Effects of Microbial Agent Application on Photosynthetic and Stress-Resistance Physiological Characteristics of *Dicranopteris dichotoma* under High Temperature Stress (Postprint)

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Abstract

To investigate the effects of Bacillus natto inoculant on the heat tolerance of Dicranopteris dichotoma, this study measured photosynthetic and high-temperature resistance physiological indicators of current-year D. dichotoma under different temperature and fertilizer treatments. The results showed that: (1) Both temperature and fertilizer could significantly affect the photosynthetic and high-temperature resistance physiology of D. dichotoma ($P<0.05$). (2) When temperature rose to 45°C, the net photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content of D. dichotoma leaves decreased significantly ($P<0.05$), and the inhibition of photosynthetic physiology in D. dichotoma by high-temperature stress belonged to non-stomatal limitation; to resist high-temperature stress, the activities of superoxide dismutase and peroxidase, as well as the contents of proline, malondialdehyde, and relative electrical conductivity of D. dichotoma increased significantly ($P<0.05$). (3) Compared with the control group and organic fertilizer group, the inoculant significantly increased the net photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, and the activities of superoxide dismutase and catalase of D. dichotoma leaves, while decreasing the intercellular CO2 concentration, malondialdehyde content, and relative electrical conductivity ($P<0.05$). (4) Using an evaluation method combining principal component analysis and membership function method to comprehensively evaluate the high-temperature resistance capacity of D. dichotoma, it was found that D. dichotoma had higher scores for high-temperature resistance capacity under organic fertilizer application with added inoculant. In summary, D. dichotoma possesses certain high-temperature resistance and can selectively induce stress-resistance physiological activities to adapt to high-temperature environments according to different stress conditions; Bacillus natto inoculant

alleviated the inhibition of photosynthesis in D. dichotoma by high-temperature stress, induced the enhancement of antioxidant enzyme activities to mitigate cellular damage, reduced the pressure of osmotic regulation, and effectively improved the resistance capacity of D. dichotoma to high-temperature stress. The findings of this study hold certain significance for ecological restoration and soil and water conservation in southern red soil erosion areas and similar regions.

Full Text

Effects of Bacterial Agent Application on Photosynthetic and Stress-Resistance Physiological Characteristics of *Dicranopteris pedata* Under High Temperature Stress

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Abstract: To investigate the influence of *Bacillus natto* agent on the heat tolerance capacity of *Dicranopteris pedata*, this study measured photosynthetic and high-temperature resistance physiological indicators of annual *D. pedata* under different temperature and fertilizer treatments. The results showed that: (1) Both temperature and fertilizer significantly affected the photosynthesis and high-temperature resistance physiology of *D. pedata* ($P < 0.05$). (2) When temperature increased to 45°C, the net photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content of *D. pedata* leaves decreased significantly ($P < 0.05$), with the inhibition of photosynthetic physiology by high-temperature stress being non-stomatal limitation. To resist high-temperature stress, the superoxide dismutase and peroxidase activities, as well as proline and malondialdehyde content and relative electrical conductivity, increased significantly ($P < 0.05$). (3) Compared with the control and organic fertilizer groups, the bacterial agent significantly increased the net photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, and activities of peroxide dismutase and catalase, while decreasing intercellular CO_2 concentration, malondialdehyde content, and relative electrical conductivity ($P < 0.05$). (4) Using a combined evaluation method of principal component analysis and membership function, the high-temperature resistance capacity of *D. pedata* was comprehensively evaluated, revealing higher scores under organic fertilizer application with bacterial agent addition. In conclusion, *D. pedata* possesses certain high-temperature resistance and can selectively induce stress-resistance physiological activities to adapt to high-temperature environments. *Bacillus natto* agent alleviates the inhibition of photosynthesis by high-temperature stress, induces increased antioxidant enzyme activity to mitigate cellular damage, reduces osmotic regulation pressure, and effectively enhances *D. pedata*' s resistance to

high-temperature stress. These findings hold significance for ecological restoration and soil-water conservation in southern red soil erosion areas and similar regions.

Keywords: *Dicranopteris pedata*; high temperature stress; *Bacillus natto*; photosynthetic characteristics; stress resistance physiology

Introduction

Plant growth, development, and dispersal are highly sensitive to temperature variations (Wahid et al., 2007). Under global warming, high temperature acts as an abiotic stress factor that damages plant photosynthetic systems and reduces photosynthetic efficiency (Hayat et al., 2009), while disrupting the balance of reactive oxygen species (ROS) that destroy plant cell membranes, attack biological macromolecules, and hinder normal cellular functions (Janicka-Russak et al., 2012), ultimately affecting plant growth and even causing death. In response to high-temperature stress, plants activate defense mechanisms such as inducing antioxidant enzyme activity to eliminate excess ROS and accumulating osmotic regulation substances to balance intracellular and extracellular water potential, thereby mitigating damage and protecting cells (Zhang et al., 2023).

Beyond plants' intrinsic defense mechanisms, microorganisms can enhance plant thermotolerance in response to high-temperature stress (Zhang et al., 2020). Beneficial bacteria and fungi particularly can improve plant physiological performance under stress, strengthen stress resistance, and help plants overcome abiotic stress damage (Levy et al., 1983). Consequently, bacterial agent application has become an effective measure to induce plant stress-resistance physiological expression and enhance plant capacity to respond to high-temperature stress (Shen et al., 2016). Research has identified *Bacillus natto* as a probiotic with strong thermostability and stability that enhances immunity and regulates intestinal flora by producing antimicrobial substances, vitamins, and antioxidants (Yin and Xu, 2011). Moreover, *Bacillus natto* agent offers advantages of low preparation cost, high benefit, and environmental friendliness, and can assist organic and chemical fertilizers to achieve better application effects (Liu et al., 2022), aligning with sustainable development in modern ecological environments.

Dicranopteris pedata, a perennial fern widely distributed in southern China, serves as an important pioneer plant for soil erosion control and ecological restoration in southern red soil erosion areas (Liang et al., 2021). Due to sparse understory vegetation or even lack of canopy cover in these erosion areas (Yuan et al., 2020), surface temperatures rise substantially during summer, exposing *D. pedata* to high-temperature stress that limits its growth and spread. However, how *D. pedata*' s photosynthetic and high-temperature resistance physiology respond to such stress, and whether *Bacillus natto* can enhance its thermotolerance, remain unknown scientific questions with scarce relevant research, rep-

resenting a gap in soil-water conservation research in southern red soil erosion areas. Therefore, under global warming, understanding *D. pedata*' s response to high-temperature stress and promoting its stress resistance may become crucial for soil-water conservation and ecological restoration in these regions.

To investigate *D. pedata*' s response to high-temperature stress, this study hypothesized that *Bacillus natto* agent could induce physiological and biochemical changes in *D. pedata* to resist high-temperature stress. We examined *D. pedata*' s high-temperature resistance capacity from perspectives of photosynthetic physiology and stress resistance physiology, aiming to provide theoretical basis and scientific support for *D. pedata*' s adaptive growth under high-temperature environments and ecological management of soil erosion areas in southern red soil regions and similar areas.

Materials and Methods

1.1 Experimental Materials The experimental material consisted of one-year-old *D. pedata* transplanted from Gushan, Fuzhou City, Fujian Province (119°22 -119°25 E, 25°20 -26°05 N). The tested soil was mountainous red soil developed from granite in the Gushan Scenic Area. The bacterial agent was *Bacillus natto* prepared by the Fujian Institute of Microbiology using the following method: *Bacillus natto* strains were inoculated into Luria-Bertani broth (LB: 0.5% yeast extract, 1% peptone, 0.5% NaCl, pH 7.2–7.4) and potato dextrose agar (PDA: 20% potato, 2% glucose, 2% agar), then cultured in a constant-temperature shaking incubator at 37°C and 150 $r \cdot min^{-1}$. After 24 h, the cultured seed liquid was mixed into solid fermentation medium (1 kg wheat bran, 8 kg soybean meal, 6.5 L water) at 1% inoculation volume and cultured at 37°C for 48 h. Finally, the solid fermentation product was dried at 50°C.

1.2 Experimental Design Uniformly growing annual *D. pedata* were selected from Gushan, Fuzhou, and transplanted into artificial climate incubators at the Fujian Institute of Microbiology. After two months of cultivation, three treatment groups were established: (1) no fertilizer (F1), (2) organic fertilizer application (F2, organic fertilizer/soil = 1/4, V/V), and (3) *Bacillus natto* agent + organic fertilizer (F3, agent/fertilizer/soil = 1/1/4, V/V/V). During the pre-cultivation period, the incubator simulated a 16 h day at 25°C and 8 h night at 20°C, with light intensity of 1000 $mol \cdot m^{-2} \cdot s^{-1}$.

One month after fertilization, short-term high-temperature stress experiments were conducted. The diurnal pattern remained unchanged while temperature regimes were set at 25°C/20°C, 35°C/30°C, and 45°C/40°C for 48 h. The 25°C/20°C treatment served as the control, while 35°C/30°C and 45°C/40°C represented high-temperature stress groups. To prevent drought, all treated materials were watered daily at 7:00 AM to maintain soil water content at 60%–70%. Photosynthetic parameters were measured between 9:00–11:00 the day

after short-term high-temperature stress treatment. Each treatment included three replicated *D. pedata* plants, with two mature leaves per plant measured (six total measurements per treatment). Leaves used for photosynthesis measurement were then cut and stored at low temperature for subsequent indoor physiological indicator measurement, with three replicates per treatment.

1.3 Detection Methods A portable photosynthesis system (Li-6800, USA) was used to measure photosynthetic parameters of *D. pedata* leaves under constant indoor light source: net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci). Measurement conditions were set as follows: chamber flow rate 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, pressure valve 0.1 kPa, light intensity 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, CO_2 concentration 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, leaf temperature 25°C, and chamber humidity 55%–60%. Chlorophyll (Chl) content was determined using the acetone method. Stress physiological indicators were measured as follows: superoxide dismutase (SOD) activity by nitroblue tetrazolium method, peroxidase (POD) activity by guaiacol method, catalase (CAT) activity by UV spectrophotometry, proline (Pro) content by ninhydrin method, malondialdehyde (MDA) content by thiobarbituric acid method, and relative electrical conductivity (REC) by conductivity meter.

1.4 Data Processing All measured physiological indicator data were entered into Microsoft Excel 2019 for organization and calculation. SPSS 22 software was used for two-way ANOVA and one-way ANOVA to examine the effects of fertilization treatment and temperature changes on *D. pedata* photosynthetic and high-temperature resistance physiology. For one-way ANOVA, the S-N-K method was used for post-hoc multiple comparisons when homogeneity of variance was satisfied, while Dunnett' s C test was used when homogeneity of variance was not met.

A combined method of principal component analysis and fuzzy mathematics membership function (Zhu et al., 2022) was used to evaluate the high-temperature tolerance of *D. pedata* under different stress treatments. SPSS 22 software was used for Pearson correlation analysis, Kaiser-Meyer-Olkin test, and Bartlett' s sphericity test to verify the feasibility of principal component analysis. Principal component analysis was then performed using SPSS 22 to obtain eigenvalues, contribution rates, and principal component loadings, from which principal component scores (PCS) were calculated. Membership function values of PCS were calculated, and combined with principal component contribution rate ratios to obtain comprehensive scores for *D. pedata* high-temperature resistance under three temperature stresses. The calculation formulas were:

$$UPCS_j = \frac{x_j - x_{min}}{x_{max} - x_{min}} \quad W_j = \frac{P_j}{\sum_{j=1}^{n} P_j} \quad j = 1, 2, 3, \ldots, n$$

$$D = \sum_{j=1}^{n} UPCS_j \times R_j \quad j = 1, 2, 3, \ldots, n$$

where $UPCS_j$ is the membership function value of the jth PCS; x_j is the jth PCS; x_{min} is the minimum value in the jth principal component; x_{max} is the maximum value in the jth principal component; W_j is the contribution rate ratio of the jth PCS; P_j is the contribution rate of the jth principal component; and D is the comprehensive score of *D. pedata* high-temperature tolerance, with higher D values indicating stronger high-temperature tolerance.

Origin Pro 2022 software was used for figure preparation.

Results

2.1 Photosynthetic Characteristics of *D. pedata* Leaves Two-way ANOVA results (Table 1) showed that different temperatures and fertilizers significantly affected all photosynthetic characteristics of *D. pedata* ($P < 0.05$). Additionally, the interaction between temperature and fertilizer significantly influenced all photosynthetic indicators ($P < 0.05$).

As temperature increased (Figure 1 [Figure 1: see original paper]), Pn in the F2 group first increased then decreased significantly ($P < 0.05$). When temperature rose from 25°C to 35°C, Tr, Gs, and Ci in the F2 group remained essentially unchanged, but at 45°C, Tr and Gs decreased significantly while Ci increased significantly ($P < 0.05$). In the F3 group, Pn and Gs showed similar trends to F2, while Tr first increased then decreased and Ci first decreased then increased, with significant differences ($P < 0.05$). Among the three fertilization treatments, when temperature reached 45°C, all gas exchange parameters except Ci in the F3 group were significantly higher than in F2 and F1 groups ($P < 0.05$). Chlorophyll content in all three fertilization treatments decreased significantly with rising temperature ($P < 0.05$). Compared with the control F1 group, chlorophyll content in F2 and F3 groups was significantly higher at 35°C and 45°C, with F3 group showing significantly higher chlorophyll than F2 at 45°C ($P < 0.05$).

2.2 Cell Membrane Damage in *D. pedata* Two-way ANOVA results (Table 2) demonstrated that different temperatures and fertilizers significantly affected both REC and MDA in *D. pedata* ($P < 0.05$). However, the interaction between temperature and fertilizer showed no significant effect on cell membranes.

As temperature increased (Figure 2 [Figure 2: see original paper]), REC and MDA in all three fertilization treatments increased significantly ($P < 0.05$). Compared with the F1 group, F2 showed no significant differences in REC and

MDA, while F3 exhibited significantly lower REC and MDA than F1 under 45°C high-temperature stress ($P < 0.05$).

2.3 Antioxidant and Osmotic Regulation in *D. pedata* Two-way ANOVA results (Table 3) revealed that different temperatures and fertilizers significantly affected both antioxidant enzyme activities and osmotic regulation substances ($P < 0.05$). Except for POD, the interaction between temperature and fertilizer significantly influenced SOD, CAT, and Pro ($P < 0.05$).

With increasing temperature (Figure 3 [Figure 3: see original paper]), SOD and POD activities in all fertilization treatments increased significantly. CAT in the F3 group continued to increase significantly with temperature, while F2 showed a trend of first increasing then decreasing significantly ($P < 0.05$). Among different fertilization treatments, CAT activity in F3 at 35°C and SOD and CAT activities in F3 at 45°C were significantly higher than in F1 and F2 groups ($P < 0.05$). Pro content in all three treatments increased significantly with temperature ($P < 0.05$). Inter-treatment comparisons showed that Pro content in F2 and F3 was significantly higher than F1 at 25°C, significantly lower in F3 than F1 and F2 at 35°C ($P < 0.05$), but showed no significant differences among the three groups at 45°C ($P > 0.05$).

2.4 Evaluation of High-Temperature Tolerance Pearson correlation analysis (Figure 4 [Figure 4: see original paper]A) showed that all indicators were significantly correlated ($P > 0.05$) except for CAT, which was only significantly positively correlated with POD. The KMO test result was 0.86, and Bartlett' s sphericity test yielded $P < 0.005$, confirming the suitability of the data for principal component analysis.

Principal component analysis (Table 4 ; Figure 4B and 4C) identified two principal components with eigenvalues greater than 1. The first principal component had an eigenvalue of 8.71, contributing 79.18% of variance, while the second principal component had an eigenvalue of 1.43, contributing 13.00%, with a cumulative contribution rate of 92.18% that reflected most information regarding *D. pedata*' s high-temperature tolerance under different stresses. According to Figure 4B and the loading matrix, the first principal component included main information from ten indicators: Tr, Pn, Gs, Ci, SOD, POD, Pro, MDA, REC, and Chl, while the second principal component included only CAT information. Using x_1-x_{11} to represent Tr, Pn, Gs, Ci, SOD, POD, CAT, Pro, MDA, REC, and Chl, and y_1 and y_2 to represent first and second principal component scores, formulas (4) and (5) were obtained from the loading matrix and eigenvalues. Based on y_1 and y_2 values, membership function values U_j were calculated, and using principal component contribution ratio W_j as weight, comprehensive scores for *D. pedata*' s high-temperature tolerance under three temperature stresses were obtained (Table 5). Evaluation results showed that *D. pedata* under F3 treatment exhibited superior high-temperature tolerance compared to F2 treatment.

Discussion

3.1 High-Temperature Stress Inhibits *D. pedata* Photosynthesis Plant photosynthesis is a complex biochemical process extremely sensitive to external temperature changes (Zhang et al., 2011), making photosynthetic characteristics effective indicators of high-temperature damage. Pn, Tr, Gs, and Ci represent four fundamental photosynthetic parameters, while Chl content also reflects photosynthetic capacity. In this study, as temperature increased, Pn, Tr, and Gs in the F1 group decreased significantly at 45°C, indicating that *D. pedata* can resist moderate high-temperature stress but experiences reduced photosynthetic rates under severe stress. These findings align with previous research (Du et al., 2012; Feng et al., 2014). Pn, Tr, Gs, and Ci are closely related, as high-temperature stress reduces plant Gs, consequently decreasing Pn and Tr (An et al., 2010). This suggests that *D. pedata* responds to high-temperature stress by appropriately closing leaf stomata. However, at 45°C stress, *D. pedata* showed significantly increased Ci, likely because reduced photosynthetic rate resulted in less CO_2 assimilation than that from external sources or respiration. This indicates that high-temperature stress inhibition of *D. pedata* photosynthesis is non-stomatal limitation (Drake et al., 2017). Correlation analysis revealed extremely significant negative correlations between Ci and photosynthetic indicators including Pn, Tr, and Gs, further confirming non-stomatal limitation of photosynthesis in *D. pedata* under high-temperature stress. High-temperature stress damages photosynthetic reaction sites, electron transport chains, and PSII oxygen-evolving complexes in *D. pedata* leaves, reducing mesophyll cell photosynthetic activity, inhibiting leaf photosynthetic capacity, decreasing CO_2 entry into mesophyll cells, increasing intercellular CO_2 accumulation, and consequently raising Ci.

3.2 High-Temperature Tolerance Capacity of *D. pedata* REC and MDA are commonly used as indicator indices for evaluating plant cell membrane damage (Zhao et al., 2015; Zhang et al., 2022). In this study, REC and MDA in F1 group *D. pedata* leaves were significantly higher than the control only when stress temperature reached 45°C, indicating that *D. pedata* cell membranes can maintain integrity and normal physiological functions to a certain degree. However, under severe stress, cell membranes remain threatened by ROS oxidation and sustain damage. Numerous studies have demonstrated that plants can rapidly regulate antioxidant enzymes such as SOD, POD, and CAT, along with osmotic regulation substances like Pro, to resist stress threats (Ul Hassan et al., 2021). At 35°C stress, POD, CAT, and Pro played key roles in resisting high-temperature stress, enabling *D. pedata* to maintain relatively low REC and MDA with minimal damage. At 45°C, SOD and POD activities and Pro concentration jointly resisted high-temperature stress, but CAT activity decreased significantly while REC and MDA increased substantially. This indicates that despite enhanced antioxidant enzyme and osmotic regulation activ-

ity, *D. pedata* still suffered considerable damage under severe high-temperature stress. Additionally, different response measures were observed under varying stress intensities, demonstrating that *D. pedata* can selectively enhance antioxidant enzyme activity according to different stress environments to adapt to high temperatures—an intrinsic plant stress response (Liu and Fang, 2020).

3.3 Bacterial Agent Application Promotes *D. pedata* Photosynthetic Capacity Under High-Temperature Stress Photosynthesis is crucial for plant biomass accumulation and photosynthate translocation (Xue et al., 2010) and represents an important metabolic process in plants. Enhanced photosynthetic physiology can improve plant stress resistance (Chen et al., 2013). Microbial agents contain abundant beneficial live bacteria that can improve soil while enhancing plant physiological performance (Wang, 2019). Compared with F1 and F2 treatments, *D. pedata* under F3 treatment at 45°C stress showed higher Pn, Tr, Gs, and Chl. Combined with two-way ANOVA results, this indicates that organic fertilizer combined with *Bacillus natto* agent enhanced *D. pedata*' s photosynthetic capacity under high-temperature stress, thereby strengthening its high-temperature resistance. The severity of high-temperature stress damage to plant photosynthetic physiology depends on the impact on enzyme activities such as Rubisco and functional integrity of photosynthetic organs like PSII in thylakoid membranes (Ye et al., 2023). Based on the non-stomatal limitation of high-temperature stress inhibition on *D. pedata* photosynthetic physiology, our results demonstrate that organic fertilizer with added *Bacillus natto* agent effectively alleviated damage to photosynthesis-related enzyme activities and leaf photosynthetic organs, protecting *D. pedata* leaf photosynthetic activity. Correlation analysis revealed extremely significant positive correlations among Pn, Tr, Gs, and Chl, indicating close relationships between Chl synthesis and *D. pedata* photosynthetic capacity, and further suggesting that bacterial agent application enhances *D. pedata* photosynthesis by promoting Chl synthesis.

Organic fertilizer with *Bacillus natto* showed significantly better promotion of *D. pedata* photosynthetic physiology than organic fertilizer alone. Comparison between F1 and F2 groups under 45°C stress revealed that organic fertilizer could enhance *D. pedata* photosynthetic capacity but remained significantly lower than F3 treatment. This may be because active substances secreted by *Bacillus natto* and nutrients in organic fertilizer jointly promoted accumulation of chlorophyll synthesis precursors, significantly increasing Chl and thereby enhancing *D. pedata*' s light energy conversion and utilization capacity. Similar findings were reported by Chen et al. (2013), who found that maize inoculated with AM fungi showed stronger photosynthetic capacity, and by Wang et al. (2021), who reported that adding microbial agents to organic substrates regulated leaf stomata and increased Pn and Chl. Additionally, microorganisms produce relevant hormones and signaling molecules that assist *D. pedata* in inducing stomatal opening/closing, photosynthetic electron transport, and other metabolic pathways, thereby increasing Pn (Zhang et al., 2019; Wang et al., 2021)—another important reason why F3 treatment with *Bacillus natto* showed stronger photo-

synthetic capacity than F2 treatment with organic fertilizer alone.

3.4 Bacterial Agent Application Induces Stress-Resistance Physiology in *D. pedata* Under High-Temperature Stress Numerous studies have shown that microbial fertilizers can improve soil structure and microbial environment, possess potassium-solubilizing, phosphorus-releasing, and nitrogen-fixing functions, and significantly promote crop growth and enhance plant stress resistance (Fan et al., 2021). Correlation analysis revealed extremely significant positive correlations between SOD and POD, and between CAT and POD, under 45°C stress, strongly demonstrating the key role of SOD-POD synergy and POD-CAT synergy in resisting ROS oxidation of cell membranes. Specifically, SOD and CAT in F3 group under 45°C stress were generally higher than in F1, and particularly when CAT activity decreased in F1 and F2 groups, F3 group showed significantly increased CAT activity. This indicates that under high-temperature stress, *Bacillus natto* agent induces higher enzyme activity in *D. pedata* to eliminate harmful substances produced under stress, strengthening SOD-POD and POD-CAT synergistic effects. Although Pro, REC, and MDA in *D. pedata* all increased with high-temperature stress intensity, the findings that Pro in F3 treatment was significantly lower than F1 and F2 at 35°C, and MDA and REC in F3 were significantly lower than F1 at 45°C, further demonstrate that *Bacillus natto* agent can effectively improve *D. pedata* thermotolerance and alleviate high-temperature stress damage (Yang et al., 2013). High-temperature stress tends to increase plant transpiration, causing cells to lose water easily and resulting in water stress. As an osmotic regulator, Pro should play a key role, but experimental results showed that bacterial agent application did not significantly induce Pro synthesis to resist water stress. However, reports indicate that natto fermentation produces γ-polyglutamic acid with good water retention and biological solubility (Zhang, 2014), which can induce accumulation of hydrophilic osmotic regulation substances (Zhu et al., 2010), thereby reducing cellular osmotic potential and enhancing *D. pedata* resistance.

3.5 Comprehensive Evaluation of Bacterial Agent-Induced High-Temperature Stress Tolerance in *D. pedata* Principal component analysis revealed that the first principal component contained 79.2% of information on *D. pedata* photosynthetic and stress-resistance physiology. POD, REC, SOD, Pro, MDA, and Ci showed positive correlations with the first principal component, while Pn, Gs, Tr, and Chl showed negative correlations. Since POD, SOD, and Pro are important plant stress-resistance biochemical substances, this further demonstrates that POD and SOD activity induction and Pro synthesis accumulation are important defense mechanisms for *D. pedata* in response to high-temperature stress, consistent with previous conclusions. Indicator indices of cell membrane damage, REC and MDA, also provided strong evidence of damage to *D. pedata* cells under high-temperature stress. From different fertilization treatment perspectives, the unfertilized F1 group and organic fertilizer-only F2 group overlapped considerably, indicating

that organic fertilizer alone cannot enhance *D. pedata*' s high-temperature stress resistance. However, the F3 treatment with added *Bacillus natto* showed distinct differences from F1 and F2 groups. Figure 4C further revealed that different temperature treatments caused obvious changes in *D. pedata* photosynthetic and high-temperature resistance physiology, with the F3 treatment group in the first quadrant and F1 and F2 groups in the fourth quadrant at 45°C. These results strongly demonstrate that organic fertilizer with added *Bacillus natto* effectively enhanced *D. pedata* photosynthetic capacity and high-temperature tolerance.

However, evaluating high-temperature tolerance using single physiological indicators has limitations, as high-temperature stress affects multiple aspects of plants and plant regulation of various physiological activities to resist stress is extremely complex. Currently, combining principal component analysis with membership function method is a popular approach for comprehensive plant stress-resistance evaluation (Zhu et al., 2022). Therefore, to evaluate the effect of *Bacillus natto* agent on *D. pedata* high-temperature stress resistance, this study employed this method to comprehensively evaluate high-temperature tolerance under F2 and F3 treatments. Evaluation results showed that *D. pedata* under F3 treatment exhibited stronger high-temperature tolerance than F2 treatment across all three temperature regimes, indicating that *Bacillus natto* agent effectively improved *D. pedata* high-temperature tolerance. Furthermore, two-way ANOVA revealed significant interactive effects of temperature changes and different fertilizer applications on *D. pedata* photosynthetic and stress-resistance physiology. However, this interaction showed no significant effect on REC and MDA, because high temperature inhibited *D. pedata* photosynthetic characteristics, forcing stress-resistance physiological regulation, while *Bacillus natto* agent enhanced *D. pedata* photosynthesis and assisted stress-resistance physiological regulation to improve high-temperature tolerance, further demonstrating that *D. pedata* effectively alleviated high-temperature stress damage under *Bacillus natto* agent action.

Conclusion

This study investigated *D. pedata*' s response to high-temperature stress and the capacity of *Bacillus natto* agent combined with organic fertilizer to induce high-temperature stress resistance. The findings indicate that *D. pedata* possesses certain high-temperature tolerance, but suffers severe damage under excessive temperature stress, leading to non-stomatal limited photosynthetic rate decline and cell membrane oxidation. Under such conditions, *D. pedata* can regulate its antioxidant enzyme activities and osmotic regulation substances according to different stress situations to resist high-temperature stress. Moreover, compared with organic fertilizer alone, *Bacillus natto* agent combined with organic fertilizer can alleviate high-temperature stress inhibition of *D. pedata* photosynthesis, induce increased antioxidant enzyme activity to mitigate cellular damage,

reduce osmotic regulation pressure, and effectively enhance *D. pedata*' s resistance to high-temperature stress.

References

AN Jiajia, LI Xinguo, LI Shaopeng, et al., 2010. Effects of short-term high temperature on photosynthesis and related physiological indices of Brazil banana plantlets [J]. Chinese Agricultural Science Bulletin, 26(20): 362-365.

CHEN Xiaoying, SONG Fengbin, ZHU Xiancan, et al., 2013. Effect of arbuscular Mycorrhizal fungi on photosynthetic characteristics in maize plants under high temperature stress [J]. Acta Agriculturae Boreali-Sinica, 28(2): 108-113.

DU Yaodong, LI Jianling, WANG Hua, et al., 2012. Effects of high temperature stress on the flag leaf photosynthesis and Chlorophyll fluorescence parameters of rice [J]. Chinese Journal of Ecology, 31(10): 2541-2548.

DRAKE JE, POWER SA, DUURSMA RA, et al., 2017. Stomatal and non-stomatal limitations of photosynthesis for four tree species under drought: A comparison of model formulations [J]. Agricultural and Forest Meteorology, 247: 454-466.

FAN Na, PENG Zhidong, BAI Wenbin, et al., 2021. Influences of microbial agents on soil enzyme activity and sorghum growth [J]. Journal of Agricultural Science and Technology, 23(2): 185-192.

FENG B, LIU P, LI G, et al., 2014. Effect of heat stress on the photosynthetic characteristics in flag leaves at the grain-filling stage of different heat-resistant winter wheat varieties [J]. Journal of Agronomy and Crop Science, 200(2): 143-155.

HAYAT S, MASOOD A, YUSUF M, et al., 2009. Growth of Indian mustard (*Brassica juncea* L.) in response to salicylic acid under high-temperature stress [J]. Brazilian Journal of Plant Physiology, 21(3): 187-195.

JANICKA-RUSSAK M, KABALA K, WDOWIKOWSKA A, et al., 2012. Response of plasma membrane H+-ATPase to low temperature in cucumber roots [J]. Journal of Plant Research, 125(2): 165-170.

LEVY Y, DODD J, KRIKUN J, 1983. Effect of irrigation, water salinity and rootstock on the vertical distribution of vesicular-arbuscular mycorrhiza in citrus roots [J]. New Phytologist, 95(3): 397-403.

LIANG Meixia, CHEN Zhibiao, CHEN Zhiqiang, et al., 2021. Ecological restoration effects of soil and *Dicranopteris pedata* under different activities in Benggang [J]. Research of Soil and Water Conservation, 28(6): 138-143.

LIU Min, FANG Yulin, 2020. Effects of heat stress on physiological indexes and ultrastructure of grapevines [J]. Scientia Agricultura Sinica, 53(7): 1444-1458.

LIU Y, LI C, ZHAO B, et al., 2022. Inoculation of prickly pear litter with microbial agents promotes the efficiency in aerobic composting [J]. Sustainability, 14(8): 4084824.

SHEN Jie, CAI Yan, ZHANG Yuyu, et al., 2016. Effect of effective microorganisms fertilizer on growth and enzyme activities of tobacco seedlings [J]. Journal of Northwest A&F University (Natural Science Edition), 44(4): 48-56.

Ul HASSAN M, RASOOL T, IQBAL C, et al., 2022. Linking plants functioning to adaptive responses under heat stress conditions: a mechanistic review [J]. Journal of Plant Growth Regulation, 41(7): 2596-2613.

WAHID A, GELANI S, ASHRAF M, et al., 2007. Heat tolerance in plants: an overview [J]. Environmental and Experimental Botany, 61(3): 199-223.

WANG Guipeng, MA Lele, FAN Binghua, et al., 2021. Effects of microbial agents of nutrient transformation, yield and quality of tomato with organic nutrition cultivation [J]. Journal of Northwest A&F University (Natural Science Edition), 49(9): 118-128.

WANG Zhichao, 2019. Effect of adding microbial fertilizer and magnesium fertilizer on yield and quality of new rice variety Yangengruanyu 1 [D]. Shenyang Agricultural University, 35-37.

XUE Yixia, LI Yaling, WEN Xiangzhen, 2010. Effects of air humidity on the photosynthesis and fruit-set of tomato under high temperature [J]. Acta Horticulturae Sinica, 37(3): 397-404.

YANG Qian, CHAI Wenjuan, ZHANG Chunlin, et al., 2013. Effects of a microbial fertilizer on Sorghum bicolor L. seed germination, seeding growth and stress tolerance [J]. Journal of Inner Mongolia Agricultural University (Natural Science Edition), 34(6): 102-109.

YIN Cong, XU Xiao, 2011. Research progress of natto microecologics [J]. Feed and Animal Husbandry, (10): 38-42.

YUAN Zaijian, MA Dongfang, NIE Xiaodong, et al., 2020. Progress in research on prevention and control of soil erosion under forest in Red Soil Hilly Region of South China [J]. Acta Pedologica Sinica, 57(1): 12-21.

YE Xiaoqing, ZENG Jianan, ZOU Yong, et al., 2023. Research progress on the physiological mechanism and related regulation of temperature stress on Flue-cured Tobacco [J]. Guangdong Agricultural Sciences, 50(8): 105-117.

ZHANG Chunnan, ZHANG Ruifang, WANG Hong, et al., 2020. Effects of arbuscular mycorrhizal fungi on abiotic stress tolerance in crops: a review [J]. Microbiology China, 47(11): 3880-3891.

ZHANG He, AO Pingxing, ZHAO Yan, 2022. Effect of 5-aminolevulinic acid on leaf physiology of three cultivars of medicago under high temperature stress [J]. Acta Agrestia Sinica, 30(5): 1178-1184.

ZHANG Shuntang, ZHANG Guilian, CHEN Liyun, et al., 2011. Effects of high temperature stress on net photosynthetic rate and Chlorophyll fluorescence parameters of flag leaf in rice [J]. Chinese Journal of Rice Science, 25(3): 335-338.

ZHANG Ting, LI Ping, WEI Jiali, 2023. Physiological responses of four Hedera plants to high temperature tolerance [J/OL]. Molecular Plant Breeding, 1-12[2023-12-25]. http://kns.cnki.net/kcms/detail/46.1068.S.20231027.0926.002.html.

ZHANG Wen, 2014. The preparation and agricultural application experiment of γ-Poly-Glutamic Acid [D]. Chinese Academy of Agricultural Sciences: 63-64.

ZHANG Zhongxue, FENG Zijia, QI Zhijuan, et al., 2019. Effects of compound microbial organic fertilizer with water-saving irrigation on photosynthetic and yield of rice [J]. Transactions of the Chinese Society for Agricultural Machinery, 50(7): 313-321.

ZHAO Yan, CHE Weiguang, BI Yufen, et al., 2015. Thermotolerance comprehensive evaluation of Medicago sativa L. 'Deqin' on seeding stage [J]. Northern Horticulture, (20): 69-73.

ZHU Anting, JIANG Youwu, XIE Guosheng, et al., 2010. Effects of exogenous poly γ-glutamic acid on drought tolerance and osmotic adjustment of rice seedlings [J]. Journal of Nuclear Agricultural Sciences, 24(6): 1269-1273.

ZHU Yadi, WANG Huiqin, WANG Hongzhang, et al., 2022. Evaluation and identification index of heat tolerance in different summer maize varieties at V12 stage [J]. Acta Agronomica Sinica, 48(12), 3130-3143.

Note: Figure translations are in progress. See original paper for figures.