

Article

Resistance Evaluation of Pear Ring Rot on Detached Leaves and Genetic Model Analysis in Four Pear F₁ Populations

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Abstract

Pear ring rot, caused by *Botryosphaeria kuwatsukai*, is a major threat to pear production. The resistance of four pear F₁ populations to three *B. kuwatsukai* isolates was evaluated using detached leaf inoculations, assessed by the Area Under the Disease Progress Curve (AUDPC) and average lesion diameter (ADL). Cluster analysis based on these metrics established a five-level resistance rating scale. All hybrid combinations exhibited clear segregation for resistance, with continuous phenotypic variation and coefficients of variation exceeding 50%, suggesting polygenic inheritance. Broad-sense heritability (H^2) of lesion diameter, estimated from replicated inoculations using a linear mixed-model approach, ranged from 0.32 to 0.71 across populations and isolates, indicating that the phenotypic variation was largely under genetic control. Genetic model analysis using the SEA v2.0 package identified a two-major-gene additive-dominant (2MG-AD) model as the best fit for the data across all combinations and isolates, with additive effects predominating. Isolate-specific responses were detected in the ‘Doyenne du Comice’ × ‘Huangguan’ population, and reciprocal differences between ‘Zhongai 1’ × ‘Zaosu’ and its reciprocal cross suggested potential cytoplasmic or maternal effects on resistance expression. Collectively, these findings suggest that pear leaf resistance to *B. kuwatsukai* is consistent with a two-gene additive-dominant model, supported by moderate-to-high heritability estimates. However, independent validation with additional populations and molecular markers is needed. These results highlight the value of multi-isolate screening, appropriate selection of resistant and susceptible parents, and the use of reciprocal crossing in breeding for broad-spectrum and durable resistance.

Keywords: *Pyrus*; *Botryosphaeria kuwatsukai*; heritability; genetic patterns

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1. Introduction

Pear ring rot, caused by *Botryosphaeria dothidea* and *B. kuwatsukai*, primarily affects the leaves, branches, and fruit of pears, leading to significant reductions in yield and quality and causing substantial economic losses to the industry [1,2]. Breeding resistant varieties is the most effective way to control this disease, and understanding the genetic basis of resistance is crucial for disease resistance breeding.

Currently, there are still several limitations in the genetic research on pear ring rot resistance. For example, the number of hybrid combinations and varieties studied is limited, and resistance evaluation is often conducted using only a single pathogen isolate, thereby neglecting the potential differences in pathogenic characteristics among different

isolates [3–5]. Therefore, the experiment needs to further expand the scale of hybrid combinations and parental diversity, and adopt a multi-isolate inoculation strategy. This will more comprehensively reveal the genetic patterns of pear ring rot resistance and help select breeding varieties that are stable, broad-spectrum, and resistant to pathogen variability [6,7].

Early studies were based on hybrid experiments and phenotypic statistics. For instance, a field survey on branch ring rot disease in 827 hybrid seedlings from 49 pear varieties and 10 hybrid combinations suggested that resistance to pear ring rot is likely controlled by multiple genes, exhibiting quantitative trait inheritance [8]. After evaluating the resistance of 223 F1 individual pear seedlings to ring rot disease, Zhu found that the F1 resistance phenotypes followed a normal distribution [9]. Through investigating the resistance to branch ring rot in 1652 individual seedlings from eight pear varieties and their F1 hybrids, Zhang also concluded that pear ring rot resistance is a complex, polygenic quantitative trait [10]. The resistance showed a partially dominant inheritance pattern, and the resistance alleles from the resistant parent were inherited by the progeny to some degree, although hybrid vigor was not evident.

Moreover, evaluations are mainly focused on branches and fruit; however, it is difficult to obtain fruit and dormant branch materials in the early stages of breeding. In contrast, the detached leaf inoculation method offers the advantages of being rapid, efficient, and using easily accessible materials [11]. Furthermore, the correlation between leaf resistance and that of branches and fruit has been confirmed in disease resistance evaluations for other fruit tree diseases, including kiwifruit canker [12], apple ring rot [13], pear scab [14], and pear rotting diseases [15]. Studies have also shown that mycelial plug inoculation is more efficient than the traditional spore suspension spraying method in terms of infection efficiency [14,16].

It should be noted, however, that detached leaf assays are preliminary in nature. Resistance observed in excised leaves does not always correlate perfectly with field performance, as whole-plant responses involve systemic defense mechanisms and environmental interactions that are absent in detached tissues [17,18]. Therefore, the results obtained from this detached leaf study should be considered as an initial evaluation, and confirmation under in planta conditions—ideally through multi-year and multi-site field trials—will be necessary before breeding decisions can be made.

Additionally, considering the significant variation in resistance to ring rot among different pear cultivars [2], the resistance expression in hybrid offspring is influenced by multiple factors, including the parental genotype and pathogen isolate specificity [19,20]. Therefore, this study adopts a multi-population, multi-isolate, and multi-time-point approach for leaf mycelial plug inoculation.

Existing resistance rating indicators for ring rot primarily include ADL [21], average lesion area [9], or growth rate over a single time period. However, these single indicators cannot fully quantify the cumulative damage and may overlook the genotype's early resistance potential to pathogen invasion, as well as the screening of related defense genes. Therefore, this experiment introduced the Area Under the Disease Progress Curve (AUDPC), which, by quantifying the time dynamics of disease progression throughout the entire disease cycle, offers a key advantage over measurements at a single time point, providing a more comprehensive and accurate assessment of disease resistance [22]. AUDPC has been widely applied in disease resistance evaluations and epidemiological studies in over 30 major crops and horticultural plants.

In this study, hybrid populations from four resistant-parent combinations (including a reciprocal cross) and multiple pathogen isolates were used. Resistance was evaluated using a dual-parameter clustering approach that integrated ADL and AUDPC to establish

resistance rating standards. To assess the reliability of phenotypic data, broad-sense heritability was estimated from replicated inoculations using a linear mixed model. We then analyzed resistance-susceptibility segregation ratios, fitted genetic models, compared reciprocal crosses, and tested for isolate specificity. The aim was to elucidate the genetic basis of pear leaf ring rot resistance and provide practical guidance for parent selection and hybrid breeding.

2. Materials and Methods

2.1. Plant Materials

The F1 populations were derived from four pear hybrid combinations: ‘Zaosu’ × ‘Korla Xiangli’ (169 plants), ‘Doyenne du Comice’ × ‘Huangguan’ (169 plants), ‘Zhongai 1’ × ‘Zaosu’ (75 plants), and ‘Zaosu’ × ‘Zhongai 1’ (106 plants). These populations are located in the pear breeding experimental field of the Institute of Pomology, Chinese Academy of Agricultural Sciences (IP, CAAS), Liaoning Province, China. All F1 populations consisted of four-year-old trees grown under uniform field conditions with standard training and pruning. Fully expanded mature leaves were sampled from the 3rd–5th node of current-season shoots during the morning hours, ensuring comparable physiological age across genotypes and populations. Leaf sampling was balanced across blocks to minimize environmental variation.

2.2. Culture Medium

Potato Dextrose Agar (PDA): potato (peeled) 200 g/L, glucose 20 g/L, agar 20 g/L.

2.3. Test Pathogens

Three isolates of pear ring rot pathogens used in the experiment, all belonging to *B. kuwatsukai*, were selected from a set of 21 previously isolated pear ring rot isolates by the pear breeding research group. The isolates were LN-SDGZ (from pear leaves, subsequently numbered as 7), HZ-SZ2162 (from branch ring rot at Huazhong Agricultural University, subsequently numbered as 18), and HB-ZX (from pear branch ring rot in Shijiazhuang, Hebei Province, subsequently numbered as 21) [23]. In the preliminary work, DNA from all test isolates was extracted using the Chelex-100 method, and the ITS, TEF, and TUB regions were amplified and sequenced. BLAST (MEGA7.0) analysis against NCBI showed > 99% homology with *B. kuwatsukai* and *B. dothidea*. Phylogenetic analysis combining ITS–TEF–TUB sequences, together with 58 reference isolates of Botryosphaeriaceae, was performed using MEGA7.0, clustered all test isolates in the *B. kuwatsukai* clade, confirming their species identity and close kinship with *B. dothidea*. For this experiment, the three isolates were re-activated and all inoculations were performed using first- or second-generation cultures to ensure stable pathogenicity. All isolates were cultured on Potato Dextrose Agar (PDA) medium prior to inoculation [24].

2.4. Inoculation

From May to August 2025, 15 healthy leaves were collected from each F1 single plant. All leaves were taken from the 3rd to 5th nodes of current-season shoots and were fully expanded, with leaf length ranging from 6 to 8 cm and leaf width from 4 to 6 cm (measured along the midrib and at the widest point, respectively). The leaves were surface-sterilized with 75% ethanol and rinsed thoroughly with sterile water. Two inoculation points were established on either side of the main leaf vein, and each point was pricked three times. For each genotype, four leaves were inoculated with each fungal isolate, totaling 16 inoculation points per genotype to ensure effective pathogen infection.

An excised leaf pin-prick inoculation method was employed. Fungal isolates, cultured on PDA medium at 28 °C in the dark for 5 days, were sampled as 5 mm mycelial plugs and inverted onto the leaf inoculation points. Control leaves were inoculated with blank PDA medium. After inoculation, all leaves were placed in a humid incubator at 28 °C in the dark. Lesion measurements were conducted in a blind manner for both genotype and fungal isolate. Lesion diameters were measured on days 3, 5, 7, and 9 post inoculation, and the ADL per leaf was calculated for subsequent AUDPC calculation and genetic analysis.

Data quality control was performed using Levene's test to assess homogeneity of variances before further statistical analyses.

The Area Under the Disease Progress Curve (AUDPC) was calculated to quantify disease progression over time for each genotype. Lesion diameters measured on days 3, 5, 7, and 9 post inoculation were first averaged across the four leaves per genotype to obtain a mean ADL for each time point. AUDPC was then calculated directly from these mean ADL values using the trapezoidal formula [22]:

$$AUDPC = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where Y_i is the mean ADL at day t_i , and n is the number of observation days (4 in this study: 3, 5, 7, 9 DPI). This calculation approach allowed AUDPC to capture the cumulative disease severity over time while reflecting the average response of each genotype. The resulting AUDPC values were used for resistance classification, clustering analysis, and subsequent genetic model fitting.

2.5. Resistance Evaluation

Lesion diameters were measured using the cross method on days 3, 5, 7, and 9 post inoculation. Resistance evaluation was based on lesion size, with both the AUDPC and the ADL on days 7 and 9 serving as the primary resistance indicators. To classify resistance levels, hierarchical clustering analysis was performed in Origin 2025b using Euclidean distance and the average linkage method. The dendrogram revealed five natural groupings corresponding to different levels of disease severity. To ensure that the five resistance categories accurately reflected the continuous variation and maintained balanced sample sizes, the cluster boundaries were refined based on the frequency distribution of ADL values and cluster centers. The final classification defined five resistance levels:

This approach allowed the five-level classification to reflect both the overall disease progression and the continuous variation in lesion size among the tested materials [9]. This combined data-driven clustering with distribution-based refinement, providing a robust and interpretable framework for resistance evaluation across all F1 populations and pathogen isolates. The resulting resistance categories were subsequently used for AUDPC comparison, chi-square tests, and genetic modeling.

2.6. Estimation of Broad-Sense Heritability

To assess the reliability of the phenotypic data, broad-sense heritability (H^2) was estimated for lesion diameter in each hybrid combination under each isolate treatment using the lesion diameter measurements described above.

H^2 was estimated using a mixed linear model with genotype as a random effect, implemented with the lme4 package (v1.1-35) in R. The model was specified as:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij}$$

where Y_{ij} is the lesion diameter of the j -th inoculation site from the i -th genotype, μ is the overall mean, G_i is the random effect of the i -th genotype with $G_i \sim N(0, \sigma_G^2)$, and ε_{ij} is the

residual error with $\varepsilon_{ij} \sim N(0, \sigma_E^2)$. A model was fitted using the lme4 package (v1.1-35) in R, and variance components were estimated using restricted maximum likelihood (REML) [25]. Broad-sense heritability was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$$

where σ_G^2 is the genotypic variance and σ_E^2 is the environmental (residual) variance [26]. H^2 was estimated separately for lesion diameter at 3, 5, 7, and 9 days post inoculation to examine the temporal stability of the phenotype.

2.7. Genetic Analysis

Based on the results of in vitro resistance testing in hybrid offspring and parents, the number of resistant and susceptible plants in each hybrid offspring population was counted. The segregation ratio for resistance and susceptibility was analyzed, and genetic analysis methods were applied. For example, comparing the F₁ trait mean (F), the disease level of the maternal parent (P₁), the paternal parent (P₂), and the mid-parent value (MP) was used to analyze the genetic patterns of resistance to pear ring rot, where F represents the mean phenotype value, σ represents the standard deviation, CV represents the coefficient of variation, P₁ represents the phenotype of the maternal parent, P₂ represents the phenotype of the paternal parent, MP represents the mid-parent value, H represents the dominance deviation, F/MP represents heritability, and LL represents the low parent rate.

$$F = \frac{1}{n} \sum_{i=1}^n x_i; \sigma = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - F)^2}; CV = \frac{\sigma}{F} \times 100\%; H = F - MP; F/MP = \frac{F}{MP} \times 100\%$$

Genetic model analysis was performed using the SEA v2.0 R package based on the mixed major-gene plus polygene inheritance model for quantitative traits. For each hybrid combination and isolate treatment, the phenotypic data (ADL at 9 DPI) from the F₁ population were analyzed using the F₂ population model (SEA-F₂) implemented in the software, as the mixed major-gene plus polygene segregation analysis module of SEA v2.0 adopts a maximum likelihood method for parameter estimation by fitting the phenotypic value distribution under different genetic hypotheses, which does not require raw data to conform to a normal distribution. A total of 24 genetic models were evaluated for each dataset. Candidate models were first selected based on the minimum Akaike Information Criterion (AIC) value, and the final optimal model was determined through a series of goodness-of-fit tests, including U_1^2 , U_2^2 , and U_3^2 (uniformity tests), nW^2 (Smirnov test), and Dn (Kolmogorov test). Major-gene additive and dominant effects were then estimated using the least squares method [27].

2.8. Pathogenicity Difference Analysis of Isolates

The ADL on the 9th day and AUDPC data for each population and treatment were imported into Origin2025b software to test for normal distribution. It was found that nearly all groups did not follow a normal distribution. Therefore, the data were log-transformed and then tested for normal distribution again. After that, for the populations that met normal distribution, homogeneity of variance tests, one-way ANOVA, and post hoc multiple comparisons were conducted. For populations that did not meet normal distribution, non-parametric statistics—Kruskal–Wallis H test—were applied to analyze whether there were significant differences in pathogenicity within the same population.

For each hybrid combination, (3 × 5) contingency tables were constructed with isolates (7, 18, 21) as rows and resistance grades (0–4) as columns to analyze whether the distribution of resistance differed significantly among isolates. Because some resistance

grades had low counts, resulting in expected frequencies below 5, Monte Carlo simulation with 5000 replicates was used to obtain robust p -values. Furthermore, resistance grades were grouped into resistant (0–2) and susceptible (3–4) to form 3×2 contingency tables for chi-square testing; when any expected count was below 5, results from Fisher's exact test were used. Statistical significance was set at $p = 0.05$.

2.9. Reciprocal Cross Difference Analysis of Isolates

To evaluate differences in resistance distribution between direct and reciprocal crosses, (2×5) contingency tables (cross type $\times$ resistance grade 0–4) were constructed for each inoculated isolate (isolates 7, 18, and 21). Pearson's chi-square test was used to analyze the consistency of resistance grade distributions. Since some expected frequencies were less than 5, the chi-square approximation may be inaccurate; therefore, exact p -values were calculated using Monte Carlo simulation with 5000 replicates. Additionally, to reflect the common breeding evaluation standard, resistance grades were grouped into resistant (0–2) and susceptible (3–4) categories to form 2×2 contingency tables, which were then analyzed by chi-square test. When any expected count was less than 5, Fisher's exact test was used. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Detached Leaf Resistance Evaluation

After inoculation with the three isolates of the ring rot pathogen, the lesions on pear leaves gradually expanded over time. The resistance of hybrid combinations showed significant segregation, with some exhibiting higher resistance levels. Disease symptoms of each parent inoculated with isolate 18 at 9 days post inoculation are shown in Figure 1.

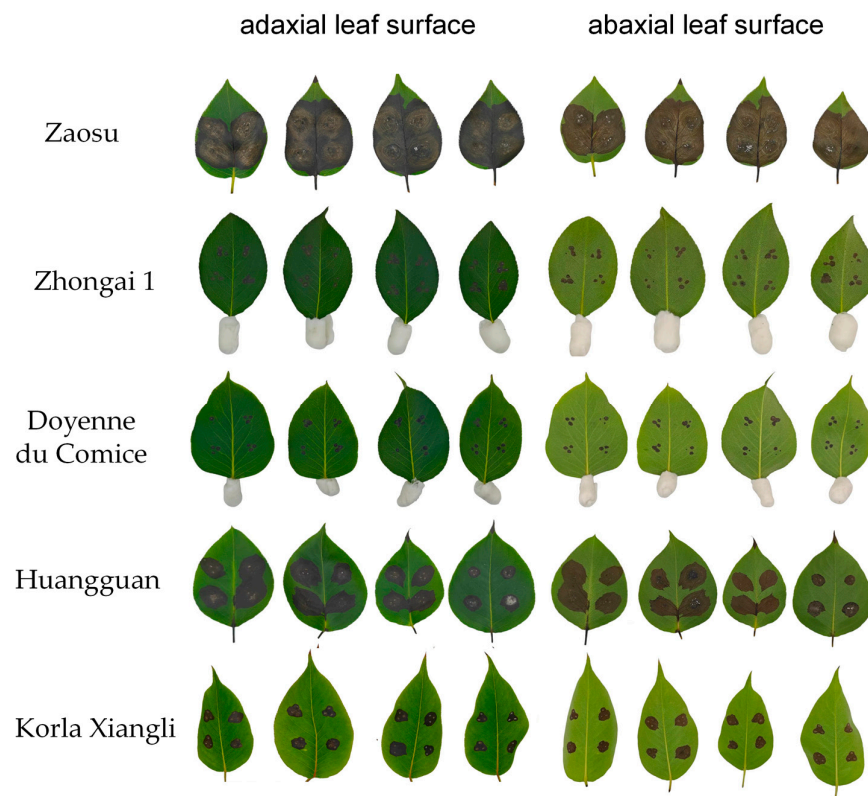


Figure 1. Disease symptoms of each parent inoculated with isolate 18 at 9 days post inoculation.

Overall, ADL increased progressively with days post inoculation (DPI) in all combinations, but clear differences in resistance were observed among combinations and isolates.

In the ‘Zaosu’ × ‘Korla Xiangli’ combination, the progeny showed moderate resistance levels, with ADL at 9 DPI ranging from 1.08 to 1.10 cm and AUDPC from 3.29 to 3.40. No substantial differences were detected among the three isolates.

The ‘Doyenne du Comice’ × ‘Huangguan’ combination exhibited isolate-specific responses. Disease severity was lowest when inoculated with isolate 18 (9 DPI ADL = 1.07 cm, AUDPC = 2.74), whereas isolate 21 caused the highest severity (9 DPI ADL = 1.29 cm, AUDPC = 3.56), indicating greater susceptibility to isolate 21 in this cross.

Both the reciprocal crosses ‘Zhongai 1’ × ‘Zaosu’ and ‘Zaosu’ × ‘Zhongai 1’ showed relatively high resistance. The direct cross exhibited 9 DPI ADL values of only 0.52–0.56 cm and AUDPC of 1.34–1.61, which were significantly lower than those of the reciprocal cross (9 DPI ADL = 0.74–0.77 cm, AUDPC = 2.15–2.34). These results suggest stronger resistance transmission when ‘Zhongai 1’ is used as the maternal parent.

Large standard deviations (SD) within each combination reflect extensive segregation of resistance among individuals, consistent with the quantitative nature of the trait. The evaluation of resistance evaluations for all hybrid combinations and isolates is summarized in Table 1.

Table 1. Temporal changes in resistance to different isolates among different hybrid combinations.

Hybrid Combination	Inoculated Isolate	D3-ADL (cm)	D5-ADL (cm)	D7-ADL (cm)	D9-ADL (cm)	AUDPC
‘Zaosu’ × ‘Korla Xiangli’	7	0.19 ± 0.15	0.38 ± 0.23	0.67 ± 0.36	1.10 ± 0.53 a	3.40 ± 1.74 a
	18	0.15 ± 0.17	0.36 ± 0.34	0.70 ± 0.54	1.08 ± 0.63 a	3.35 ± 2.45 a
	21	0.16 ± 0.17	0.36 ± 0.31	0.66 ± 0.46	1.08 ± 0.61 a	3.29 ± 2.21 a
‘Doyenne du Comice’ × ‘Huangguan’	7	0.11 ± 0.10	0.36 ± 0.22	0.62 ± 0.39	1.09 ± 0.63 a	3.16 ± 1.84 ab
	18	0.08 ± 0.06	0.27 ± 0.22	0.52 ± 0.40	1.07 ± 0.69 a	2.74 ± 1.89 a
	21	0.09 ± 0.08	0.36 ± 0.28	0.73 ± 0.51	1.29 ± 0.67 b	3.56 ± 2.22 b
‘Zhongai 1’ × ‘Zaosu’	7	0.08 ± 0.08	0.13 ± 0.16	0.27 ± 0.29	0.52 ± 0.40 a	1.40 ± 1.31 a
	18	0.10 ± 0.13	0.17 ± 0.21	0.31 ± 0.32	0.56 ± 0.43 a	1.61 ± 1.52 a
	21	0.07 ± 0.02	0.12 ± 0.07	0.25 ± 0.21	0.53 ± 0.39 a	1.34 ± 0.93 a
‘Zaosu’ × ‘Zhongai 1’	7	0.11 ± 0.09	0.28 ± 0.20	0.47 ± 0.31	0.74 ± 0.44 a	2.34 ± 1.49 a
	18	0.10 ± 0.09	0.23 ± 0.20	0.43 ± 0.33	0.74 ± 0.52 a	2.15 ± 1.59 a
	21	0.10 ± 0.08	0.24 ± 0.18	0.45 ± 0.32	0.77 ± 0.46 a	2.24 ± 1.47 a

NOTE: Within each hybrid combination and within the same column, different letters indicate significant differences among isolates ($p < 0.05$), while the same letters indicate no significant difference. Letters are assigned in ascending order of mean values (i.e., from resistant to susceptible), where a denotes the lowest value (most resistant), b the highest (most susceptible), and ab indicates an intermediate level not significantly different from either. Letters in D9-ADL and AUDPC columns are assigned independently.

Violin plots revealed clear phenotypic variation in lesion diameter among individuals within each hybrid population under the three inoculation isolates (7, 18, and 21), indicating quantitative segregation for disease response. Across populations, the distributions were generally unimodal but differed in central tendency and dispersion, suggesting both population-specific resistance levels and isolate-dependent disease severity. Notably, the ‘Zaosu’ × ‘Korla Xiangli’ population showed broader distributions with a pronounced upper tail compared with the other crosses, consistent with greater within-population variability in lesion development. Violin and box plots for resistance to multiple isolates among different hybrid combinations are listed in Figure 2.

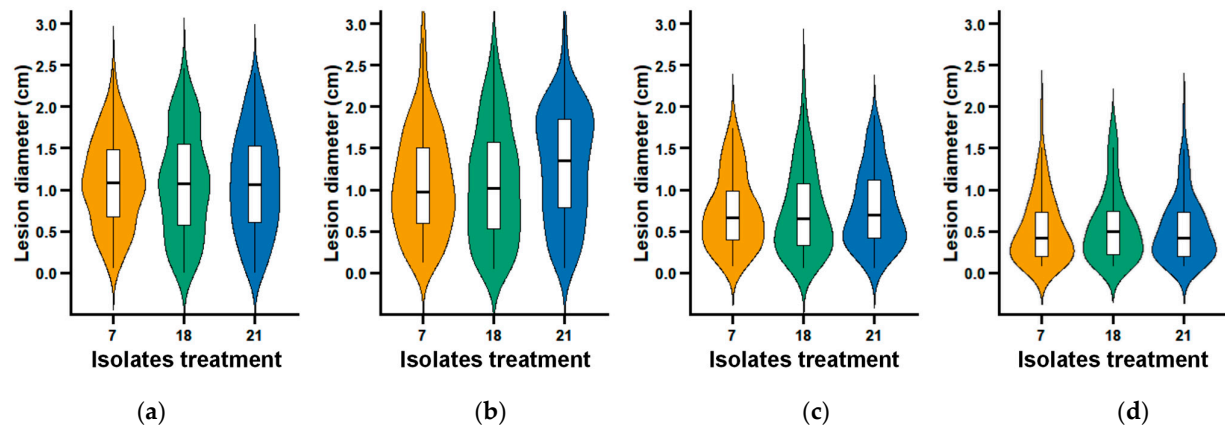


Figure 2. Violin and box plots for resistance to multiple isolates among different hybrid combinations: (a) 'Zaosu' × 'Korla Xiangli'; (b) 'Doyenne du Comice' × 'Huangguan'; (c) 'Zhongai 1' × 'Zaosu'; (d) 'Zaosu' × 'Zhongai 1'.

Scatter plots demonstrated a strong positive association between lesion diameter and AUDPC in all four populations. In each population, AUDPC increased approximately linearly with lesion diameter, supporting lesion diameter as a reliable proxy for cumulative disease progression. The fitted regression lines for isolates 7, 18, and 21 were largely parallel within populations, indicating a broadly consistent scaling between instantaneous lesion size and disease progress across inoculation isolates. However, differences in slope and scatter among isolates were evident in some populations, implying isolate-specific disease dynamics and potential population-by-isolate interactions. Scatterplots of AUDPC and 9dADL for individual plants across hybrid combinations under different isolates are listed in Figure 3.

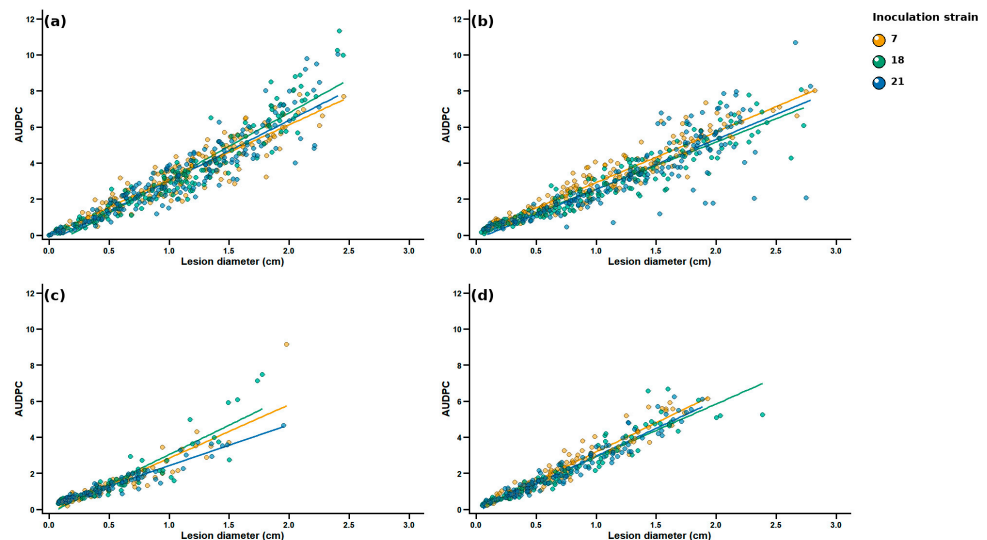


Figure 3. Scatterplots of AUDPC and 9dADL for individual plants across hybrid combinations under different isolates: (a) 'Zaosu' × 'Korla Xiangli'; (b) 'Doyenne du Comice' × 'Huangguan'; (c) 'Zhongai 1' × 'Zaosu'; (d) 'Zaosu' × 'Zhongai 1'.

3.2. Resistance Rating and Clustering Analysis

Scatterplots of AUDPC versus ADL for individual plants across the hybrid combinations under different inoculated isolates showed an approximately linear relationship, indicating that final ADL values effectively capture the overall disease progression. To classify resistance levels, we used the Area Under the Disease Progress Curve (AUDPC) together with the ADL on days 7 and 9 as the primary indicators. Hierarchical clustering analysis was performed in Origin 2025b using Euclidean distance and the average linkage method. Five clusters were obtained from the hybrid population's distribution, and these centers, together with the ADL frequency distribution, were used to define the boundaries for five categories: highly resistant, resistant, moderately resistant, susceptible, and highly susceptible. Frequency histograms of ADL demonstrated that the sample sizes within each category were reasonably balanced and that the overall distribution approximated normality, supporting the robustness of this classification. This standard allows rapid evaluation of resistance based on a single-time-point ADL measurement while retaining continuous trait variation for quantitative genetic analyses and is applicable across different inoculation isolates and hybrid combinations. A resistance rating scale was established to categorize the pear plants based on lesion diameter and disease progression, Level 0: highly resistant, Level 1: resistant, Level 2: moderately resistant, Level 3: susceptible, Level 4: highly susceptible. The evaluation criteria for resistance to leaf ring rot disease in pear trees are listed in Table 2. Subsequently, the disease levels of individual plants within each population were classified based on the established grading standards. The evaluation criteria for resistance to leaf ring rot disease in pear trees are listed in Table 3.

Table 2. Evaluation criteria for resistance to leaf ring rot.

Resistance Class	9 d ADL (cm)
0	$0 \leq \text{ADL} < 0.29$
1	$0.29 \leq \text{ADL} < 0.69$
2	$0.69 \leq \text{ADL} < 1.2$
3	$1.2 \leq \text{ADL} < 1.62$
4	$1.62 \leq \text{ADL}$

Table 3. Evaluation of resistance of F1 generation leaves to ring rot under different isolates of four hybrid combinations.

Hybrid Combination	Inoculated Isolate	Resistance Combination	Total Plants	0	1	2	3	4	Resistance Segregation Ratio
'Zaosu' × 'Korla Xiangli'	7	4 × 2	168	11	32	58	39	28	1.51:1
	18	4 × 2	169	24	30	49	26	40	1.56:1
	21	4 × 2	166	16	32	48	34	36	1.37:1
'Doyenne du Comice' × 'Huangguan'	7	2 × 4	169	20	31	52	29	37	1.56:1
	18	1 × 4	166	27	31	37	34	37	1.34:1
	21	2 × 4	167	13	25	36	32	61	0.80:1
	7	1 × 4	73	27	25	15	5	1	11.17:1
'Zhongai 1' × 'Zaosu'	18	1 × 4	75	23	31	14	5	2	9.71:1
	21	1 × 4	75	24	29	16	5	1	11.50:1
	7	4 × 1	106	18	36	32	16	4	4.30:1
'Zaosu' × 'Zhongai 1'	18	4 × 1	106	23	37	25	15	6	4.05:1
	21	4 × 1	106	13	40	31	15	7	3.82:1

In the 'Zaosu' × 'Korla Xiangli' combination, the female parent 'Zaosu' is highly susceptible, whereas the male parent 'Korla Xiangli' is resistant. As expected for a cross between resistant and susceptible parents, the progeny exhibited clear segregation for resistance. When inoculated with three different isolates, the ratio of resistant to susceptible individuals ranged from 1.37:1 to 1.51:1. The majority of individuals showed moderate resistance, with only a few displaying strong resistance. This segregation pattern suggests that resistance in this population may be controlled by genes with additive or partially recessive effects. Furthermore, the fact that a resistant parent ('Korla Xiangli') contributed to the inheritance of resistance, while the susceptible parent ('Zaosu') also likely harbors minor-effect additive alleles, highlights the complexity and polygenic nature of genetic resistance to pear ring rot.

In the 'Doyenne du Comice' × 'Huangguan' combination, the resistance of 'Doyenne du Comice' varied from moderate to high resistance across different isolates. As the pathogenicity of the isolates increased, the resistance-to-susceptibility segregation ratio decreased. When treated with isolate 21, the segregation ratio dropped to 0.8:1, and the number and proportion of highly susceptible plants increased significantly. This suggests that pear ring rot resistance may have isolate specificity.

In the 'Zhongai 1' × 'Zaosu' combination, the resistance-to-susceptibility segregation ratio was extremely high under all three treatments, and strong resistance was shown to all three isolates. The 'Zaosu' × 'Zhongai 1' combination showed the same level of resistance as the reciprocal combination, but the resistance-to-susceptibility segregation ratio was still significantly lower than the reciprocal group. There was a significant difference between the reciprocal cross groups, and when 'Zhongai 1' was used as the maternal parent, the offspring exhibited stronger resistance. This also suggests the possible involvement of cytoplasmic or maternal effects, although this interpretation remains speculative without direct molecular or cytogenetic evidence [28,29].

In the 'Zaosu' × 'Korla Xiangli' combination, the majority of the population exhibited resistance or moderate resistance to all three isolates (7, 18, 21), with only a small proportion showing high resistance or high susceptibility. The observed frequency closely resembled a normal distribution, indicating that resistance is stable across the different isolates, with little influence from isolate variation.

For the 'Doyenne du Comice' × 'Huangguan' combination, the resistance distribution exhibited isolate-specific differences: while isolates 7 and 18 followed a normal distribution, isolate 21 caused a significant increase in the frequency of 'highly susceptible' plants, which deviated from the normal distribution curve. This suggests that the resistance to isolate 21 is notably weaker compared to isolates 7 and 18, supporting the possibility of isolate-specific resistance.

In the 'Zhongai 1' × 'Zaosu' and 'Zaosu' × 'Zhongai 1' combinations, the resistance distribution across all three isolates (7, 18, 21) was highly consistent. The proportion of highly susceptible plants was very low in all treatments, and the frequency closely matched the central part of the normal distribution, with the tail approaching zero. This indicates strong and consistent resistance to isolates 7, 18, and 21, with little influence from isolate variation. The frequency distribution and normal fitting curve of different resistance categories are shown in Figure 4.

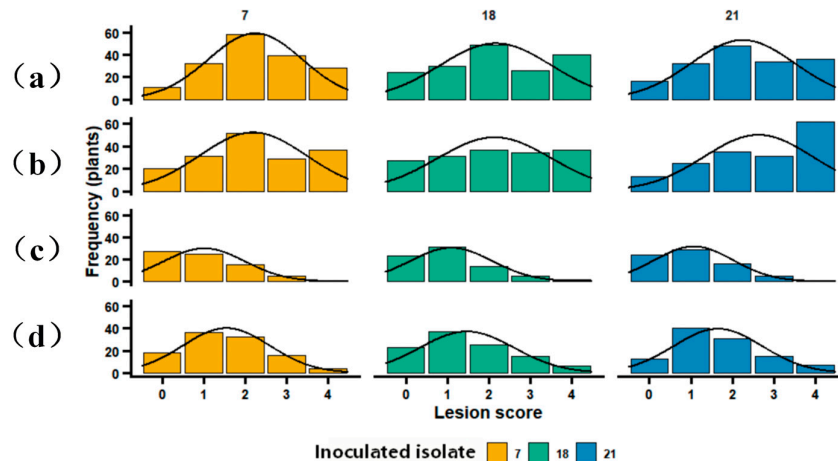


Figure 4. Frequency histograms of individual plants by disease severity class across hybrid combinations under different isolates, overlaid with normal distribution fits: (a) ‘Zaosu’ × ‘Korla Xiangli’; (b) ‘Doyenne du Comice’ × ‘Huangguan’; (c) ‘Zhongai 1’ × ‘Zaosu’; (d) ‘Zaosu’ × ‘Zhongai 1’.

3.3. Broad-Sense Heritability

Broad-sense heritability (H^2) of lesion diameter was estimated for the four hybrid combinations under three *Botryosphaeria kuwatsukai* isolates at 5, 7, and 9 DPI using linear mixed-effects models (R ‘lme4’ v1.1-35) with REML. Variance components for genotype (σ_G^2) and residual (σ_E^2) were obtained and used to calculate H^2 .

H^2 values varied among hybrid combinations, isolates, and observation times. In the ‘Zaosu’ × ‘Korla Xiangli’ population, H^2 increased from 0.324 at 5 DPI to 0.460 at 9 DPI under isolate 7, and from 0.476 to 0.631 under isolate 18, indicating that the proportion of phenotypic variance attributable to genetics increased over time. Isolate 21 showed slightly lower heritability (0.451–0.569), suggesting a larger environmental contribution for this isolate.

For ‘Doyenne du Comice’ × ‘Huangguan’, H^2 was generally higher, ranging from 0.511 to 0.707 across isolates and time points, with the highest value observed at 9 DPI under isolate 7 (0.707), indicating strong genetic control. The reciprocal crosses ‘Zhongai 1’ × ‘Zaosu’ and ‘Zaosu’ × ‘Zhongai 1’ displayed H^2 values from 0.219 to 0.684 and 0.409–0.589, respectively, with heritability generally increasing over DPI, highlighting the additive genetic contribution to resistance.

Overall, all populations exhibited moderate-to-high broad-sense heritability, particularly at later DPI, suggesting that lesion diameter is largely genetically determined and that selection for resistant individuals in breeding programs is feasible. Variation in H^2 among isolates indicates that environmental and isolate-specific effects also influence phenotypic expression. These moderate-to-high H^2 values across populations and isolates suggest that the phenotypic data are reliable for subsequent quantitative genetic and major-gene model analyses to dissect the inheritance of pear leaf ring rot resistance. The broad-sense heritability (H^2) of lesion diameter in four hybrid combinations under different isolates is listed in Table 4.

Table 4. Broad-sense heritability (H^2) of lesion diameter in four hybrid combinations under different isolates.

Hybrid Combination	Inoculated Isolate	5DPI			7DPI			9DPI		
		σ_G^2	σ_E^2	H^2	σ_G^2	σ_E^2	H^2	σ_G^2	σ_E^2	H^2
'Zaosu' × 'Korla Xiangli'	7	0.0420	0.0877	0.3239	0.1010	0.1776	0.3625	0.2367	0.2776	0.4602
	18	0.0902	0.0993	0.4759	0.2398	0.1642	0.5936	0.3604	0.2112	0.6305
	21	0.0767	0.0933	0.4512	0.1753	0.1691	0.5090	0.2999	0.2276	0.5686
'Doyenne du Comice' × 'Huangguan'	7	0.0462	0.0334	0.5809	0.1416	0.0821	0.6330	0.3879	0.1608	0.7070
	18	0.0427	0.0410	0.5102	0.1598	0.1109	0.5904	0.4180	0.2454	0.6301
	21	0.0694	0.0415	0.6255	0.2308	0.1143	0.6688	0.2308	0.1143	0.6688
'Zhongai 1' × 'Zaosu'	7	0.0264	0.0106	0.7144	0.0818	0.0378	0.6841	0.1542	0.0819	0.6530
	18	0.0417	0.0188	0.6892	0.0961	0.0612	0.6111	0.1753	0.1247	0.5844
	21	0.0041	0.0147	0.2193	0.0407	0.0461	0.4689	0.1468	0.1128	0.5655
'Zaosu' × 'Zhongai 1'	7	0.0352	0.0317	0.5256	0.0877	0.0740	0.5423	0.1754	0.1388	0.5581
	18	0.0321	0.0351	0.4773	0.0898	0.0803	0.5279	0.2286	0.1594	0.5892
	21	0.0269	0.0389	0.4094	0.0807	0.0966	0.4553	0.1657	0.1642	0.5023

3.4. Genetic Analysis of Resistance

The coefficient of variation (CV) shows a broad distribution, with all CV values greater than 50%, indicating substantial variation in the resistance to ring rot within these populations. Additionally, while the CV values across different isolate treatments did not show significant differences, notable variations were observed between the populations. This highlights the complexity and variability in the genetic makeup underlying resistance to pear ring rot.

From the perspective of genetic transmission ability, the transmission ability under different isolate treatments for different populations is mostly concentrated between 40% and 70%, indicating that pear ring rot resistance is influenced by both genetic and environmental factors.

In terms of dominance rate, only the 'Doyenne du Comice' × 'Huangguan' combination under isolate 7 exhibited a positive value, while the remaining combinations showed negative values. This suggests that the resistance in the hybrid offspring is more likely to follow a resistant inheritance pattern, possibly due to additive effects. The genetic tendencies for each combination under different treatments are summarized in Table 5.

Table 5. Genetic tendencies of each combination under different treatments.

Hybrid Combination	Inoculated Isolate	F	σ	CV	P1	P2	MP	H	F/MP
'Zaosu' × 'Korla Xiangli'	7	2.24	1.14	50.65%	4	2	3.00	−25.20%	74.80%
	18	2.17	1.35	62.27%	4	2	3.00	−27.81%	72.19%
	21	2.24	1.26	56.08%	4	2	3.00	−25.35%	74.65%
'Doyenne du Comice' × 'Huangguan'	7	2.19	1.29	59.00%	2	4	3.00	−27.02%	72.98%
	18	2.14	1.38	64.70%	1	4	2.50	−14.46%	85.54%
	21	2.63	1.32	50.17%	2	4	3.00	−12.25%	87.75%
'Zhongai 1' × 'Zaosu'	7	1.01	0.92	90.88%	1	4	2.50	−59.45%	40.55%
	18	1.09	1.00	91.06%	1	4	2.50	−56.27%	43.73%
	21	1.07	0.89	83.82%	1	4	2.50	−57.33%	42.67%
'Zaosu' × 'Zhongai 1'	7	1.55	1.06	68.27%	4	1	2.50	−38.11%	61.89%
	18	1.47	1.14	77.65%	4	1	2.50	−41.13%	58.87%
	21	1.65	1.07	65.00%	4	1	2.50	−33.96%	66.04%

NOTE: F/MP (%) represents the ratio of progeny mean to mid-parent value, reflecting the degree of transmission of parental phenotypes to offspring.

The phenotypic data for each combination under different treatments were imported into the R software package SEA v2.0 for genetic model prediction. The model calculates the maximum likelihood value (MLV) and converts it to the AIC value. The lower the AIC value, the better the model fit. Therefore, the three models with relatively smaller AIC values under each combination and treatment were selected as alternatives. All candidate models and their MLV and AIC values are listed in Table 6.

Table 6. All candidate models and their MLV and AIC values.

Hybrid Combination	Inoculated Isolate	Model	MLV	AIC
'Zaosu' × 'Korla Xiangli'	7	2MG-AD	−119.2393	250.4786
		1MG-A	−125.8729	257.7457
		2MG-EA	−126.6579	259.3159
	18	2MG-AD	−139.7001	291.4002
		2MG-EA	−145.2823	296.5646
		1MG-AD	−145.7439	299.4878
	21	2MG-AD	−133.6061	277.5746
		2MG-EA	−135.7873	279.2122
		1MG-A	−145.2835	296.567
	7	2MG-AD	−140.1037	292.2073
		2MG-EA	−146.3555	298.7109
		2MG-A	−149.1464	306.2927
'Doyenne du Comice' × 'Huangguan'	18	2MG-AD	−140.1901	292.3802
		2MG-EA	−152.7282	311.4565
		2MG-A	−155.4445	318.889
	21	2MG-AD	−152.0049	316.0099
		2MG-EA	−159.3512	324.7024
		1MG-AD	−159.4232	326.8464
	7	2MG-AD	−9.2239	30.4478
		2MG-EA	−17.9969	41.9937
		1MG-AD	−17.2183	42.4367
	18	2MG-AD	−17.5925	47.1849
		1MG-AD	−21.9529	51.9058
		2MG-EA	−24.6021	55.2043
'Zhongai 1' × 'Zaosu'	21	2MG-AD	−9.2408	30.4817
		1MG-AD	−12.0287	32.0574
		2MG-A	−12.7117	33.4234
	7	2MG-AD	−47.8244	107.6488
		1MG-AD	−52.9721	113.9442
		2MG-EA	−54.6611	115.3223
	18	2MG-AD	−59.6137	131.2275
		2MG-EA	−65.0314	136.0628
		1MG-AD	−64.5478	137.0957
	21	2MG-AD	−49.8287	111.6573
		2MG-EA	−55.4993	116.9987
		1MG-AD	−56.2934	120.5868

The three alternative models under different combinations and treatments were subjected to $U1^2$, $U2^2$, $U3^2$ (uniformity tests), nW^2 (Smirnov test), and Dn^2 (Kolmogorov test). All tests showed no significant differences with $p > 0.05$.

Therefore, based on the principle of minimizing the AIC value, the 2MG-AD model was selected as the optimal model for all hybrid combinations across all isolates. This

model assumes that resistance is controlled by two major loci, each exhibiting both additive and dominance effects, while the remaining genetic variation is attributed to polygenic background effects. The positive and relatively large additive effect estimates d_a and d_b indicate that the presence of resistance alleles from either parent progressively reduces lesion diameter in a dosage-dependent manner. The dominance effects h_a and h_b , by contrast, were generally smaller in magnitude than the additive effects, suggesting that dominance plays a secondary role in shaping the resistance phenotype. The non-zero variance component Var estimated under the 2MG-AD model reflects residual variation, which may arise from minor-effect polygenes, genotype-by-environment interactions, or experimental noise, but its magnitude was considerably smaller than the contribution of the two major genes. In practical terms, this genetic architecture implies that resistance can be enhanced by accumulating favorable alleles from both parents through recurrent selection, even when neither parent is highly resistant by itself.

In the ‘Zaosu’ × ‘Korla Xiangli’ combination, different results were observed under different treatments. The dominance effect fluctuated significantly. Under isolate 7, the dominance effect was that of susceptibility. Under isolate 18, the resistance of the first main gene pair nearly failed and shifted towards weak susceptibility dominance. Isolate 21 showed a very weak resistance dominance. The genetic structure of the combination is relatively complex, and it is speculated that the resistance-to-susceptibility segregation ratio may be due to the accumulation of minor-effect resistance genes.

In the ‘Doyenne du Comice’ × ‘Huangguan’ combination, dominant resistance was observed with isolates 7 and 18. However, when exposed to isolate 21, the first main gene pair showed a shift towards susceptibility dominance. This indicates that the resistance of the first main gene pair may be ineffective against isolates with specific pathogenic characteristics. This also helps explain the reversal of resistance-to-susceptibility segregation and the transgressive inheritance pattern observed when the population encounters isolate 21.

In the ‘Zhongai 1’ × ‘Zaosu’ combination, both h_a and h_b were negative under all three isolates, meaning that the dominant effect of the main genes reduced the trait value, i.e., the lesion diameter, resulting in resistance dominance within the population. This further explains the extremely high resistance-to-susceptibility segregation ratio and dominance rate within the population.

In the reciprocal cross ‘Zaosu’ × ‘Zhongai 1’ combination, the first main gene pair remained the same as in the orthogonal cross, maintaining dominant resistance. However, for the second main gene pair, small positive values of h_b were observed under isolates 7 and 18, suggesting the presence of weak isolate specificity. Compared to the orthogonal cross combination, it was also found that the values of $|d_b|$ and $|h_b|$ for the second main gene pair were generally lower, and the dominance of resistance was even weakened or reversed.

In all hybrid combinations, the additive effect of the first main gene pair was greater than that of the second main gene pair and greater than zero. By analyzing the ratios of $|h_a|/|d_a|$ and $|h_b|/|d_b|$, it was found that the ratios for all combinations were less than 1, indicating that the genes controlling pear ring rot resistance are primarily governed by additive effects. The estimation of genetic parameters of the optimal model for each combination is listed in Table 7 and the Suitability test results of genetic models for each combination are listed in Table 8.

Table 7. Estimation of genetic parameters of the optimal model for each combination.

Hybrid Combination	Inoculated Isolate	Model	m	da(d)	db	ha(h)	hb	Var
'Zaosu' × 'Korla Xiangli'	7	2MG-AD	0.9323	0.5762	0.2374	0.2486	0.0564	0.1785
	18	2MG-AD	0.9793	0.8088	0.0909	−0.065	0.2727	0.1042
	21	2MG-AD	1.1091	0.5197	0.3953	−0.0205	−0.0502	0.1743
'Doyenne du Comice' × 'Huangguan'	7	2MG-AD	1.1598	0.7859	0.2196	−0.0899	−0.0507	0.3299
	18	2MG-AD	1.1756	0.8493	0.2476	−0.0867	−0.0867	0.0874
	21	2MG-AD	1.1622	0.6832	0.3359	0.2868	−0.0192	0.0085
'Zhongai 1' × 'Zaosu'	7	2MG-AD	0.7129	0.4229	0.197	−0.2053	−0.136	0.0410
	18	2MG-AD	0.6996	0.4947	0.1138	−0.1658	−0.0512	0.2521
	21	2MG-AD	0.7456	0.4005	0.1629	−0.1855	−0.2069	0.0573
'Zaosu' × 'Zhongai 1'	7	2MG-AD	0.8457	0.5589	0.1603	−0.1759	0.0138	0.0912
	18	2MG-AD	0.836	0.5762	0.1459	−0.2018	0.0456	0.0025
	21	2MG-AD	0.9159	0.5364	0.0515	−0.1506	−0.1614	0.0140

NOTE: da, db: These represent the additive effects of the first and second main gene pairs, respectively. The larger the value, the greater the difference between the homozygous genotypes AA and aa for that gene. ha, hb: These represent the dominant effects of the first and second main gene pairs, respectively.

Table 8. Suitability test results of genetic models or each combination.

Hybrid Combination	Inoculated Isolate	Model	P(U1 ²)	P(U2 ²)	P(U3 ²)	P(nW ²)	P(Dn ²)
'Zaosu' × 'Korla Xiangli'	7	2MG-AD	0.9658	0.9921	0.8992	1.0002	1
	18	2MG-AD	0.9988	0.9967	0.9821	0.9999	0.9996
	21	2MG-AD	0.9966	0.9981	0.9945	1.0002	1
'Doyenne du Comice' × 'Huangguan'	7	2MG-AD	0.9948	0.9852	0.9609	1	0.999
	18	2MG-AD	0.9495	0.9595	0.9663	1.001	1
	21	2MG-AD	0.9696	0.9371	0.8663	1	0.9999
'Zhongai 1' × 'Zaosu'	7	2MG-AD	0.9775	0.98	0.9927	1.0001	1
	18	2MG-AD	0.9804	0.9986	0.9296	0.9998	0.9995
	21	2MG-AD	0.9917	0.9857	0.9751	0.999	0.9986
'Zaosu' × 'Zhongai 1'	7	2MG-AD	0.9597	0.9543	0.9734	1.0001	0.9978
	18	2MG-AD	1	0.9929	0.9717	1.0001	1
	21	2MG-AD	0.9893	0.9792	0.9581	1.0024	1

3.5. Pathogenicity Difference Analysis of Isolates

The analysis results show that there were no significant differences between the isolate treatments for the other populations. Only in the 'Doyenne du Comice' × 'Huangguan' combination under the three treatments, the overall AUDPC distribution showed significant differences, with the difference mainly coming from the comparison between isolate 18 and isolate 21. Additionally, the ADL on the 9th day under isolate 21 treatment was significantly larger than that under isolates 7 and 18. This suggests that under isolate 21 treatment, regardless of early disease invasion or late-stage disease expansion, the 'Doyenne du Comice' × 'Huangguan' population exhibits isolate-specific susceptibility to isolate 21. The Kruskal–Wallis H test results are listed in Tables 9 and 10.

Table 9. Pathogenicity differences in isolates under 9d ADL values in the ‘Doyenne du Comice’ × ‘Huangguan’ combination: Kruskal–Wallis H test.

Comparison Group	Mean Rank Difference	<i>p</i>	Sig
7 vs. 18	3.29736	1	0
7 vs. 21	−46.4165	0.01004	1
18 vs. 21	−49.71386	0.00257	1

Note: Sig = 1 indicates significant differences at the 0.05 level, while Sig = 0 indicates no significant difference.

Table 10. Pathogenicity differences in isolates under AUDPC values in the ‘Doyenne du Comice’ × ‘Huangguan’ combination: Kruskal–Wallis H test.

Comparison Group	Mean Rank Difference	<i>p</i>	Sig
7 vs. 18	35.11194	0.07598	0
7 vs. 21	−19.60191	0.44597	0
18 vs. 21	−54.71385	0.00172	1

Note: Sig = 1 indicates significant differences at the 0.05 level, while Sig = 0 indicates no significant difference.

To compare the pathogenicity differences among isolates on each hybrid combination, leaf reaction classes 0–2 (HR, R, MR) were classified as resistant (R), and classes 3–4 (MS, HS) as susceptible (S). Chi-square tests were performed for isolates 7, 18, and 21 across the four hybrid combinations.

The results are summarized in Table 11. For the hybrid ‘Zaosu’ × ‘Korla Xiangli’, neither the (3 × 5) ($X^2 = 11.001$, $p = 0.211$) nor the (3 × 2) ($X^2 = 0.362$, $df = 2$, $p = 0.835$) analyses indicated significant differences among isolates. In contrast, ‘Doyenne du Comice’ × ‘Huangguan’ showed significant differences in both (3 × 5) ($X^2 = 18.503$, $p = 0.016$) and (3 × 2) analyses ($X^2 = 10.290$, $df = 2$, $p = 0.0058$), indicating a clear variation in pathogenicity among the three isolates. Both ‘Zhongai 1’ × ‘Zaosu’ (direct) and ‘Zaosu’ × ‘Zhongai 1’ (reciprocal) exhibited no significant differences in either (3 × 5) or (3 × 2) analyses (all $p > 0.7$), suggesting similar pathogenicity profiles across isolates in these crosses.

These results indicate that the pathogenicity of isolates is isolate- and hybrid-specific, with certain hybrids, such as ‘Doyenne du Comice’ × ‘Huangguan’, showing pronounced differences among isolates, whereas other hybrids remain largely unaffected. Using both (3 × 5) and (3 × 2) analyses allows for detection of both subtle grade differences and overall resistant/susceptible trends, providing a robust assessment of isolate pathogenicity.

Table 11. Chi-square tests of differences in resistance-class distribution among hybrid combinations under different inoculated isolates.

Hybrid Combination	Test Type	X^2	df	<i>p</i>	Sig
‘Zaosu’ × ‘Korla Xiangli’	3 × 5	11.001	8	0.211	ns
	3 × 2	0.3615	2	0.8346	ns
‘Doyenne du Comice’ × ‘Huangguan’	3 × 5	18.503	8	0.0164	*
	3 × 2	10.290	2	0.00583	**
‘Zhongai 1’ × ‘Zaosu’	3 × 5	1.6116	8	0.9926	ns
	3 × 2	0.09815	2	0.9521	ns
‘Zaosu’ × ‘Zhongai 1’	3 × 5	4.8521	8	0.7776	ns
	3 × 2	0.1188	2	0.9423	ns

NOTE: Significance levels are indicated as follows: ns, $p \geq 0.05$; *, $0.01 \leq p < 0.05$; **, $0.001 \leq p < 0.01$.

3.6. Assessment of Reciprocal Effects in Direct and Reciprocal Crosses

To evaluate the reciprocal effects of different isolates on the hybrid combinations, leaf reaction scores were analyzed using both the original 0–4 scale (2×5) and a simplified resistant/susceptible classification (2×2). The 2×5 chi-square test used the theoretical chi-square distribution without Monte Carlo simulation, while the 2×2 analysis summed classes 0–2 as resistant (R) and 3–4 as susceptible (S).

The results are summarized in Table 12. For isolate 7, both the (2×5) analysis ($X^2 = 11.812$, $p = 0.0154$) and the (2×2) analysis ($X^2 = 3.9483$, $df = 1$, $p = 0.0469$) indicated significant differences between the direct (Ortho) and reciprocal (Recip) crosses, suggesting a notable reciprocal effect. Isolate 18 showed no significant difference in the 2×5 analysis ($X^2 = 5.4834$, $p = 0.2444$), and the (2×2) analysis was also not significant ($X^2 = 3.6877$, $df = 1$, $p = 0.0548$), indicating little or no reciprocal effect. Isolate 21 exhibited a significant difference in both (2×5) ($X^2 = 14.425$, $p = 0.0048$) and (2×2) ($X^2 = 5.4643$, $df = 1$, $p = 0.0194$) analyses, demonstrating a clear reciprocal effect.

Table 12. Chi-square tests of the effects of reciprocal crosses on resistance-class distribution under different inoculated isolates.

Isolate	Test Type	X^2	df	p	Sig
7	2×5	11.812	4	0.0154	*
	2×2	3.9483	1	0.0469	*
18	2×5	5.4834	4	0.2444	ns
	2×2	3.6877	1	0.0548	ns
21	2×5	14.425	4	0.0048	**
	2×2	5.4643	1	0.0194	*

NOTE: Significance levels are indicated as follows: ns, $p \geq 0.05$; *, $0.01 \leq p < 0.05$; **, $0.001 \leq p < 0.01$.

These results indicate that reciprocal effects vary among isolates, with isolates 7 and 21 showing significant differences between direct and reciprocal crosses, while isolate 18 does not. Using both the full-scale (2×5) and simplified resistant/susceptible (2×2) analyses provides a robust assessment of reciprocal effects, capturing both subtle grade differences and overall resistance trends.

4. Discussion

Across all hybrid combinations examined, resistance phenotypes displayed continuous variation with coefficients of variation exceeding 50%, and the segregation ratios of resistant to susceptible individuals were consistent with a quantitative trait. Genetic model analysis using the mixed major-gene plus polygene inheritance approach identified the two-major-gene additive-dominant (2MG-AD) model as the most parsimonious fit, with additive effect estimates consistently larger than dominance effect estimates. These findings suggest that pear leaf resistance to *B. kuwatsukai* is primarily governed by two major genes and that the additive action of alleles constitutes the principal genetic component of resistance. This inference aligns with the additive or dosage-dependent modes of action documented for several defense-related genes. The incremental enhancement of lignin deposition mediated by *PcMYB44* and the direct antifungal activity of *PbrChiA* may collectively produce the continuous phenotypic distributions observed in segregating populations [30,31]. The non-zero residual variance estimated under the 2MG-AD model further implies that minor-effect polygenes or environmental factors also contribute to phenotypic variation, a pattern that aligns with the complex, multi-tiered defense network—involving hormone crosstalk, ROS homeostasis, and secondary metabolism—that has been extensively documented in pear–Botryosphaeria interaction studies [32–35].

Notably, the ‘Doyenne du Comice’ × ‘Huangguan’ population exhibited substantially greater susceptibility when challenged with isolate 21. The resistance-to-susceptibility segregation ratio declined from approximately 1.5:1 under isolates 7 and 18 to 0.8:1 under isolate 21, and the Area Under the Disease Progress Curve was significantly elevated. This isolate-specific response suggests that recognition or defense mechanisms in this cross may be differentially effective against distinct pathogen genotypes. In the prevailing framework of plant–pathogen interactions, resistance often involves both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) [36]. It is plausible that isolate 21 possesses effector proteins capable of evading or suppressing the major resistance gene(s) segregating in this hybrid background. This would compromise gene-for-gene resistance in this specific cross. The existence of isolate-specific pathogenicity underscores the importance of employing multiple, genetically diverse isolates during resistance screening to avoid selecting for narrow-spectrum resistance that could be rapidly eroded under field conditions.

Reciprocal differences were evident between the ‘Zhongai 1’ × ‘Zaosu’ and ‘Zaosu’ × ‘Zhongai 1’ populations. When the resistant parent ‘Zhongai 1’ served as the maternal parent, progeny exhibited markedly higher resistance, with resistance-to-susceptibility ratios of approximately 11:1 compared with 4:1 in the reciprocal cross. This asymmetry indicates that resistance to ring rot may be influenced not only by nuclear genes but also by cytoplasmic genetic factors or maternal effects [37,38]. However, it is important to emphasize that this interpretation remains speculative at this stage, as the current dataset does not include cytogenetic or molecular evidence that would be required to definitively attribute the observed reciprocal differences to cytoplasmic inheritance. To date, functionally characterized resistance genes in pear, such as *PcMYB44*, *PbrChiA*, and *Pbr3RAV2*, are all nuclear-encoded, and information regarding the potential involvement of organellar genomes in ring rot defense is scarce [21,39]. Therefore, while the reciprocal differences provide a rationale for further investigation, they should not be interpreted as proof of cytoplasmic inheritance without additional experimental validation.

An observation of considerable practical importance was the recovery of resistant individuals from the cross between a susceptible parent and a resistant parent. This outcome is compatible with the 2MG-AD model and suggests that the resistant parent contributes major resistance alleles, while the susceptible parent may also harbor unexpressed or minor-effect resistance alleles that can be recombined and accumulated in offspring to generate transgressive resistance phenotypes. From a breeding perspective, this finding highlights the value of including both resistant and susceptible parents in crosses, as even susceptible cultivars may carry minor-effect alleles that enhance resistance when combined with major genes from resistant parents, provided that sufficiently large populations are generated and subjected to rigorous phenotypic selection.

Several limitations of the present study should be acknowledged. Broad-sense heritability estimates ranged from 0.32 to 0.71 across populations and isolates, indicating that detached leaf assays, while efficient, remain subject to microenvironmental variation. Most importantly, resistance observed in detached leaves represents only a preliminary screening, definitive evaluation of disease resistance must be conducted on whole plants under field conditions. Therefore, future evaluations should be complemented by multi-year, multi-location field assessments on branches and fruit to confirm the stability of resistance phenotypes. Furthermore, the 2MG-AD model, although statistically well-supported, represents an inference rather than a direct demonstration of the existence of two major genes. Confirmation of the physical location and identity of these putative major loci require QTL mapping or genome-wide association studies. Acknowledging these caveats does not weaken the central conclusions, but rather sharpens the priorities for follow-up investigations.

The findings of this study point to several priorities for future research, including QTL mapping to validate and locate the major genes implicated by the 2MG-AD model, the development of molecular markers to facilitate marker-assisted selection, and further investigation of cytoplasmic contributions to resistance using the reciprocal crosses.

In summary, this study provides a population genetic characterization of pear leaf resistance to *B.kuwatsukai*, indicating that the trait is polygenic and that a two-major-gene additive-dominant model offers a parsimonious description of the observed segregation patterns. The predominance of additive effects, together with moderate-to-high broad-sense heritability estimates, suggests that resistance can be progressively improved through recurrent selection and the accumulation of favorable alleles. The detection of isolate-specific responses and reciprocal effects further highlights the complexity of the resistance phenotype and the need for multi-isolate screening and careful consideration of cross direction in breeding programs. Collectively, these findings establish a population genetic framework that connects the extensive molecular knowledge of pear–*Botryosphaeria* interactions with practical strategies for breeding ring rot-resistant cultivars.

5. Conclusions

In summary, detached leaf assays across four F_1 populations indicate that pear leaf ring rot resistance is a polygenic trait for which the two-major-gene additive-dominant (2MG-AD) model provides the best fit to the present data. Additive effects predominated, suggesting that resistance can be progressively enhanced by accumulating favorable alleles through recurrent hybridization and selection. Importantly, moderate-to-high broad-sense heritability estimates ($H^2 = 0.32\text{--}0.71$) derived from replicated inoculations support that the observed variation in lesion diameter is largely genetically determined, strengthening the confidence in subsequent genetic model fitting.

Isolate-specific responses in the ‘Doyenne du Comice’ × ‘Huangguan’ combination, and significant differences between the reciprocal crosses ‘Zhongai 1’ × ‘Zaosu’ and ‘Zaosu’ × ‘Zhongai 1’, underscore the importance of both pathogen variation and the need to consider possible cytoplasmic or maternal influences on resistance expression. Nevertheless, the latter interpretation remains speculative and requires corroboration through molecular or cytogenetic studies.

Together, these results offer an effective method for breeding pears resistant to pear ring rot. It is suggested that multi-isolate detached leaf screening should be included in routine selection procedures, and that reciprocal crosses should be carefully planned to take advantage of potential maternal or cytoplasmic effects. Implementing these approaches should speed up the creation of pear varieties with strong, long-lasting resistance to ring rot.

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Abbreviations

The following abbreviations are used in this manuscript:

AUDPC	Area Under the Disease Progress Curve
ADL	Average Lesion Diameter
DPI	Days Post Inoculation
F	Mean Phenotype Value
σ	Standard Deviation
CV	Coefficient of Variation
P1	Phenotype of the Maternal Parent
P2	Phenotype of the Paternal Parent
MP	Mid-Parent Value
H	Dominance Deviation
Ta	Heritability
LL	Low Parent Rate
MLV	Maximum Likelihood Value
2MG-AD	Two Main Gene Pairs with Additive-Dominant Genetic Effects
1MG-A	One Main Gene with Additive Effects
2MG-EA	Two Main Gene Pairs with Additive-Environmental Genetic Effects
P	Prob
Sig	Significance

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