zhemi 6' was the most susceptible. 'Chenlu 182' displayed high resistance to vine decline but slight resistance to anthracnose and *Fusarium* wilt, while 'Aihua 2' showed high resistance to anthracnose but moderate resistance to other diseases. These cultivar-specific resistance patterns highlight the importance of multi-pathogen screening in breeding. Our findings provide actionable guidance for selecting disease-resistant watermelon varieties, supporting sustainable cultivation and reducing reliance on chemical controls.

Key words: Watermelon cultivars; Disease resistance; Resistance breeding; Pathogen-specific resistance

Abbreviations used: HR (high resistance), MR (moderate resistance), SR (slight resistance), DAI (days post-inoculation).

Introduction

The declining quality of watermelon crops has been increasingly linked to several key factors, including severe disease outbreaks, seed degeneration issues, and challenges associated with continuous cropping practices. These problems have created a growing demand for watermelon varieties with enhanced disease resistance and improved storage tolerance in modern agricultural production (Miao *et al.*, 2010). Disease resistance evaluation represents a critical component in both watermelon breeding programs and variety assessment. This identification process constitutes a substantial and ongoing research effort, requiring systematic screening of resistant cultivars from extensive germplasm collections (Han *et al.*, 2016).

Current methodologies for assessing watermelon disease resistance are generally classified into three approaches: field-based evaluation, controlled environment (indoor) testing, and in vitro techniques (An *et al.*, 2009; Yadav *et al.*, 2021). Among these methods, field identification remains the most widely adopted and reliable approach for evaluating plant disease resistance. This method offers direct observation of plant-pathogen interactions under natural conditions, providing the most representative and ecologically relevant data for disease resistance assessment (Wang *et al.*, 2016; Chen *et al.*, 2020). Recent studies have systematically integrated seedling-stage artificial inoculation with field-based evaluations using disease nursery continuous cropping systems, enabling comprehensive assessment of both seedling and adult-plant resistance to *Fusarium* wilt in watermelon resources (Li *et al.*, 2020). The watermelon variety 'Zhongmi 1' has been validated through multi-year field production trials following artificial inoculation, confirming its moderate

resistance to *Fusarium* wilt and tolerance to continuous cropping (Zhou *et al.*, 2025). Moreover, recent studies have successfully employed field-based disease nursery systems combined with controlled inoculation to identify watermelon germplasm with broad-spectrum resistance to three fungal diseases (Bai *et al.*, 2024). The authenticity of field conditions allows for comprehensive evaluation of resistance traits that may not be fully expressed in controlled environments, making it an indispensable tool in resistance breeding programs.

To further evaluate the resistance of four watermelon varieties against four major diseases, this study conducted systematic indoor resistance assessments against four major watermelon diseases—powdery mildew (caused by *Podosphaera xanthii*), anthracnose (*Colletotrichum orbiculare*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *niveum*), and vine decline (*Didymella bryoniae*)—using four cultivated varieties ('Jike 20', 'Chenlu 182', 'Zhemi 6', and 'Aihua 2') as comparative controls. The findings provide valuable insights for disease-resistant variety selection and sustainable watermelon cultivation.

Material and Methods

Plant materials and experimental setup: The seeds of four cultivated watermelon varieties ('Jike 20', 'Chenlu 182', 'Zhemi 6', and 'Aihua 2') were obtained from Changchun Dafu Agricultural Seedling Trading Co., Ltd. Pathogen inoculum, including conidial suspensions of *Podosphaera xanthii* (powdery mildew), *Colletotrichum orbiculare* (anthracnose), *Fusarium oxysporum* f. sp. *niveum* (*Fusarium* wilt), and *Didymella bryoniae* (vine decline), were provided by Wobao Biological Technology Co., Ltd.

Equipment and growth conditions: The experiment utilized sandy soil as the growth medium, with standard horticultural tools (shovel, scissors, and knife) for plant maintenance. Disease assessment and data recording were performed using a notebook and pen for manual observations. Laboratory equipment, including a 1 L beaker and an electronic balance (precision: 0.01 g), were employed for precise measurement of inoculum concentrations and other experimental parameters.

Experimental period and site description: The disease resistance evaluation of the four watermelon varieties was conducted from April to October 2019 at the Experimental Base of Jilin Agricultural Science and Technology University. The plants were grown in sandy loam soil with the following chemical properties: 1.5 g/kg total nitrogen, 15 mg/kg available phosphorus, and 75 mg/kg exchangeable potassium. This soil type was selected for its optimal physical characteristics (excellent drainage and aeration) and balanced fertility, which are particularly suitable for watermelon cultivation while minimizing confounding effects on disease development.

Seed germination and seedling preparation: Seeds from the four watermelon varieties were surface-sterilized and soaked in distilled water at room temperature for 8–10 h. Germination was carried out in a constant-temperature incubator at 28°C until 80% of seeds had sprouted. The germinated seeds were then sown in nutrient bowls, with

400 seeds per variety (totaling 1,600 seeds across all four varieties). Seedlings were maintained under controlled conditions until reaching the 4–5 true-leaf stage.

Pathogen inoculation: Spore suspensions of the four pathogens (*Podosphaera xanthii*, *Colletotrichum orbiculare*, *Fusarium oxysporum* f. sp. *niveum*, and *Didymella bryoniae*) were prepared at a standardized concentration of 100 mg/L. Inoculation was performed using the seedling root immersion method, where roots of uniformly grown seedlings were submerged in the respective spore suspensions for 10 min. This method ensures uniform pathogen exposure at the seedling stage, while still allowing standardized evaluation of all four pathogens under controlled conditions.

Greenhouse cultivation and disease assessment: After inoculation, seedlings were transplanted into a greenhouse with a randomized block design. Each variety was planted in separate rows containing 100 plants, with protective barrier rows between treatments to prevent cross-contamination. Greenhouse conditions were maintained at 25–28°C with optimal humidity to facilitate disease development.

Data collection and resistance evaluation: Disease progression was monitored at 5-day intervals, with four total observations. Diseased and dead seedlings were recorded, and the disease incidence rate was calculated as:

$$\text{Disease incidence rate (\%)} = \frac{(\text{Number of diseased plants} + \text{Number of dead plants})}{\text{Total number of plants}} \times 100\%$$

Resistance levels were classified at 20 days after inoculation (DAI) according to national standards: high resistance (HR): 0–20% incidence, moderate resistance (MR): 21–50% incidence, slight resistance (SR): 51–80% incidence, susceptible (S): 81–100% incidence. This standardized methodology ensured consistent and reproducible evaluation of disease resistance across all tested varieties.

Statistical analysis: Experimental data were systematically processed and analyzed using Microsoft Excel 2010 for preliminary data organization and calculation. Final figures were prepared using Adobe Photoshop (v23.0.2). Statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted to determine significant differences among treatment groups, followed by Duncan's new multiple range test for post-hoc comparisons at a 95% confidence level ($p < 0.05$). All values are presented as mean $\pm$ standard error (SE) unless otherwise specified.

Results

Evaluation of powdery mildew resistance in watermelon varieties: The temporal progression of powdery mildew infection revealed distinct resistance patterns among the four watermelon cultivars (Fig. 1). Initial symptoms appeared as early as 5 days after

inoculation (DAI), with ‘Zhemi 6’ showing the highest susceptibility (9% incidence), followed by ‘Chenlu 182’, ‘Aihua 2’, and ‘Jike 20’. Statistical analysis indicated no significant difference ($p > 0.05$) between ‘Aihua 2’ and ‘Jike 20’ at this stage, though all other cultivars differed significantly from ‘Jike 20’. Disease progression showed increasing divergence among varieties: at 10 DAI: ‘Aihua 2’ demonstrated peak susceptibility (23%), exceeding ‘Zhemi 6’ (18%), ‘Chenlu 182’ (15%), and ‘Jike 20’ (12%); At 15 DAI: ‘Zhemi 6’ emerged as most susceptible (36%), followed by ‘Aihua 2’ (32%), ‘Chenlu 182’ (28%), and ‘Jike 20’ (21%); At 20 DAI: Disease severity peaked, with ‘Zhemi 6’ reaching 68% incidence, significantly higher ($p < 0.05$) than ‘Aihua 2’ (58%), ‘Chenlu 182’ (49%), and ‘Jike 20’ (35%). The comprehensive resistance ranking, based on cumulative disease incidence, was established as: ‘Jike 20’ > ‘Chenlu 182’ > ‘Aihua 2’ > ‘Zhemi 6’. These results demonstrate ‘Jike 20’ maintained consistently superior resistance throughout the evaluation period, while ‘Zhemi 6’ showed progressive susceptibility to powdery mildew infection.

Evaluation of anthracnose resistance in watermelon varieties:

The temporal progression of anthracnose infection revealed distinct resistance patterns among the four watermelon cultivars (Fig. 2). Initial symptoms at 5 days after inoculation (DAI) showed ‘Chenlu 182’ as the most susceptible variety with 15% disease incidence, significantly higher ($p < 0.05$) than ‘Zhemi 6’ (12%), ‘Jike

20' (9%), and 'Aihua 2' (6%). Disease severity increased progressively, with 'Chenlu 182' reaching 26% incidence at 10 DAI, while 'Aihua 2' maintained the lowest infection rate at 10%. Although no significant difference ($p>0.05$) was observed between 'Zhemi 6' and 'Jike 20' at this stage, both cultivars showed intermediate susceptibility between 'Chenlu 182' and 'Aihua 2'.

By 15 DAI, disease pressure intensified with 'Chenlu 182' exhibiting 48% infection, significantly exceeding other varieties. The resistance hierarchy became more pronounced at 20 DAI, with 'Chenlu 182' showing severe susceptibility (75%), followed by 'Zhemi 6' (58%), 'Jike 20' (45%), and the consistently resistant 'Aihua 2' (32%). Statistical analysis confirmed significant differences ($p<0.05$) among all cultivars at both 15 and 20 DAI. The overall resistance ranking established 'Chenlu 182' as most susceptible, followed by 'Zhemi 6', 'Jike 20', and 'Aihua 2', which demonstrated stable resistance throughout the evaluation period. These findings highlight 'Aihua 2' as a promising candidate for anthracnose resistance breeding programs.

Evaluation of *Fusarium* wilt resistance in watermelon varieties: The temporal progression of *Fusarium oxysporum* f. sp. *niveum* infection revealed distinct resistance patterns among the four watermelon cultivars (Fig. 3). Initial symptoms at 5 days after inoculation (DAI) showed 'Zhemi 6' as the most susceptible variety with 13% disease incidence, followed by 'Chenlu 182' (11%), 'Aihua 2' (8%), and 'Jike 20' (6%). While no significant difference ($p>0.05$) was observed between 'Zhemi 6' and 'Chenlu 182', both cultivars showed significantly higher susceptibility than 'Aihua 2' and 'Jike 20'.

Disease progression exhibited cultivar-specific patterns, with 'Zhemi 6' demonstrating consistently high susceptibility throughout the evaluation period, reaching 46% incidence by 20 DAI. Interestingly, 'Jike 20' showed intermediate susceptibility (35% at 20 DAI), while 'Aihua 2' (28%) and 'Chenlu 182' (24%) maintained relatively stable resistance. Significant differences ($p<0.05$) among all cultivars were observed at 10, 15 and 20 DAI, establishing the following resistance ranking: 'Jike 20' > 'Aihua 2' > 'Chenlu 182' > 'Zhemi 6'. The superior resistance of 'Chenlu 182' to *Fusarium* wilt contrasts with its susceptibility to anthracnose, highlighting the cultivar-specific nature of disease resistance mechanisms in watermelon. These results provide valuable benchmarks for resistance breeding programs targeting *Fusarium* wilt in watermelon.

Evaluation of vine decline resistance in watermelon varieties: The progression of vine decline infection demonstrated significant varietal differences among the four watermelon cultivars (Fig. 4). Initial symptoms at 5 days after inoculation (DAI) revealed 'Zhemi 6' as the most susceptible variety (13% incidence), followed by 'Jike 20' (10%), 'Aihua 2' (7%), and 'Chenlu 182' (5%). Disease severity increased progressively, with 'Zhemi 6' showing consistently highest susceptibility at each evaluation interval (21% at 10 DAI, 33% at 15 DAI, and 46% at 20 DAI). 'Jike 20' maintained intermediate susceptibility throughout the study period, while 'Aihua 2'

and particularly 'Chenlu 182' exhibited stable resistance. Statistical analysis confirmed significant differences ($p<0.05$) among all cultivars at every evaluation point (5, 10, 15, and 20 DAI). The comprehensive resistance assessment established a clear susceptibility hierarchy: 'Zhemi 6' > 'Jike 20' > 'Aihua 2' > 'Chenlu 182', with 'Chenlu 182' emerging as the most resistant cultivar against vine decline. These findings highlight the potential of 'Chenlu 182' as a valuable genetic resource for breeding programs targeting vine decline resistance in watermelon.

Table 1. Comparative resistance classification of four watermelon varieties against major fungal diseases.

Diseases	Varieties	Disease resistance level
Powdery mildew	'Jike 20'	HR
	'Chenlu 182'	MR
	'Zhemi 6'	SR
	'Aihua 2'	MR
Anthracnose	'Jike 20'	HR
	'Chenlu 182'	SR
	'Zhemi 6'	MR
	'Aihua 2'	HR
<i>Fusarium</i> wilt	'Jike 20'	HR
	'Chenlu 182'	SR
	'Zhemi 6'	SR
	'Aihua 2'	MR
Vine decline	'Jike 20'	MR
	'Chenlu 182'	HR
	'Zhemi 6'	MR
	'Aihua 2'	MR

Note: Resistance levels are defined based on disease incidence at 20 DAI: HR (high resistance, 0–20%), MR (moderate resistance, 21–50%), SR (slight resistance, 51–80%)

Comparative analysis of disease resistance in watermelon varieties: Comprehensive evaluation of disease resistance profiles revealed distinct patterns among the four watermelon cultivars (Table 1). 'Jike 20' demonstrated superior resistance, showing high resistance (HR) to powdery mildew, anthracnose, and *Fusarium* wilt, along with moderate resistance (MR) to vine decline. 'Chenlu 182' exhibited variable resistance, displaying moderate resistance to powdery mildew (MR), slight resistance to anthracnose (SR), high resistance to vine decline (HR), and slight resistance to *Fusarium* wilt (SR). 'Zhemi 6' showed relatively weaker resistance overall, with slight resistance to powdery mildew (SR), moderate resistance to anthracnose (MR), slight resistance to *Fusarium* wilt (SR), and moderate resistance to vine decline (MR). Interestingly, 'Aihua 2' presented a unique resistance profile, combining moderate resistance to powdery mildew (MR) and *Fusarium* wilt (MR) with high resistance to anthracnose (HR), though showing moderate resistance (MR) to vine decline. These differential resistance patterns highlight the complex genetic basis of disease resistance in watermelon cultivars and provide valuable information for targeted breeding programs.

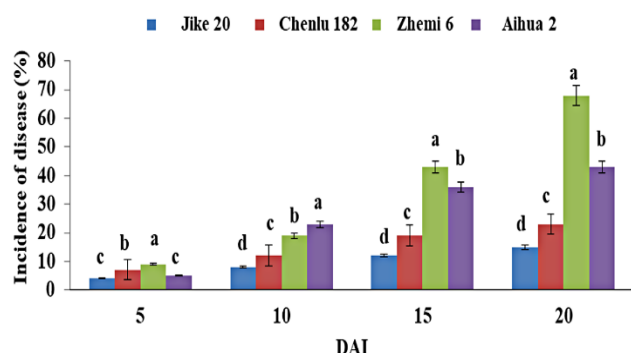


Fig. 1. Comparative resistance of watermelon varieties to powdery mildew infection. The x-axis indicates days after inoculation (DAI), while the y-axis shows disease incidence (%). Column colors represent different cultivars: blue for 'Jike 20', orange for 'Chenlu 182', gray for 'Zhemi 6', and yellow for 'Aihua 2'. Data are presented as mean values $\pm$ standard error (SE) of three independent biological replicates. Lowercase letters above columns indicate statistically significant differences (Duncan's test, $p < 0.05$), with distinct letters denoting significant variation among varieties at each time point.

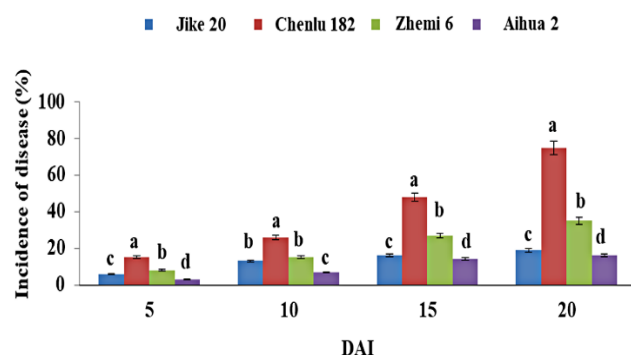


Fig. 2. Temporal progression of anthracnose disease incidence in four watermelon varieties following artificial inoculation. The x-axis indicates days post-inoculation (DAI), while the y-axis shows disease incidence (%). Color-coded columns represent different cultivars: red ('Chenlu 182'), blue ('Zhemi 6'), green ('Jike 20'), and yellow ('Aihua 2'). Data are presented as means $\pm$ standard error (SE) of three independent biological replicates. Distinct lowercase letters (a-d) above columns indicate statistically significant differences among varieties at each time point (Duncan's multiple range test, $p < 0.05$). The dashed horizontal line marks the 50% disease incidence threshold for moderate resistance classification.

Discussion

Previous research has employed indoor identification techniques to evaluate *Fusarium* wilt resistance in eight watermelon varieties (Zhang *et al.*, 2015). Notably, 'Chenlu 182' displayed differential resistance patterns, showing moderate susceptibility at the seedling stage but developing high resistance during fruiting - a phenomenon not reported in earlier studies (Li *et al.*, 2015), suggesting limitations in previous resistance assessments. Parallel investigations using in vitro methods screened 45 watermelon germplasm accessions, with 'Aihua 2' demonstrating moderate anthracnose resistance (Li *et al.*, 2015), contrasting with our current findings. This discrepancy likely stems from methodological differences: while prior studies utilized tissue culture-based in vitro approaches with direct bacterial inoculation, our investigation implemented controlled indoor evaluations employing root immersion inoculation

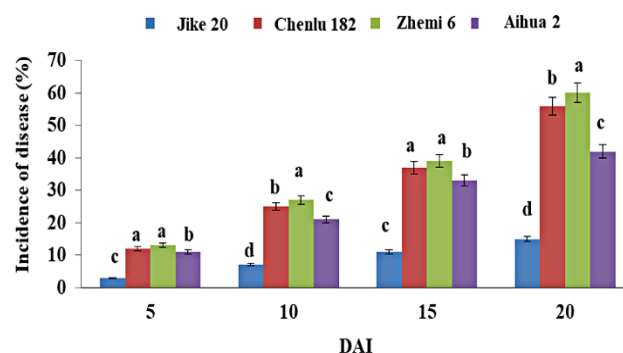


Fig. 3. Comparative resistance of watermelon varieties to *Fusarium oxysporum* f. sp. *niveum* infection. The x-axis shows days post-inoculation (DAI), while the y-axis indicates disease incidence (%). Color-coded columns represent cultivars: purple ('Zhemi 6'), orange ('Jike 20'), green ('Aihua 2'), and blue ('Chenlu 182'). Data points reflect means $\pm$ standard error (SE) from three biological replicates. Distinct lowercase letters (a-d) above columns indicate statistically significant differences among varieties at each time point ($p < 0.05$, Duncan's test). The dotted horizontal line at 30% marks the threshold for moderate resistance classification.

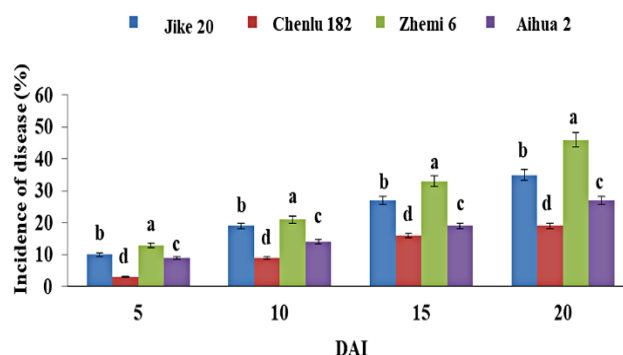


Fig. 4. Disease progression of vine decline in four watermelon varieties following artificial inoculation. The x-axis indicates days post-inoculation (DAI: 5, 10, 15, 20), while the y-axis shows disease incidence (%). Color-coded columns represent cultivars: red ('Zhemi 6'), blue ('Jike 20'), green ('Aihua 2'), and yellow ('Chenlu 182'). Data are presented as means $\pm$ standard error (SE) of three independent biological replicates. Distinct lowercase letters (a-d) above columns indicate statistically significant differences among varieties at each time point (Duncan's multiple range test, $p < 0.05$). The dashed horizontal line at 25% marks the threshold for moderate resistance classification.

techniques. Recent technical methods for evaluating watermelon disease resistance have been developed, integrating field validation with controlled-environment testing to ensure reproducibility and reliability across different experimental settings (Liu *et al.*, 2022). Furthermore, comprehensive reviews on cucurbit *Fusarium* wilt resistance have emphasized that integrating multiple assessment methods—including field natural identification, seedling-stage artificial inoculation, and molecular marker-assisted screening—is essential for accurately characterizing resistance profiles and avoiding method-dependent (Chen *et al.*, 2025). These contrasting methodologies—tissue culture versus whole-plant systems with root inoculation—may account for the observed variations in resistance phenotypes, highlighting the need for standardized protocols and further comparative studies to elucidate method-dependent effects on disease resistance assessment outcomes.

While previous research has demonstrated stage-specific resistance patterns in watermelon germplasm, with distinct responses observed between seedling and fruiting stages (Li *et al.*, 2018), the present study focused exclusively on seedling-stage evaluation of four cultivars. Comprehensive evaluations of watermelon germplasm have revealed that resistance to major diseases often varies between seedling and adult-plant stages (Liu *et al.*, 2022; Zhang *et al.*, 2023). This single-stage assessment, though informative, presents limitations in fully characterizing the disease resistance profiles of ‘Jike 20’, ‘Chenlu 182’, ‘Zhemi 6’, and ‘Aihua 2’. The absence of vegetative (extension) and reproductive stage evaluations means our current understanding of their resistance dynamics remains incomplete, particularly for diseases like *Fusarium* wilt that may manifest differently across developmental phases. To establish comprehensive resistance benchmarks with practical agricultural value, future investigations should incorporate multi-stage assessments spanning the entire growth cycle, as cultivars showing susceptibility at the seedling stage may develop higher resistance later in development, and vice versa. Such expanded studies would not only validate these preliminary findings but also provide more reliable guidance for cultivar selection in commercial watermelon production systems.

The four cultivars exhibited distinct resistance profiles, with ‘Jike 20’ showing broad-spectrum resistance, ‘Zhemi 6’ the highest susceptibility, and ‘Chenlu 182’ and ‘Aihua 2’ displaying contrasting pathogen-specific patterns. The disease incidence evaluation showed consistent susceptibility rankings across pathogens. The broad-spectrum resistance of ‘Jike 20’ to multiple pathogens suggests possible activation of basal defense pathways, while the contrasting pathogen-specific profiles of ‘Chenlu 182’ and ‘Aihua 2’ imply the presence of race-specific resistance loci, such as NLR family genes, which have been documented in Cucurbitaceae species (Wang *et al.*, 2026). Our findings are consistent with the recent studies demonstrating independent genetic control of *Fusarium* wilt resistance in different watermelon cultivars (Zhang *et al.*, 2015; Wang *et al.*, 2019), further supporting the pathogen-specific patterns observed in our cultivars. These differential resistance profiles provide valuable insights for developing targeted breeding strategies and highlight the importance of cultivar-specific disease management approaches in watermelon production systems. For breeding programs, this implies that pyramiding multiple resistance genes is necessary to achieve broad-spectrum resistance as seen in ‘Jike 20’, whereas cultivar-specific disease management and targeted breeding for individual pathogens may be more practical for varieties with narrow resistance profiles such as ‘Chenlu 182’ and ‘Aihua 2’. Integrating genetic resistance with biological control strategies—such as the use of antagonistic bacteria like *Bacillus marisflavi* XJ-04 (Zhu & Wang, 2026)—may further enhance sustainable watermelon production by reducing reliance on chemical fungicides. Future work should integrate genetic or transcriptomic analyses to uncover the molecular mechanisms underlying these resistance patterns, thereby enhancing the global relevance of these findings.

Conclusions

The study revealed significant varietal differences in resistance to four major watermelon diseases. ‘Jike 20’ demonstrated superior resistance, making it a valuable genetic resource for breeding programs. In contrast, ‘Zhemi 6’ showed consistent susceptibility, necessitating targeted management strategies. The differential resistance profiles of ‘Chenlu 182’ and ‘Aihua 2’ highlight the complexity of pathogen-specific resistance mechanisms. These results emphasize the need for integrated disease management and the development of multi-resistant cultivars to enhance watermelon production sustainability. Multi-pathogen resistance screening, as demonstrated here, serves as an efficient breeding tool to identify cultivars with broad-spectrum or pathogen-specific resistance, thereby reducing the need for multiple single-disease screening cycles. Future research should expand evaluations to include additional growth stages and field trials to validate these findings under natural conditions.

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