

Article

Field Resistance to Powdery Mildew in *Lycium barbarum* Germplasm, Associated Leaf Anatomical Traits, and Temporal Physiological Responses in Ningqi-1

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Abstract

Lycium barbarum L. is a characteristic agricultural crop in Ningxia. Powdery mildew is a serious fungal disease affecting *Lycium barbarum*. Resistance analysis of *Lycium barbarum* varieties is rarely reported, and comparative information on field resistance and associated anatomical and physiological responses remains limited. This study evaluated powdery mildew resistance in 26 *Lycium barbarum* germplasm accessions under natural field conditions over two years, compared leaf anatomical traits in eight representative accessions, and characterized early physiological responses in the genotype Ningqi-1 after inoculation. Based on the two-year mean disease index (DI), the 26 accessions were assigned to four descriptive resistance categories. Four sigmoidal equations were fitted to the observed disease-progress data; these equations were used only as descriptive curves and were not treated as independently validated predictive models. Across the eight representative accessions, the 2021 DI was negatively correlated with the number of palisade tissue layers, palisade tissue thickness, and leaf compactness, but positively correlated with spongy tissue thickness (two-tailed Pearson correlation, $n = 8$). In Ningqi-1, inoculation induced time-dependent changes in antioxidant-enzyme activities, malondialdehyde content, defense-related enzyme activities, lignin content, soluble protein content, and soluble sugar content during the first 48 h. The increase in malondialdehyde content suggested enhanced membrane lipid peroxidation during infection, which may reflect oxidative stress associated with powdery mildew infection. Overall, this study characterized field disease responses, candidate anatomical traits associated with DI, and early physiological responses in Ningqi-1. These findings provide preliminary information for future resistance screening, but validation in larger germplasm populations and under controlled inoculation conditions is still required.



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Keywords: *Lycium barbarum* L.; powdery mildew; germplasm accessions; resistance evaluation; leaf anatomy; physiological response

1. Introduction

Lycium barbarum L. is a perennial, multi-branched, thorny deciduous shrub belonging to the Solanaceae family and the *Lycium* genus. Its leaves are simple, alternate, or clustered in groups of 2–4 [1]. In China, the genus *Lycium* comprises seven species and three varieties [2]. It is mainly cultivated in the Northwest region, such as Ningxia, Qinghai, and Gansu. *Lycium barbarum* has the characteristics of being salt-tolerant, drought-tolerant, and suitable for growing in sandy areas [3]. *Lycium barbarum* leaves are commonly known as Tianjingcao, Dixianmiao, and Tiancai [4]. *Lycium barbarum* branches and leaves contain alkaloids, scopolamine, and other pharmacologically active substances, as well as vitamins and various amino acids. They have functions such as soothing the liver and clearing heat, lowering blood pressure, and anti-inflammation. *Lycium barbarum* leaves are both food and medicine. They can be used as vegetables or as traditional Chinese medicine. In the local area, *Lycium barbarum* leaves have also developed into a series of by-products such as edible goji berries and *Lycium barbarum* bud tea. A planting model integrating production and commerce has been formed in the local area, which is of great significance to the development of the Northwest region [5]. Plant diseases, as one of the greatest threats to plant growth, are typically composed of three basic elements: pathogens, susceptible hosts, and environmental conditions. With the development and encroachment of human society, human production and social activities have become the fourth element in the occurrence of plant diseases. Most plant diseases occur because human activities disrupt the natural ecological balance, such as changes in farming methods, crop monoculture, and alterations in cultivation practices. Powdery mildew (PM) is a fungal disease caused by powdery mildew fungi. There are many species of PM fungi that are obligate parasitic pathogens on angiosperms. Powdery mildew fungi are obligate biotrophic fungal pathogens that establish a persistent interaction with host tissues by forming haustoria in plant cells. Currently, there are 700 species of powdery mildew fungi that can colonize approximately 10,000 plant species [6,7]. Previous regional reports have identified *Arthrocladiella mougeotii* as a causal agent of *Lycium barbarum* powdery mildew. However, the inoculum used in the present study was not subjected to ITS sequencing or species-specific molecular identification; therefore, it is referred to as the powdery mildew fungus in the experimental sections [8]. Typical symptoms include the formation of a white, powdery fungal layer on the leaf surface; severe infection may lead to leaf curling, chlorosis, and premature defoliation. The germination of powdery mildew conidia, appressorium formation, haustorium establishment, and subsequent expansion of secondary hyphae follow a distinct temporal sequence [9–14]. However, systematic comparisons of field resistance and disease progression among different *Lycium barbarum* germplasm accessions remain limited. After plants are infected by powdery mildew, a series of physiological changes occur, such as reactive oxygen species (ROS), malondialdehyde (MDA), and related enzymes, all of which are involved in the response to this process. Reactive oxygen species include superoxide anion (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2), which act as signaling molecules in cells but are also considered to be the unavoidable toxic byproducts of aerobic metabolism [15]. Under normal plant growth conditions, the plant ROS content maintains a balance between ROS production and ROS elimination, and the plant maintains a low level of ROS concentration [16]. Under adverse conditions, ROS will accumulate, damaging biomolecules such as proteins, nucleic acids, and lipids, thereby affecting plant growth and

development [17]. During plant–microbe interactions, a large amount of reactive oxygen species accumulates at sites where hypersensitive responses (HRs) occur [18]. Malondialdehyde (MDA) is usually produced as a byproduct of lipid peroxidation and is therefore considered an indicator of cell damage [19]. The degree of lipid peroxidation in plant cell membranes can be determined by the MDA content, thereby understanding the degree of pathogen infection. In order to reduce the toxic effects of oxidative damage, plants activate antioxidant systems and select a series of complex detoxification mechanisms to combat the accumulation and toxicity of ROS [20]. These include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which mainly participate in maintaining the redox balance of cells under various stresses. After peas are infected with powdery mildew, both the highly resistant genotype and the susceptible genotype show higher levels of SOD and total phenols and lower MDA content [21]. After zucchini is infected with powdery mildew, the activities of POD, SOD, and CAT all increase immediately to remove excess ROS and free radicals [22]. After *Cucurbita moschata* genotypes were inoculated with powdery mildew, ROS increases rapidly and then gradually decreases. At the same time, it inhibits the development of powdery mildew by rapidly increasing the activities of POD, SOD, and CAT [23]. In addition to regulating the development of plant diseases through changes in ROS, plants also secrete various enzymes to resist disease infection. The production of antioxidant enzymes leads to cell wall thickening and structural deformation, resulting in callose deposition and phenolic compound accumulation [24]. Plant defense-related enzymes include phenylalanine ammonia-lyase, polyphenol oxidase, chitinase, and β -1,3-glucanase. When pathogens infect the plant epidermis, the plant surface releases a large amount of chitinase and β -1,3-glucanase, which decompose the fungal cell wall. Chitinase decomposes the main substance of the fungal cell wall into chitin oligosaccharides that can be recognized by plants, and β -1,3-glucan is decomposed into oligosaccharides, inducing a plant immune response, thereby decomposing the fungal cell wall to resist pathogen infection [25,26]. In addition, under plant stress conditions, as pathogens infect plants, the degradation of soluble proteins is promoted. The more pathogenic the strain, the faster and greater the accumulation of soluble proteins in the plant [27].

Based on this background, the study comprised three distinct components: (1) screening 26 *Lycium barbarum* germplasm accessions under natural disease conditions over two years and characterizing their disease progress curves; (2) comparing the microscopic anatomical structures of healthy and infected leaves from eight representative accessions and analyzing the correlations between anatomical traits and the disease index (DI); and (3) characterizing the early physiological responses of a single genotype, Ningqi-1, following inoculation. The study tested the hypotheses that lower DI values are associated with more highly developed palisade tissue and greater leaf tissue compactness and that inoculation induces time-dependent changes in defense-related physiological parameters in Ningqi-1. The study did not involve modeling ecological or meteorological factors, nor did it extrapolate the physiological responses of a single genotype as a general mechanism underlying resistance differences across all germplasm accessions. The disease progress equations were used solely to describe the observed data and should not be regarded as independently validated predictive tools. Overall, this study aimed to characterize the relationship between field disease patterns and relevant environmental conditions at a broader scale while examining the association between leaf microscopic anatomical traits and powdery mildew resistance at the anatomical level. The findings are expected to provide preliminary information for the evaluation and utilization of *Lycium barbarum* germplasm and for future studies of powdery mildew resistance.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

This study used 26 *Lycium barbarum* germplasm accessions for field evaluation of powdery mildew resistance. The accessions were planted in plot No. 3 of the germplasm nursery at the Luhutai Horticultural Experiment Station, Ningxia Academy of Agriculture and Forestry Sciences. Physiological responses were evaluated in Ningqi-1 rooted cuttings inoculated with the powdery mildew fungus. Because powdery mildew fungi are obligate biotrophs, inoculum was collected from naturally infected leaves of the highly susceptible accession Tianjing-3. Ningqi-1 cuttings and seedlings were maintained in the plant tissue culture laboratory of North Minzu University at 25 °C under a 12 h light/12 h dark photoperiod and a light intensity of 4000 lx.

At the time of evaluation, the 26 *Lycium barbarum* germplasm accessions were 5 years old in 2021 and 7 years old in 2023. The experiment was arranged in a randomized complete block design with three replicates. Within each block, the 26 accessions were independently randomized. Each accession was planted in a single-row plot containing eight plants, giving a total of 24 plants per accession across the three replicates. The two plants at the ends of each row served as guard plants, while the six central plants were permanently designated for disease assessment. Row and plant spacing were 3.0 m × 1.0 m. Uniform drip-irrigation, fertilization, and pruning practices were applied throughout the experimental field. Irrigation was conducted six to seven times per year according to soil moisture conditions. Well-decomposed organic manure was applied in autumn, together with nitrogen, phosphorus, and potassium fertilizers. Structural pruning was performed in winter, while bud removal, shoot pinching, and removal of root suckers were carried out during the growing season. To ensure comparability of disease-resistance evaluations, no fungicides were applied during the disease assessment periods in 2021 and 2023. Only nonchemical disease-management practices were adopted, including the removal of dead branches and fallen leaves, weed control, and improvement of canopy ventilation. Disease development occurred from August to October. During this period, the mean temperature, mean relative humidity, and cumulative precipitation were approximately 17.0 °C, 59%, and 92.9 mm, respectively, in 2021, compared with approximately 18.1 °C, 54%, and 55.8 mm, respectively, in 2023.

2.2. Research Methods

2.2.1. Resistance Evaluation of 26 *Lycium barbarum* Germplasm Accessions

Using 26 different genotypes of goji germplasm as the research object, the incidence of powdery mildew was statistically analyzed over time. Five healthy seedlings were randomly selected from each germplasm, and five branches were selected from each seedling (one branch in each of the five directions: east, south, west, north, and center, with each branch having more than 30 leaves). Each selected seedling and branch was labeled accordingly, and the location was recorded for subsequent statistical analysis. According to the “Technical Regulations for Monitoring and Forecasting of Diseases and Pests of Goji” [28] and the real-time incidence of disease in goji in field No. 3, field surveys were conducted approximately every week from 6 September 2021 to 11 October 2021 and from 1 July to 10 October 2023, respectively. The number of leaves in each disease severity category was recorded and used to calculate the disease index (DI). Because the survey periods differed between the two years, the two annual datasets were not treated as strict replicates under identical conditions. Accordingly, the DI for each year and the two-year mean were reported separately.

The 2021 survey began in September because continuous field observations were initiated only after disease symptoms had become relatively stable that year. In 2023, the

survey was advanced to July to more comprehensively document disease progression from initial appearance through development to peak severity. Consequently, the survey periods were not identical between the two years, and the corresponding data were analyzed and reported separately. In both years, disease assessments were conducted by the same evaluator using consistent criteria, and the same tagged plants were monitored throughout each annual survey. However, because winter pruning and shoot renewal prevented the use of exactly the same branches across years, branches were reselected and tagged at the beginning of each survey year. Within each year, the same set of tagged branches was continuously monitored throughout the observation period.

The number of diseased leaves at each level was recorded, and the disease index was calculated. The disease level was classified using a 0–5 level recording method (Figure 1). Grade 0: Leaves are free of disease, have no lesions, and are of normal color; Grade 1: Lesions cover less than 5% of the leaf area, and the mycelial layer is thin; Grade 2: Lesions cover 6–10% of the entire leaf area; Grade 3: Lesions cover 11–25% of the entire leaf area; Grade 4: Lesions cover 26–50% of the entire leaf area; Grade 5: Lesions cover more than 50% of the entire leaf area. The raw data were processed hierarchically. Each individual plant was treated as an independent biological replicate, whereas the five branches sampled from different canopy positions and their associated leaves were treated as subsamples within the plant. The numbers of leaves in the different disease severity categories were first pooled across the five branches to calculate the DI for each plant. The DI values of five plants per germplasm accession were then used to calculate the accession mean and measures of variation. Accordingly, the sample size used in the statistical analyses was five plants; neither individual branches nor individual leaves were treated as independent replicates, thereby avoiding pseudoreplication.

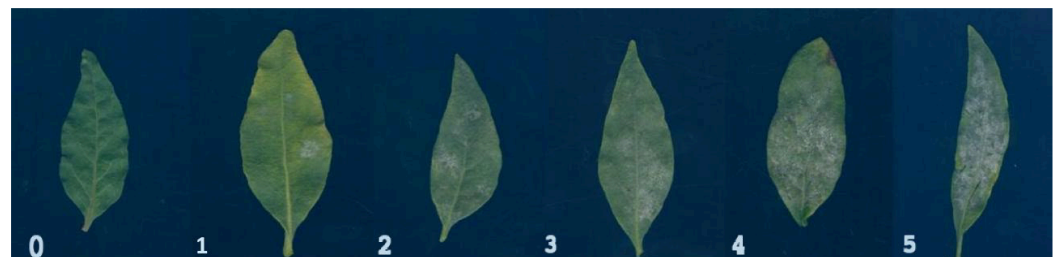


Figure 1. Classification of powdery mildew on leaves of *Lycium barbarum*.

Disease Index (DI) = $100 \times \frac{\sum(\text{number of diseased leaves at each level} \times \text{representative value at each level})}{(\text{total number of leaves surveyed} \times \text{highest representative value})}$. A preliminary evaluation was conducted based on data obtained from field surveys, and the disease index was calculated. The degree of resistance to powdery mildew in different goji germplasms was evaluated using the leaf disease index: When DI = 0, the germplasm resistance was immune (I); when $0 < \text{DI} \leq 5$, the germplasm resistance was highly resistant (HR); when $5 < \text{DI} \leq 15$, the germplasm resistance was moderately resistant (MR); when $15 < \text{DI} \leq 25$, the germplasm resistance was moderately susceptible (MS); when $25 < \text{DI} \leq 50$, the germplasm resistance was highly susceptible (HS); and when $50 < \text{DI} \leq 100$, the germplasm resistance was extremely susceptible (ES).

Nonlinear fitting of the continuously monitored disease index (DI) data was performed using MATLAB Compiler Runtime 8.3 (R2014a; The MathWorks, Inc., Natick, MA, USA) with four sigmoidal equations: (1) $\text{logistic}(x) = \frac{A1 - A2}{1 + (x/x_0)^p} + A2$; (2) $\text{slogistic1}(x) = \frac{a}{1 + \exp(-k \times (x - x_c))}$; (3) $\text{slogistic2}(x) = \frac{a}{1 + ((a - y_0)/y_0) \times \exp(-4 \times W_{\max} \times x/a)}$; and (4) $\text{slogistic3}(x) = \frac{a}{1 + b \times \exp(-k \times x)}$. In these equations, y represents the DI, and t denotes the number of days elapsed since June 30. $A1$ and $A2$ are

the lower and upper asymptotes, respectively; (x_0) and (x_c) are curve-position parameters; p and k are shape or growth-rate parameters; a is the upper asymptote; y_0 is the initial value; $W_{\max}$ is the maximum growth-rate parameter; and b is a scale parameter.

Disease progress parameters were calculated numerically from the derivatives of the fitted curves. The inflection time t^* was defined as the time at which the second derivative equaled zero, and the maximum DI increase rate was defined as the first derivative evaluated at t^* . The beginning t_1 and end t_2 of the rapid-growth phase were determined by the two time points at which the third derivative equaled zero. The duration of the rapid-growth phase was calculated as $R_R = t_2 - t_1$ (d), the DI increment during this phase as $G_R = y(t_2) - y(t_1)$, and the mean DI increase rate as $D_G = G_R/R_R$ (DI·d^{−1}). Here, GR denotes the increment in DI.

2.2.2. Microstructural Differences Between Healthy and Infected Leaves of Eight Representative *Lycium barbarum* Accessions

Eight healthy and diseased goji leaves with different resistances were taken from the field. Each treatment comprised three biological replicates, each obtained from an independent plant. For each biological replicate, 10 microscopic fields of view or section positions were randomly selected for measurement. The measurements were first averaged within each biological replicate, and these replicate means were then used for statistical analysis. Leaves with an area of about 1 cm² were cut off with dissecting scissors. They were fixed and stained according to the method of Zhang Kun [29] and observed under a microscope. The healthy and diseased leaf materials of the eight different goji germplasms were made into paraffin sections. The leaf anatomical structure was observed. The digital slides were viewed and analyzed using CaseViewer software (version 2.4; 3DHISTECH Ltd., Budapest, Hungary), and the relevant indicators of the leaf cross section were recorded: leaf mesophyll thickness, upper and lower epidermal thickness, palisade tissue thickness, and spongy tissue thickness. The study included three replicates. Ten sets of data were measured for each sample, and the average value was taken. The leaf compactness (CTR) was calculated according to the method of Jia Xiande et al. [30]: $LC (\%) = (\text{palisade tissue thickness} / \text{leaf thickness}) \times 100$. The data were analyzed using two-way analysis of variance (ANOVA), with germplasm accession and leaf health status as fixed factors, and their interaction was also tested. For trypan blue staining, three independent leaves were examined per treatment, with five fields of view randomly selected from each leaf. Images were acquired using a 20× objective at a standardized resolution of 2048 × 1536 pixels, while exposure, white balance, and other imaging parameters were kept constant. Samples were randomly coded, and observations were evaluated in a blinded manner. Trypan blue staining was used primarily for qualitative observation of hyphae, spores, and penetration structures, and no quantitative statistical analysis was performed. Representative images were selected a priori according to the following criteria: intact tissue, uniform staining, a clearly focused image plane, appropriate exposure, and the ability to reflect the typical features observed in the majority of fields for the corresponding treatment.

2.2.3. Infection Process of the Powdery Mildew Fungus in Ningqi-1 and Determination of Physiological Parameters

Two independent inoculation experiments were conducted: one for microscopic observation of the infection process and the other for physiological and biochemical measurements. Twenty-four uniform Ningqi-1 rooted cuttings, 25 d after rooting, were divided into eight groups, with three biological replicates per group. One group served as the 0 h control and was sprayed with sterile water, whereas the remaining groups were inoculated with approximately 3 mL of spore suspension per plant. The suspension was sprayed downward from a distance of 30 cm above the seedlings. Leaves were sampled at 0, 2, 4, 8,

12, 24, 48, and 60 h after inoculation. Fixation and staining were performed as described in Section 2.2.2, and the infection process was observed microscopically [31]. Preparation of spore suspension: Powdery mildew-infected leaves of Tianjing-3 were collected from the field. Conidia were gently brushed from the infected leaves into sterile water, and the concentration of the resulting suspension was determined using a hemocytometer. The suspension was adjusted to more than 2×10^6 conidia mL^{-1} , gently mixed immediately before inoculation, and sprayed uniformly onto the leaf surfaces. After inoculation, the plants were maintained at approximately 90% relative humidity. The inoculum was not subjected to ITS sequencing or species-specific molecular identification and is therefore referred to conservatively as the powdery mildew fungus throughout the experimental sections.

The physiological assays employed a staggered-inoculation, simultaneous-sampling design. Each group comprised 30 plants. Equal amounts of leaf tissue from every 10 plants were pooled to constitute one biological replicate, resulting in three biological replicates per group. The inoculated groups were sprayed with a spore suspension at 48, 24, or 12 h before the common sampling time. Sterile-water-treated groups were included at the corresponding time points as time-matched controls, while the 0 h group was sprayed with sterile water at the time of sampling. The fourth to sixth fully functional leaves were collected simultaneously from all groups, yielding samples corresponding to 0, 12, 24, and 48 h. Following inoculation, the plants were maintained at approximately 90% relative humidity. The inoculated and sterile-water control groups were placed in separate areas and physically isolated to prevent cross-contamination. Infection at each time point was confirmed by visual examination of leaf symptoms and by trypan blue staining to detect hyphae and infection structures. The inoculation experiment was independently repeated three times using identical inoculation, incubation, and sampling conditions in each experiment. Each pooled sample was divided into aliquots of approximately 0.1 g, immediately frozen in liquid nitrogen, and stored at -80°C until analysis.

The physiological and biochemical parameters measured included superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA), chitinase, β -1,3-glucanase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), lignin content, soluble protein content, and soluble sugar content. All measurements were performed by our research group using the corresponding microassay kits supplied by Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Absorbance was measured with a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Three biological replicates were included at each time point, with three technical replicates performed for each biological replicate. The mean of the three technical replicates was used as the measurement value for the corresponding biological replicate. Each parameter was normalized to the sample fresh weight or protein content, as specified in the manufacturer's instructions.

2.2.4. Statistical Analysis

Data processing and statistical analyses were performed using Python 3.13 and SciPy 1.17. For each survey date, DI was first calculated separately for each of five plants per accession ($n = 5$ biological replicates), and the plant-level values were used to calculate the accession mean and standard error. For the exploratory comparison between 2021 and 2023, the final-survey plant-level DI values were summarized as one accession-level mean for each year, and the 26 germplasm accessions were treated as paired units in a two-tailed paired *t*-test. Thus, plant-level replicates were used to estimate within-accession variation, whereas the comparison between years used one accession-level mean per accession per year. Because the survey windows differed between years and only accession-level means were used in the paired comparison, the accession $\times$ year interaction was not

estimated, and a repeated-measures mixed-effects model was not constructed. Associations between leaf traits and the final 2021 DI were evaluated using two-tailed Pearson correlation analysis based on germplasm-level means ($n = 8$). Pearson's correlation coefficient (r), two-tailed p values, and 95% confidence intervals calculated using Fisher's z transformation were reported. For each physiological and biochemical parameter, differences among the four sampling times were analyzed using one-way ANOVA followed by the actual multiple-comparison test. Differences were considered statistically significant at $p < 0.05$. Unless otherwise stated, data are presented as mean $\pm$ standard error (SE).

3. Results

3.1. Evaluation of Powdery Mildew Resistance in 26 *Lycium barbarum* Germplasm Accessions

Based on the two-year mean disease index from the final surveys conducted in 2021 and 2023, together with the standardized resistance classification criteria, the 26 *Lycium barbarum* germplasm accessions were assigned to four descriptive resistance categories (Table 1). Six accessions—Dayezihuang, Guangdongjianye, Tianjing-3, Guangdongyuanye, Zhongke-1, and Ningqicai-1—were classified as extremely susceptible. Three accessions—16-17-5-6, Ningqi-10, and 16-23-7-8—were classified as highly susceptible. Eight accessions—Ningqi-2, YX-2, Ningqi-3, 14-01, Z63, Ningqi-4, Ningqi-6, and 14-401—were classified as moderately susceptible. Nine accessions—Ningqi-1, Ningqi-5, No. 5 Huang, 16-22-8-9, Ningqi-7, 14-02, Ningqi-8, 173, and Ningnongqi-9—were classified as moderately resistant. When evaluated according to the two-year mean DI, no accession reached the immune or highly resistant category.

The mean final-survey DI values of the 26 accessions were 31.21 in 2021 and 31.89 in 2023. An exploratory paired t -test, with germplasm accession treated as the pairing unit, indicated no significant overall difference in DI between the two years. Nevertheless, the annual resistance category changed for 11 accessions, suggesting that individual germplasm accessions may have responded differently to interannual environmental variation. Because the survey periods were not identical between the two years and only one aggregated DI value was retained for each accession in each year, this paired comparison was used solely to describe the overall difference between years and cannot substitute for a formal test of the germplasm accession $\times$ year interaction. To characterize powdery mildew progression among the different germplasm accessions, four sigmoidal growth equations were fitted separately. The fitted equations and parameter estimates for each accession are provided in the Supplementary Table. All fitted curves exhibited a typical sigmoidal growth pattern, with coefficients of determination R^2 ranging from 0.9375 to 0.9985, indicating that the equations provided a good descriptive fit to the observed disease-progress data.

It should be noted that different parameterizations were used for different germplasm accessions, and the available data were insufficient for a systematic evaluation of model-selection criteria, residual distributions, parameter stability, or independent predictive performance. Therefore, the model parameters presented in Table 2 were used primarily to describe variation in powdery mildew progression among germplasm accessions and were not intended to establish a universal prediction threshold or directly guide field disease management.

Table 1. Year-specific disease indices and descriptive resistance categories of 26 *Lycium barbarum* germplasms.

Germplasm	Disease Index (2021)	Class (2021)	Disease Index (2023)	Class (2023)	Two-Year Mean DI	Descriptive Overall Class
Dayezihuang	100.00	ES	97.48	ES	98.74	ES
Guangdongjianye	99.87	ES	89.10	ES	94.48	ES
Tianjing-3	96.02	ES	88.60	ES	92.31	ES
Guangdongyuanye	79.12	ES	84.73	ES	81.92	ES
Zhongke-1	56.87	ES	66.92	ES	61.89	ES
Ningqicai-1	51.67	ES	56.17	ES	53.92	ES
16-17-5-6	24.54	MS	34.37	HS	29.46	HS
Ningqi-10	23.97	MS	31.62	HS	27.80	HS
16-23-7-8	23.76	MS	30.92	HS	27.34	HS
Ningqi-2	23.87	MS	25.39	HS	24.63	MS
YX-2	22.65	MS	21.62	MS	22.14	MS
Ningqi-3	30.56	HS	13.00	MR	21.78	MS
14-01	18.56	MS	24.13	MS	21.34	MS
Z63	18.23	MS	17.48	MS	17.85	MS
Ningqi-4	17.41	MS	18.07	MS	17.74	MS
Ningqi-6	15.51	MS	16.61	MS	16.06	MS
14-401	14.73	MR	15.95	MS	15.34	MS
Ningqi-1	20.65	MS	8.04	MR	14.35	MR
Ningqi-5	12.96	MR	15.57	MS	14.26	MR
No. 5 Huang	16.98	MS	11.52	MR	14.25	MR
16-22-8-9	13.05	MR	14.33	MR	13.69	MR
Ningqi-7	12.53	MR	14.19	MR	13.36	MR
14-02	4.65	HR	10.54	MR	7.59	MR
Ningqi-8	2.82	HR	9.59	MR	6.20	MR
173	5.23	MR	6.63	MR	5.93	MR
Ningnongqi-9	5.19	MR	6.62	MR	5.90	MR

Note: DI values are accession means calculated from five biological replicates (individual plants; $n = 5$) at the final survey in each year. I, DI = 0; HR, $0 < DI \leq 5$; MR, $5 < DI \leq 15$; MS, $15 < DI \leq 25$; HS, $25 < DI \leq 50$; ES, $50 < DI \leq 100$. The descriptive overall class was assigned from the arithmetic mean of the two year-specific accession means because the survey windows differed. No letter-based multiple comparisons are shown.

Table 2. Parameters derived from fitted disease-progress models for 26 *Lycium barbarum* germplasms.

Germplasm	Inflection Time, t^* (d)	Maximum DI Increase Rate (DI d ⁻¹)	Rapid-Phase Start, t_1 (d)	Rapid-Phase Duration, R_R (d)	DI Increment, G_R	Mean DI Increase Rate, DG (DI d ⁻¹)
Dayezihuang	75.00 ± 1.00	2.47 ± 0.68	58.00 ± 7.21	33.67 ± 14.77	56.62 ± 6.96	1.68
Guangdongjianye	39.67 ± 1.86	5.68 ± 3.03	31.00 ± 4.58	17.67 ± 6.89	51.13 ± 6.42	2.89
Tianjing-3	57.00 ± 5.13	1.27 ± 0.21	39.67 ± 0.88	47.33 ± 5.78	59.91 ± 6.71	1.27
Guangdongyuanye	72.33 ± 1.76	3.61 ± 0.56	64.33 ± 0.33	16.00 ± 3.21	46.96 ± 2.56	2.94
Zhongke-1	100.33 ± 4.25	2.70 ± 0.34	89.67 ± 2.33	20.00 ± 4.73	44.85 ± 4.77	2.24
Ningqicai-1	96.67 ± 10.72	1.60 ± 0.15	84.33 ± 11.86	21.00 ± 6.66	28.55 ± 7.12	1.36
16-17-5-6	78.33 ± 1.45	0.27 ± 0.03	60.67 ± 2.18	35.00 ± 6.43	7.97 ± 0.68	0.23
Ningqi-10	91.33 ± 12.45	1.49 ± 0.51	77.67 ± 6.69	27.00 ± 11.93	27.57 ± 10.03	1.02
16-23-7-8	105.67 ± 0.88	2.23 ± 1.04	97.00 ± 2.65	16.33 ± 6.12	21.09 ± 1.50	1.29
Ningqi-2	106.00 ± 11.01	0.55 ± 0.07	77.00 ± 8.66	36.67 ± 5.36	16.58 ± 1.49	0.45
YX-2	77.00 ± 0.58	1.24 ± 0.28	68.00 ± 1.53	18.00 ± 3.51	17.84 ± 0.20	0.99
Ningqi-3	94.33 ± 4.09	0.65 ± 0.08	81.67 ± 2.91	25.67 ± 2.33	14.69 ± 2.67	0.57
14-01	97.33 ± 3.28	0.54 ± 0.32	88.00 ± 5.57	18.33 ± 6.69	5.07 ± 0.44	0.28
Z63	108.00 ± 6.42	0.83 ± 0.03	97.00 ± 8.50	19.67 ± 7.69	14.58 ± 5.87	0.74
Ningqi-4	99.00 ± 1.73	0.73 ± 0.11	90.33 ± 0.67	17.00 ± 2.08	11.28 ± 3.21	0.66
Ningqi-6	82.67 ± 2.73	1.23 ± 0.54	75.67 ± 1.86	13.67 ± 5.36	9.66 ± 2.11	0.71
14-401	82.00 ± 2.00	0.46 ± 0.09	73.67 ± 3.18	17.00 ± 2.64	6.41 ± 0.30	0.38
Ningqi-1	86.33 ± 1.33	0.42 ± 0.04	72.33 ± 1.86	28.33 ± 0.88	10.30 ± 0.81	0.36
Ningqi-5	92.33 ± 4.06	0.66 ± 0.17	82.33 ± 2.33	20.67 ± 4.91	10.50 ± 1.24	0.51
No. 5 Huang	93.67 ± 1.86	0.45 ± 0.09	83.67 ± 2.03	20.67 ± 0.88	8.17 ± 1.67	0.40
16-22-8-9	96.33 ± 10.83	1.00 ± 0.30	89.67 ± 8.17	9.33 ± 1.33	7.47 ± 1.63	0.80
Ningqi-7	99.67 ± 0.88	3.57 ± 2.83	62.33 ± 31.17	41.67 ± 30.17	8.20 ± 1.19	0.20
14-02	99.33 ± 11.62	0.16 ± 0.04	73.33 ± 6.57	33.67 ± 11.28	3.95 ± 0.44	0.12
Ningqi-8	77.33 ± 5.33	1.15 ± 0.76	46.00 ± 23.01	38.33 ± 19.94	2.68 ± 1.08	0.07
173	85.33 ± 2.84	0.74 ± 0.41	77.00 ± 2.08	17.00 ± 8.73	6.54 ± 1.49	0.38
Ningnongqi-9	98.67 ± 1.76	0.39 ± 0.11	88.00 ± 1.15	21.00 ± 5.00	6.15 ± 0.89	0.29

Note: Values are presented as mean ± SE. t^* , inflection time; t_1 , beginning of the rapid-growth phase; R_R , duration of the rapid-growth phase; G_R , DI increment during the rapid-growth phase; DG, mean DI increase rate. No letter-based multiple comparisons are shown.

3.2. Microstructural Differences Between Healthy and Infected Leaves of Eight Representative *Lycium barbarum* Accessions

3.2.1. Trypan Blue Staining of Infected Leaves from Eight Representative *Lycium barbarum* Accessions

To encompass the four resistance categories identified on the basis of the two-year mean DI while also considering material availability, eight germplasm accessions were selected for trypan blue staining: the extremely susceptible accessions Guangdongjianye, Tianjing-3, and Ningqicai-1; the highly susceptible accession Ningqi-10; the moderately susceptible accession 14-01; and the moderately resistant accessions Ningqi-1, Ningqi-7, and 173. The corresponding results are shown in Figure 2. In diseased leaves, visible hyphae and conidial structures generally became more abundant as DI increased. However, these observations were qualitative, and neither fungal biomass nor structural density was quantified. No obvious hyphae or spores were observed in the representative field of view for accession 173, but this does not indicate immunity because its field DI was greater than zero.

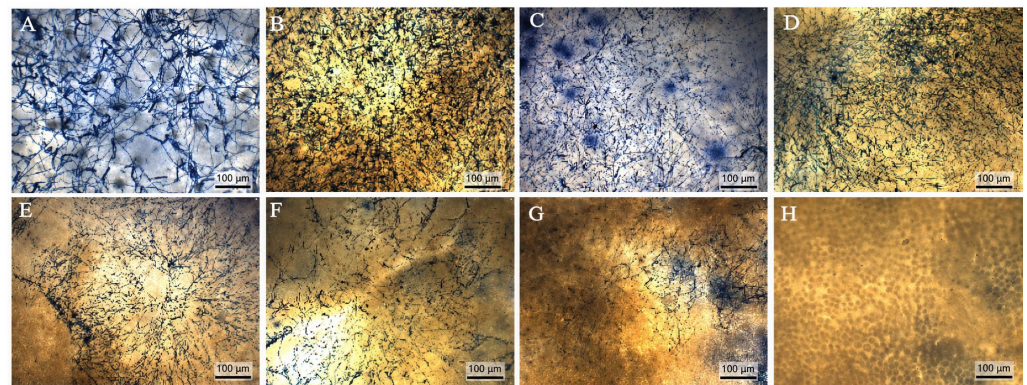


Figure 2. Representative trypan blue-stained infected leaves from eight *Lycium barbarum* germplasms: (A) Guangdongjianye; (B) Tianjing-3; (C) Ningqicai-1; (D) Ningqi-10; (E) 14-01; (F) Ningqi-1; (G) Ningqi-7; (H) 173. Fungal structures were assessed qualitatively.

3.2.2. Paraffin-Section Observations of Healthy and Infected Leaves from Eight Representative *Lycium barbarum* Accessions

Based on cross-sectional microstructure observations of different resistant germplasms, Guangdongjianye and Tianjing-3 showed ectoplanar leaves, while the other six germplasms showed isoplanar leaves with indistinct boundaries between palisade and spongy tissues. Comparison of healthy and infected leaves from the eight germplasms (Figure 3) revealed that the palisade tissues of healthy leaves were neatly arranged and tightly connected with small intercellular gaps, while the palisade tissues of diseased leaves were irregularly arranged, with wrinkled and loose cells and larger intercellular gaps.

The number of palisade tissue layers (PTL), upper epidermis thickness (UET), lower epidermis thickness (LET), palisade tissue thickness (PTT), spongy tissue thickness (STT), mesophyll thickness (MT), leaf thickness (LT), and leaf compactness (LC) were measured in healthy and infected leaves from the eight representative accessions using CaseViewer software. Following infection, LT, MT, and PTT decreased in most germplasm accessions, although the magnitude of these reductions varied markedly among accessions (Table 3).

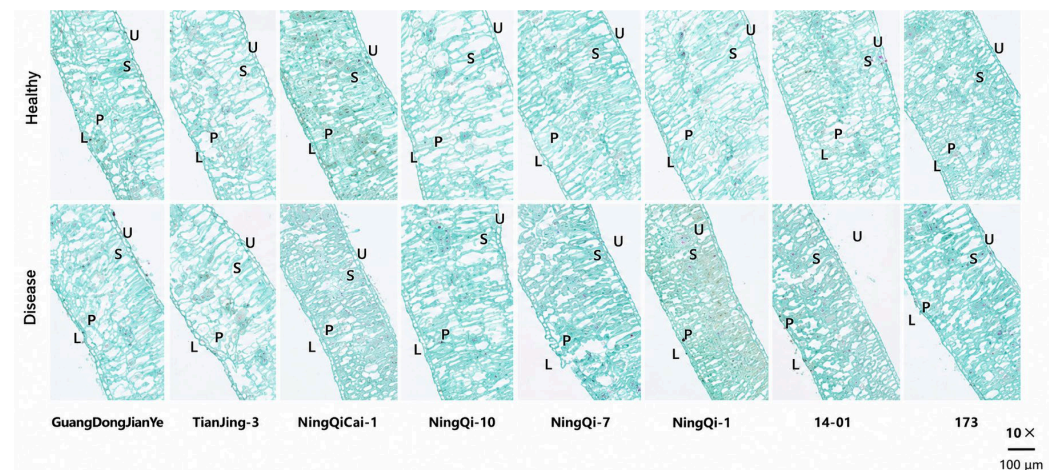


Figure 3. Paraffin sections of healthy and infected leaves from eight *Lycium barbarum* germplasms. The upper and lower rows represent healthy and infected leaves, respectively. U, upper epidermis; S, spongy tissue; P, palisade tissue; L, lower epidermis.

The leaf and mesophyll thicknesses of the eight healthy germplasms ranged from 422.3 μm to 952.6 μm and from 374.5 μm to 909.2 μm , respectively. The leaf and mesophyll thicknesses of the infected leaves of the eight *Lycium barbarum* germplasms ranged from 267.1 μm to 573.4 μm and from 239.1 μm to 530.7 μm , respectively. After powdery mildew infection, the leaf and mesophyll thicknesses of all germplasms except for the Guangdongjianye accession showed varying degrees of thinning. The palisade tissue thickness of the eight healthy germplasms ranged from 200.43 μm to 909.23 μm , and thinning occurred after infection. The palisade tissue thickness of the infected germplasms ranged from 97.13 μm to 530.73 μm , indicating that the palisade tissue thinned after infection. The stronger the resistance of the germplasm, the thicker the palisade tissue. Among the eight germplasms, the palisade tissue thickness was Ningqi-1 > 14-01 > Ningqi-7 > 173 > Ningqi-10 > Ningqicai-1 > Tianjing-3 > Guangdongjianye.

Among the eight germplasm accessions, the 2021 DI was significantly negatively correlated with the number of palisade tissue layers (PTL), palisade tissue thickness (PTT), and leaf compactness (LC) but significantly positively correlated with spongy tissue thickness (STT) (Table 4). Thus, a greater number of palisade tissue layers, thicker palisade tissue, and higher leaf compactness were associated with a lower DI and greater resistance, whereas thicker spongy tissue was associated with a higher DI and lower resistance. The correlations of upper epidermis thickness, lower epidermis thickness, leaf thickness, and mesophyll thickness with DI were not statistically significant at $p < 0.05$. In addition, correlations between leaf area-related traits of diseased leaves and the disease index were evaluated across the different *Lycium barbarum* germplasm accessions (Table 5). The leaf length-to-width ratio was significantly negatively correlated with the 2021 DI, suggesting that more elongated leaves may be associated with a lower DI. The correlations of leaf area, leaf length, leaf width, and leaf perimeter with DI did not reach statistical significance at $p < 0.05$.

Table 3. Leaf anatomical measurements of healthy and infected leaves from eight *Lycium barbarum* germplasms.

Germplasm	Status	UET (μm)	LET (μm)	LT (μm)	MT (μm)	PTL	PTT (μm)	STT (μm)	LC (%)
Guangdongjianye	Healthy	21.97 ± 2.97	19.03 ± 1.20	422.27 ± 6.45	374.47 ± 5.82	2	200.43 ± 18.32	180.33 ± 18.22	47.46
Guangdongjianye	Infected	21.63 ± 2.58	17.77 ± 2.22	460.33 ± 29.66	418.83 ± 33.63	2	204.80 ± 13.79	218.97 ± 28.34	44.49
Tianjing-3	Healthy	23.67 ± 2.60	19.97 ± 3.04	467.83 ± 24.44	444.00 ± 27.60	2	265.63 ± 20.93	158.57 ± 21.21	56.78
Tianjing-3	Infected	18.33 ± 0.47	13.80 ± 2.48	267.07 ± 21.68	239.13 ± 20.50	2	97.13 ± 11.81	141.20 ± 12.35	36.37
Ningqicai-1	Healthy	22.43 ± 1.76	18.07 ± 3.37	458.77 ± 11.60	416.00 ± 18.37	6	416.00 ± 18.37	0.00 ± 0.00	90.68
Ningqicai-1	Infected	20.27 ± 0.31	17.80 ± 0.66	367.93 ± 7.09	323.00 ± 8.58	6	323.00 ± 8.58	0.00 ± 0.00	87.79
Ningqi-10	Healthy	26.87 ± 0.90	22.63 ± 2.30	807.97 ± 3.09	757.40 ± 3.75	4	757.40 ± 3.75	0.00 ± 0.00	93.74
Ningqi-10	Infected	26.53 ± 1.59	21.50 ± 0.87	573.43 ± 10.40	530.73 ± 8.08	5	530.73 ± 8.08	0.00 ± 0.00	92.55
Ningqi-1	Healthy	25.07 ± 1.39	21.03 ± 1.18	952.63 ± 46.57	909.23 ± 46.65	5	909.23 ± 46.65	0.00 ± 0.00	95.44
Ningqi-1	Infected	21.27 ± 1.68	18.60 ± 1.35	394.87 ± 24.44	352.00 ± 25.54	6	352.00 ± 25.54	0.00 ± 0.00	89.14
Ningqi-7	Healthy	20.90 ± 2.37	17.97 ± 1.03	872.80 ± 27.80	838.30 ± 16.55	6	838.30 ± 16.55	0.00 ± 0.00	96.05
Ningqi-7	Infected	21.70 ± 0.59	16.80 ± 1.15	458.07 ± 25.59	420.13 ± 21.73	6	420.13 ± 21.73	0.00 ± 0.00	91.72
14-01	Healthy	31.07 ± 2.32	27.30 ± 5.01	930.63 ± 48.13	875.00 ± 48.84	6	875.00 ± 48.84	0.00 ± 0.00	94.02
14-01	Infected	24.07 ± 3.96	18.47 ± 1.97	445.70 ± 31.31	402.37 ± 26.31	6	402.37 ± 26.31	0.00 ± 0.00	90.28
173	Healthy	27.00 ± 2.52	22.70 ± 2.95	847.73 ± 7.42	796.63 ± 8.11	7	796.63 ± 8.11	0.00 ± 0.00	93.97
173	Infected	23.70 ± 0.36	20.80 ± 2.41	481.03 ± 16.18	436.43 ± 7.82	7	436.43 ± 7.82	0.00 ± 0.00	90.73

Note: Values are means ± SE. UET, upper epidermis thickness; LET, lower epidermis thickness; LT, leaf thickness; MT, mesophyll thickness; PTL, number of palisade tissue layers; PTT, palisade tissue thickness; STT, spongy tissue thickness; LC, leaf compactness, calculated as $PTT/LT \times 100$. LC values were calculated from the reported mean PTT and LT values.

Table 4. Pearson correlations between the 2021 disease index and infected-leaf anatomical traits in eight *Lycium barbarum* germplasms ($n = 8$).

Anatomical Trait	Abbreviation	Pearson's r with DI	Two-Tailed p	95% CI for r
Upper epidermis thickness	UET	−0.607	0.1105	[−0.919, 0.171]
Lower epidermis thickness	LET	−0.631	0.0934	[−0.925, 0.133]
Leaf thickness	LT	−0.529	0.1776	[−0.899, 0.280]
Mesophyll thickness	MT	−0.515	0.1915	[−0.895, 0.298]
Number of palisade tissue layers	PTL	−0.934	0.0007	[−0.988, −0.671]
Palisade tissue thickness	PTT	−0.884	0.0036	[−0.979, −0.476]
Spongy tissue thickness	STT	0.912	0.0016	[0.580, 0.984]
Leaf compactness	LC	−0.936	0.0006	[−0.989, −0.680]

Note: Correlations were two-tailed and were calculated using germplasm-level means from infected leaves together with the final 2021 DI ($n = 8$). Pearson's r , two-tailed p values, and 95% confidence intervals based on Fisher's z transformation are shown. The small sample size limits precision, and the correlations do not demonstrate causation. No multiple-comparison letters are used.

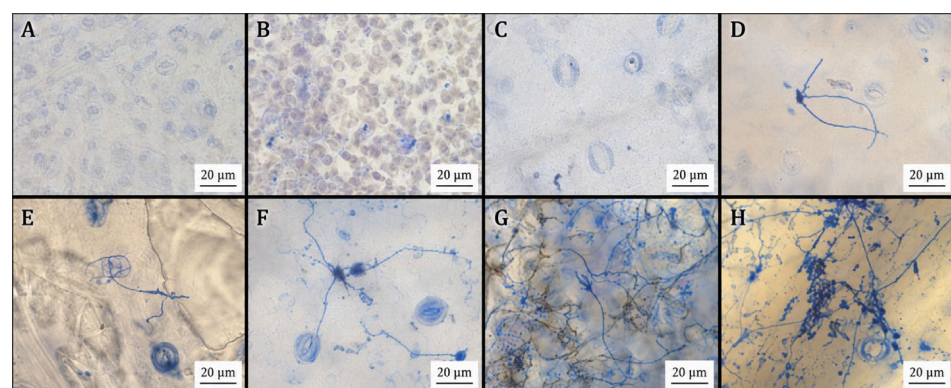
Table 5. Pearson correlations between the 2021 disease index and leaf size/shape traits in eight *Lycium barbarum* germplasms ($n = 8$).

Leaf Trait	Abbreviation	Pearson's r with DI	Two-Tailed p	95% CI for r
Leaf area	LA	0.537	0.1699	[−0.270, 0.901]
Leaf length	LL	−0.361	0.3796	[−0.850, 0.461]
Leaf length-to-width ratio	LWR	−0.783	0.0216	[−0.959, −0.175]
Leaf width	LW	0.589	0.1245	[−0.198, 0.914]
Leaf perimeter	LP	−0.280	0.5018	[−0.822, 0.529]

Note: LWR, leaf length-to-width ratio. Correlations were two-tailed and were calculated using germplasm-level means together with the final 2021 DI ($n = 8$). Only LWR was significantly associated with DI at $p < 0.05$. No multiple-comparison letters are used.

3.3. Infection Process of the Powdery Mildew Fungus and Physiological Responses of Ningqi-1

As shown in Figure 4, under a microscope, no spores were observed on the leaf surface after powdery mildew infection. At 0 h and 2 h, spores began to invade the leaf epidermal cells. At 8 h, infection structures were produced in the plant epidermal cells and invaded the surrounding cells. At 12 h, secondary spores were produced and appeared in patches in the leaves. At 24 h, the infection structures began to be dense, and multiple fungal infection structures were observed. At 48 h, the mycelium further spread in the field of view, a large number of infection structures appeared, and secondary conidiophores appeared.

**Figure 4.** Trypan blue staining of Ningqi-1 leaves at 0, 2, 4, 8, 12, 24, 48, and 60 h after inoculation with the powdery mildew fungus. Panels (A–H) represent the corresponding sampling times.

Physiological parameters were measured in leaves at 0, 12, 24, and 48 h after inoculation. POD and SOD activities changed significantly over time, indicating a dynamic

antioxidant-enzyme response during infection. CAT activity also varied over time, reaching its lowest value at 24 h before increasing at 48 h. MDA content increased progressively and was approximately 2.5-fold higher at 48 h than at 0 h, suggesting enhanced membrane lipid peroxidation during infection (Figure 5A–D). Because ROS were not directly measured, the changes in antioxidant-enzyme activities should not be interpreted as direct evidence of changes in ROS levels.

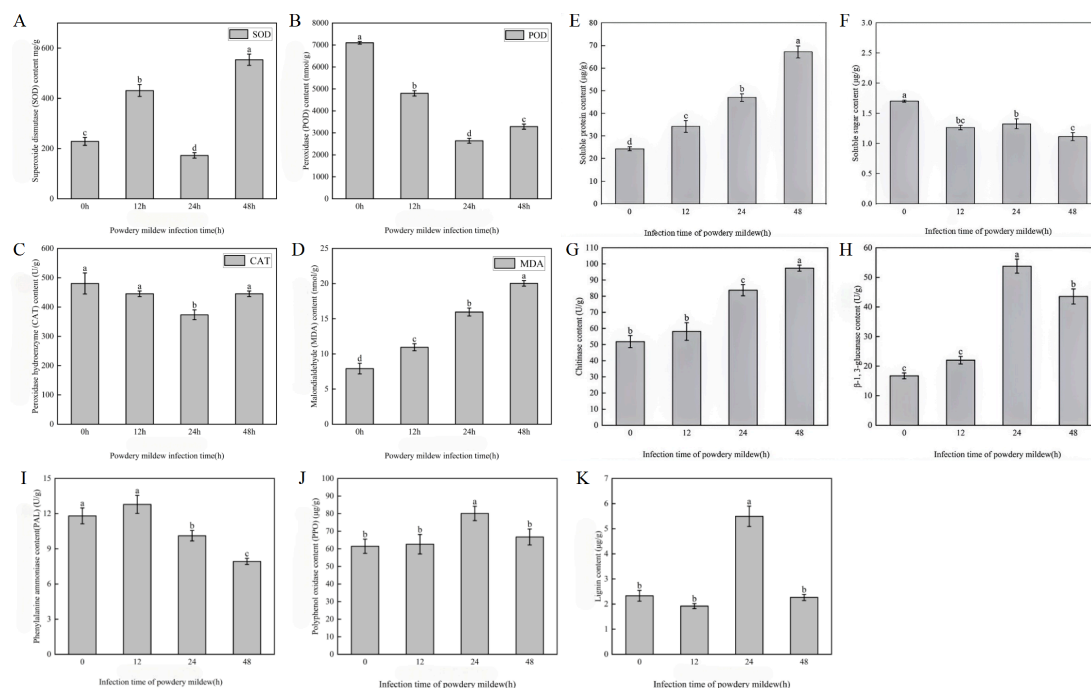


Figure 5. Physiological responses of Ningqi-1 leaves at 0, 12, 24, and 48 h after inoculation with the powdery mildew fungus. (A) Superoxide dismutase (SOD) content; (B) peroxidase (POD) content; (C) catalase (CAT) content; (D) malondialdehyde (MDA) content; (E) soluble protein content; (F) soluble sugar content; (G) chitinase content; (H) β -1,3-glucanase content; (I) phenylalanine ammonia-lyase (PAL) content; (J) polyphenol oxidase (PPO) content; and (K) lignin content. Values are presented as the mean $\pm$ SE of three biological replicates. Each biological replicate consisted of pooled leaf tissue from ten plants, and three technical measurements were averaged for each biological replicate. Error bars indicate SE. Different lowercase letters above the bars indicate significant differences among sampling times at $p < 0.05$.

Following inoculation, Figure 5E–H show that soluble protein content increased over time and reached its highest level at 48 h. Soluble sugar content generally decreased, with a slight increase at 24 h. Chitinase activity increased progressively, whereas β -1,3-glucanase activity peaked at 24 h. Compared with the 0 h levels, MDA content and chitinase activity were approximately 2.5- and 1.9-fold higher at 48 h, respectively, whereas β -1,3-glucanase activity and lignin content reached approximately 3.3- and 2.4-fold their initial levels at 24 h. These changes indicate time-dependent physiological responses in Ningqi-1 during the early stage of infection.

Phenylalanine ammonia-lyase (PAL) activity, polyphenol oxidase (PPO) activity, and lignin content initially increased and then decreased. PAL activity was slightly higher than the control at 12 h but lower at 24 and 48 h. PPO activity and lignin content reached their maximum values at 24 h (Figure 5I–K). These results represent only the physiological response of Ningqi-1 under the inoculation conditions used in this study.

4. Discussion

This study investigated the powdery mildew incidence of 26 goji germplasms from 2021 to 2023 according to the grading standards in the goji disease regulations. The powdery mildew resistance levels of these 26 germplasms were evaluated. Based on the two-year mean DI, six germplasm accessions were classified as extremely susceptible, three as highly susceptible, eight as moderately susceptible, and nine as moderately resistant; none met the criteria for immunity or high resistance. Annual resistance categories changed in 11 accessions, indicating that environmental conditions, survey timing, and epidemic duration may influence the final DI. Powdery mildew of goji can affect the quality of autumn fruit. Studies have shown that the nutritional quality of goji fruit harvested at the end of September is relatively better among the four harvest periods, with rich content of amino acids, proteins, and phenolic substances [32]. The evaluation of resistance to *Lycium barbarum* powdery mildew provides a useful reference for future research on this disease, the selection and breeding of resistant germplasm, and the broader utilization and promotion of *Lycium barbarum* germplasm resources.

The plant epidermis, as the first line of defense against external stimuli, plays a very significant role in the plant's disease resistance. After infection by pathogens, the leaf mesophyll cells, especially the palisade cells, of various types of goji leaves changed morphologically after being infected by powdery mildew. Some palisade cells broke apart to form new cells, and the palisade cells changed from plump, elongated cells to shrunken, short cells. Furthermore, through measurement and statistical analysis of goji leaves from eight different germplasms, the structural density of healthy leaves of the same germplasm was higher than that of infected leaves. Changes in leaf cell structure and morphology may be related to the disease resistance of goji.

Studies have shown that there is a significant correlation between plant resistance and its microstructure [33]. Studies have shown that there is no significant correlation between mango leaf resistance to malformation and leaf thickness [34]. In this study, measurements revealed no significant correlation between the thickness of the upper and lower epidermis, leaf thickness, and mesophyll thickness of *Lycium barbarum* leaves and powdery mildew resistance. The development of the palisade tissue in leaves reflects plant resistance to some extent. In plant cells, the more regular and compact the arrangement of epidermal cells and their outer walls, palisade tissue, and spongy tissue cells, and the smaller the gaps, the better they resist pathogen invasion [35,36]. Studies have shown a certain correlation between the thickness of the palisade tissue in strawberries and their powdery mildew resistance [37,38]. In the present study, comparisons between healthy and infected leaves showed that infection was generally accompanied by looser palisade cell arrangement, reduced tissue thickness, and decreased leaf compactness. The 2021 DI was negatively correlated with PTL, PTT, and LC, indicating that these traits were associated with a lower DI and, consequently, greater resistance. In contrast, the 2021 DI was positively correlated with STT, indicating that thicker spongy tissue was associated with a higher DI and lower resistance.

This study characterized the early physiological and biochemical responses of Ningqi-1 following inoculation with the powdery mildew fungus. The measured parameters included the antioxidant enzymes SOD, POD, and CAT; the membrane lipid peroxidation indicator MDA; the defense-related enzymes chitinase and β -1,3-glucanase; PAL and PPO associated with phenylpropanoid metabolism; lignin content; soluble protein content; and soluble sugar content. These parameters exhibited distinct temporal patterns rather than continuously increasing or decreasing during the first 48 h after inoculation. For example, SOD activity increased at 12 h, decreased at 24 h, and reached its highest level at 48 h; CAT activity decreased to its lowest level at 24 h and subsequently increased at 48 h;

and lignin-related indicators reached relatively high levels at 12 or 24 h, consistent with previous studies [39,40]. The increase in MDA content suggests enhanced membrane lipid peroxidation during infection, which may reflect oxidative stress associated with powdery mildew infection. Because ROS were not directly quantified in this study, changes in ROS levels cannot be inferred directly from the present data. Chitinase, β -1,3-glucanase, and soluble protein also showed time-dependent changes following inoculation. These results describe only the early physiological and biochemical responses of Ningqi-1 under the experimental conditions used in this study and should not be generalized as resistance mechanisms shared by all *Lycium barbarum* germplasm accessions.

5. Conclusions

This study characterized the field responses of 26 *Lycium barbarum* germplasm accessions to powdery mildew over two survey years. Among eight representative accessions, a greater number of palisade tissue layers, thicker palisade tissue, and higher leaf compactness were associated with a lower disease index (DI), whereas thicker spongy tissue was associated with a higher DI. Healthy leaves consistently exhibited greater tissue compactness than diseased leaves. These anatomical traits may be considered candidate traits associated with field DI, but their predictive value requires prospective validation. Following inoculation, Ningqi-1 exhibited time-dependent changes in multiple indicators related to redox regulation, pathogenesis-related enzymes, and phenylpropanoid metabolism during the 0–48 h period. The disease progress equations used in this study were descriptive fits. Collectively, these findings provide preliminary clues for screening resistant germplasm and identifying candidate anatomical and physiological indicators, while also laying a foundation for further investigation of the physiological and molecular mechanisms underlying powdery mildew resistance in *Lycium barbarum*.

Future studies should employ standardized field designs, larger germplasm populations, and independent datasets for validation before these indicators are applied to breeding selection or disease prediction.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16151657/s1>, Table S1. Logistic-equation parameters for *Lycium barbarum* germplasm accessions. Table S2. Slogistic3-equation parameters for *Lycium barbarum* germplasm accessions. Table S3. Slogistic2-equation parameters for *Lycium barbarum* germplasm accessions. Table S4. Slogistic1-equation parameters for *Lycium barbarum* germplasm accessions. Figure S1. Relationships between the final 2021 disease index and infected-leaf anatomical traits across eight *Lycium barbarum* germplasm accessions. (a) number of palisade tissue layers; (b) palisade tissue thickness; (c) spongy tissue thickness; and (d) leaf compactness. Points represent germplasm-level means for infected leaves. Leaf compactness was calculated as $PTT/LT \times 100$ using the values reported in Table 5.

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

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