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Physiological Responses of Four Hedera Plants to High Temperature Tolerance

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Abstract Ivy is an important horticulture plant for three-dimensional urban greening, and heat stress is one of the important environmental factors limiting the normal growth of ivy. Studying the physiological response of ivy to high temperature stress will be of great significance for screening suitable varieties of ivy and increasing the diversity of urban three-dimensional greening landscape. Two-year cutting seedlings of four ivy varieties were cultured in an artificial climate incubator (20 ℃, 16 h light / 8 h dark) for two weeks, and then treated at 40 ℃ for 7 days. The morphological changes and physiological indexes, such as heat injury index, chlorophyll content, chlorophyll fluorescence parameters, MAD, proline content, CAT activity and SOD activity, were recorded at 0, 1, 3, 5 and 7 days. The heat resistance and heat resistance mechanism of the test materials were comprehensively evaluated through the membership function method. The results showed that the heat resistance of the four ivy species was ‘Sark’>‘Ingelise’>‘Wonder’>‘Golden Ivalace’. The chlorophyll content, maximum photochemical quantum yield (F_v/F_m) and apparent quantum transfer efficiency (ETR) of the four ivy species decreased with the increase of heat stress duration. While the content of MDA continued to increase, and the increase of MDA in heat tolerant varieties was smaller than that in heat sensitive varieties. However, the proline content, CAT activity and SOD activity increased first and then decreased. The peak value of proline content in heat tolerant varieties was later than that in heat sensitive varieties, and the SOD and CAT activities in heat tolerant varieties were significantly higher than those in heat sensitive varieties ($P<0.05$).

Keywords Hedera; Heat stress; Physiological indicators; Heat resistance; Membership function

1 Introduction

Ivy is a perennial evergreen vine belonging to the Araliaceae family and the Hedera genus. It can grow by climbing, creeping, and hanging due to its well-developed aerial roots and good spreading ability of branches. It is an excellent material for urban three-dimensional greening. At present, there are over 500 species and varieties of ivy in the world, most of which are distributed in Europe. Among them, the British ivy (*Hedera helix* L.) is the largest population in the ivy genus. Ivy belongs to the shade loving plant, and its most suitable growth temperature is 18 ℃~20 ℃. It is cold resistant (Metcalfe, 2005) and afraid of extreme heat. It usually grows extremely slowly or stops growing above 30 ℃, and is prone to pest infestations and even wilting at 35 ℃ and above.

In the hot summer, high temperature becomes one of the important environmental factors affecting the normal growth of ivy. There are many types of ivy with diverse leaf colors and shapes, but there are less than 10 commonly used varieties in urban greening in China. Therefore, screening heat-resistant ivy varieties and exploring the heat resistance mechanism of ivy are of great significance for enriching the diversity of three-dimensional greening plants and improving the heat resistance of ivy.

At present, research on the abiotic stress physiology of ivy mainly focuses on drought, low temperature, heavy metal, and salt stress, with little research on its high temperature stress. For example, drought stress can lead to an increase in the relative permeability of the plasma membrane and proline content of silver edge ivy (Zhang et al., 2015), and a significant decrease in the average and maximum net photosynthetic rate (Pn) of ivy leaves under drought conditions (Xia et al., 2010). Spraying 5 mg/L abscisic acid under low temperature stress can enhance the activity of antioxidant enzymes in Chinese ivy and alleviate the damage caused by low temperature stress (Xiong et al., 2022), while Rehm et al. (2014) found in spring in Switzerland that adult leaves of English ivy in forests are more resistant to freezing than young leaves near the ground. Under low concentration cadmium stress, ivy can reduce the damage caused by stress by regulating soluble protein and proline content, and regulating catalase and peroxidase activity (Cheng et al., 2019). It also has strong tolerance to lead stress (Yang et al., 2020). Li et al. (2022) used exogenous low concentration NO treatment to enhance the antioxidant enzyme activity and osmotic regulation ability of Chinese ivy, thereby improving its salt tolerance. In addition, Pandey et al. (2015) evaluated the air pollution tolerance index of British ivy, and another study found that some green leaf varieties of British ivy have higher light saturation points and lower light compensation points than some flowering leaf varieties (Zhang, 2019). Overall, there is currently no research on the physiological and biochemical responses of ivy to high temperature stress.

This study used four types of ivy cuttings as test materials, and recorded their morphological changes under artificial high temperature stress. Physiological indicators such as chlorophyll content, chlorophyll fluorescence index, malondialdehyde (MDA) content, proline content, superoxide dismutase (SOD) activity, and catalase (CAT) activity in their leaves were measured to analyze and compare the differences in heat resistance among the four ivy varieties, screen ivy varieties with better heat resistance, and provide theoretical reference for the breeding and cultivation of ivy heat-resistant varieties in the later stage.

2 Results and Analysis

2.1 The effect of high temperature stress on the morphology of different ivy varieties

The plants and leaves of ‘Golden Ivalace’ were most severely damaged, while ‘Sark’ performed the best. The leaves of ‘Ingelis’ and ‘Wonder’ showed significant wilting (Table 1). ‘Golden Ivalace’ is most sensitive to high temperature stress, and on the third day of heat treatment, it first shows wilting of young leaves, while ‘Sark’, ‘Ingelis’, and ‘Wonder’ do not show significant changes in morphology. On the 5th day of heat treatment, ‘Golden Ivalace’ suffered severe damage from high temperature stress, with a heat damage index of 57.2%, while ‘Sark’ did not show significant morphological damage, and the young leaves of the apical meristematic tissue of ‘Ingelis’ and ‘Wonder’ began to wilt. On the 7th day, the heat damage index of ‘Golden Ivalace’ and ‘Wonder’ reached 84.2% and 78.2% respectively. The tender leaves of ‘Ingelise’ showed obvious withering, while most of the mature leaves were healthy. However, ‘Sark’ had good growth except for a few leaves that withered, demonstrating strong resistance to high temperature stress.

Table 1 Heat index of 4 species of ivy under different heat stress durations

Number of heat treatment days	Heat index			
	Sark	Ingelise	Wonder	Golden Ivalace
0 d	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1 d	13.99±1.56	13.15±1.26	18.08±2.36	28.12±2.63
3d	16.69±1.20	28.33±3.37	28.91±3.18	39.11±5.19
5 d	24.44±2.30	33.17±0.22	37.35±1.17	57.20±7.57
7 d	36.63±6.15	66.63±7.14	78.18±2.21	84.22±5.35

2.2 The effect of high temperature on chlorophyll content in different varieties of ivy

Except for the first day of high-temperature treatment, with the increase of high-temperature stress time, the chlorophyll a and total chlorophyll content of the four ivy leaves showed an overall decreasing trend (Figure 1). On the first day of high-temperature treatment, except for ‘Golden Ivalace’, the chlorophyll a, chlorophyll b, and total chlorophyll content of the other three varieties ‘Sark’, ‘Ingelise’, and ‘Wonder’ increased slightly compared to the control. With the extension of high-temperature treatment time, the chlorophyll a content of all ivy varieties showed varying degrees of decrease. On the 7th day of high temperature stress, the chlorophyll a content in ‘Sark’ and ‘Ingelis’ decreased by 37.7% and 29.6% respectively compared to the control, while the chlorophyll a content in ‘Golden Ivalace’ and ‘Wonder’ decreased by 72% and 69.2% respectively. The chlorophyll b changes of the four varieties are irregular. The total chlorophyll content of Golden Ivalace decreased the most compared to the control, reaching 41.1%, while the total chlorophyll content of Ingelis decreased the least compared to the control, at 24.8%.

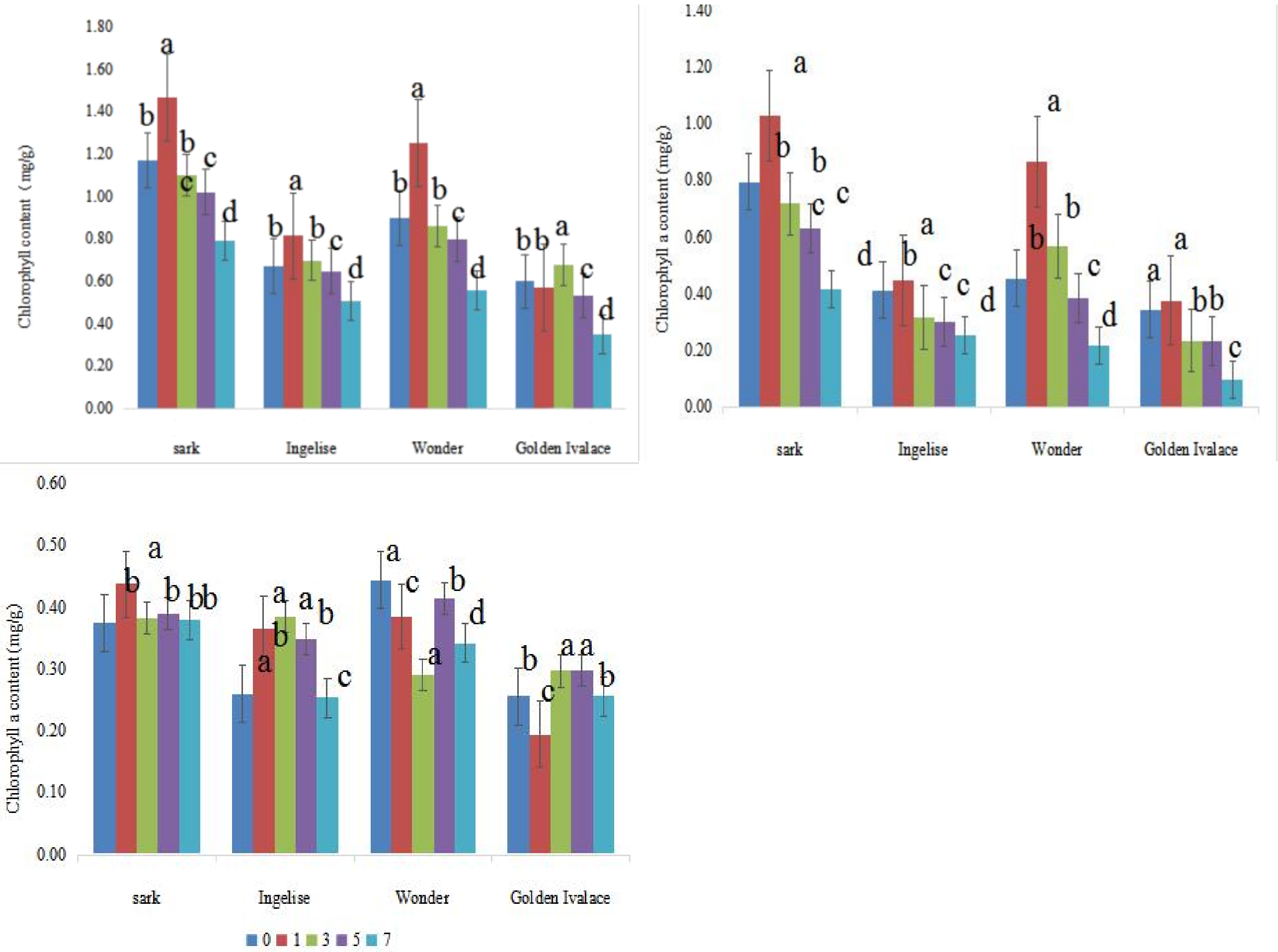

Figure 1 Changes of chlorophyll content of ivy under heat stress

Note: Different lowercase letters indicate significant differences between different treatments ($P<0.05$)

Under high temperature stress, the maximum photochemical efficiency Fv/Fm of the four ivy varieties showed a decreasing trend overall, with significant differences in the degree of decrease among different varieties ($P<0.05$) (Figure 2A). When not subjected to high temperature treatment, there was no significant difference in Fv/Fm values among the four types of ivy, ‘Sark’, ‘Ingelise’, ‘Golden Ivalace’, and ‘Wonder’. When subjected to high temperature stress for one day, the Fv/Fm value of ‘Golden Ivalace’ slightly decreased.

On the third day of heat treatment, the Fv/Fm values of all varieties significantly decreased compared to the control group ($P<0.05$). Among them, the Fv/Fm values of ‘Golden Ivalace’ and ‘Wonder’ decreased by 65.9% and 67.8%, respectively, while ‘Sark’ and ‘Ingelish’ only decreased by 15.0% and 16.7%, respectively. On the 5th day of heat treatment, the Fv/Fm values of all varieties decreased more compared to the control, but the Fv/Fm values of ‘Sark’ and ‘Ingelise’ were still higher than those of ‘Golden Ivalace’ and ‘Wonder’. On the 7th day, the Fv/Fm values of ‘Sark’, ‘Ingelise’, ‘Golden Ivalace’, and ‘Wonder’ decreased by 34.8%, 49.5%, 88.7%, and 69.4%, respectively, compared to the control group.

The apparent photosynthetic electron transfer rate (ETR) of four ivy varieties increased slightly during high temperature stress and then decreased significantly. On the first day, the ETR of all four ivy varieties showed varying degrees of increase, reaching its highest value on the third day, and significantly decreasing compared to the control on the fifth and seventh days (Figure 2B) ‘Sark’ and ‘Ingelise’ showed a decrease of 62.3% and 62.5% compared to the control, while ‘Golden Ivalace’ and ‘Wonder’ showed a decrease of 70.0% and 76.5% compared to the control. The ETR level of ‘Sark’ at each stage was significantly higher than that of other varieties ($P<0.05$). As the duration of stress increases, high temperature affects the electron transfer ability of the PS Ⅱ reaction center in ivy.

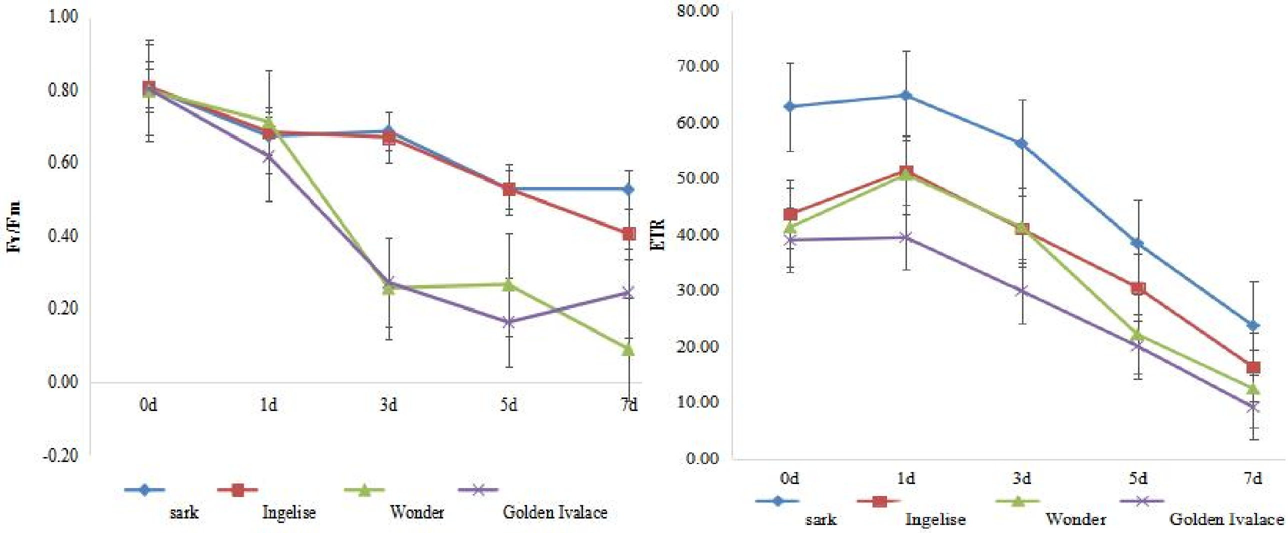

Figure 2 Changes of maximum photochemical efficiency Fv/Fm (A) and photosynthetic electron transfer rate ETR (B) of ivy leaves under high temperature stress

The MDA content of four ivy plants showed an increasing trend with the duration of high temperature stress (Figure 3A). On the first day of high temperature stress, compared with the control, there was no detailed change in the MDA content of the four ivy varieties. However, as the duration of high temperature stress increased, the MDA content of the four ivy varieties continued to increase and the trend of change was roughly the same. By the 7th day, the MDA content of ‘Sark’ and ‘Ingelise’ increased by 67.9% and 71.7% respectively, with a significantly lower growth rate than ‘Golden Ivalace’ (107.0%) and ‘Wonder’ (128.7%).

The Proline content in the leaves of four types of ivy showed a trend of first increasing and then decreasing after being subjected to high temperature stress, and the peak time of different varieties varied (Figure 3B). When high temperature stress was not applied, the proline content in the leaves of the tested ivy was relatively low. As the duration of high temperature stress prolongs, the proline content in the leaves gradually accumulates, and the degree of accumulation varies among different varieties.

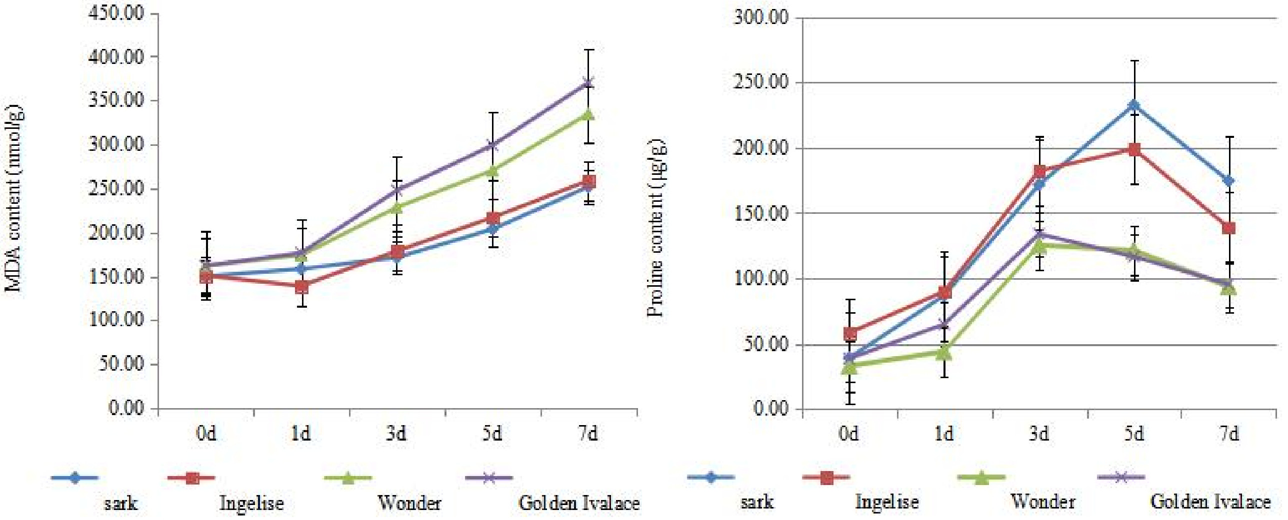

Figure 3 Effect of high temperature stress on malondialdehyde (A) and proline (B) contents in ivy leaves

The proline content in the leaves of ‘Sark’ and ‘Ingelis’ reached its highest level on day 5 of high temperature stress, which was 5.93 and 3.41 times that of the control, respectively, and then decreased; The proline content in the leaves of ‘Wonder’ and ‘Golden Ivalace’ reached its highest level on the third day of high temperature stress, which was 3.81 and 3.46 times that of the control, respectively, and then began to decrease.

2.3 The effect of high temperature on SOD and CAT activities in ivy

The SOD activity of four ivy varieties reached its peak on the third day of high temperature stress and decreased (Figure 4A) ‘Sark’ and ‘Ingelise’ showed a gradual increase in SOD activity from high temperature stress, reaching their highest levels on the third day of stress, which were 2.18 and 2.16 times higher than the control, respectively. ‘Wonder’ also reached its peak SOD activity on the third day after a slight decrease on the first day of high temperature stress, which was 2.33 times higher than the control. On the third day of stress, the highest peak of SOD activity reached by ‘Golden Ivalace’ was only 1.19 times that of the control, and then began to decline, reaching only 41.1% of the control on the seventh day. Its SOD activity remained at a lower level compared to the other three varieties, and the change was relatively small.

The trend of CAT activity changes and SOD activity changes in the four Ivy varieties are similar, both showing an initial increase followed by a decrease (Figure 4B). On the third day, the CAT activity of ‘Sark’, ‘Ingelise’, ‘Wonder’, and ‘Golden Ivalace’ increased to their highest values, which were 2.11, 2.40, 3.02, and 1.47 times higher than the control, respectively, before gradually decreasing thereafter. On the 7th day, the CAT activity of ‘Ingelise’, ‘Wonder’, and ‘Golden Ivalace’ decreased significantly (*P*<0.05) to the lowest level, reaching 72.9%, 81.8%, and 61.3% of the control, respectively.

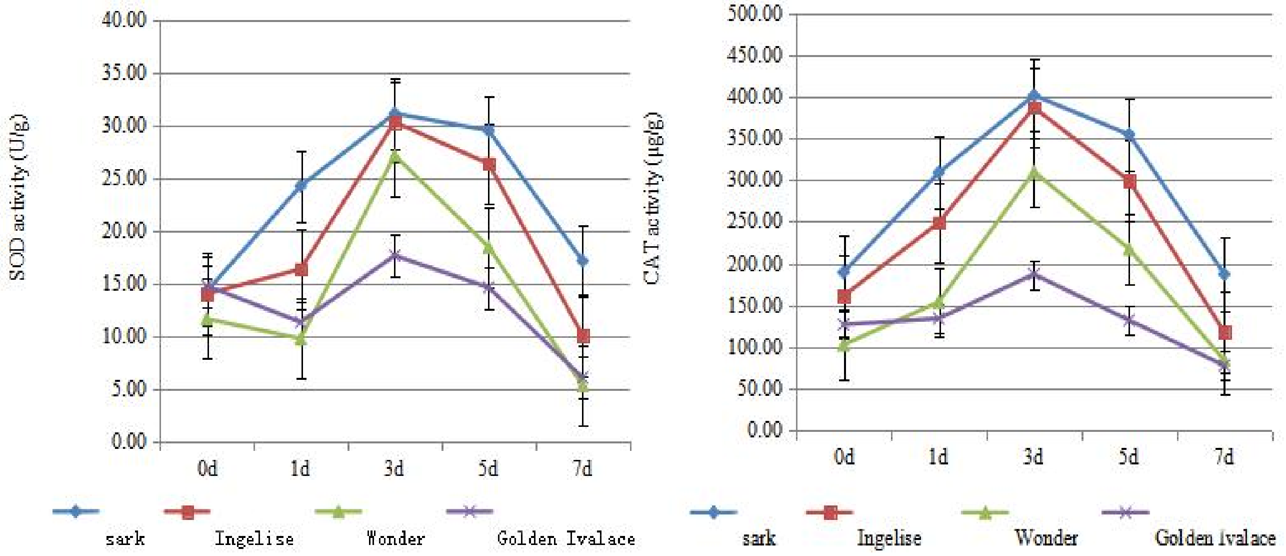

Figure 4 Effect of high temperature stress on SOD activity (A) and CAT activity (B) in ivy leaves

2.4 Comprehensive evaluation of heat resistance of four types of ivy

This experiment uses the average membership function values of four physiological indicators of ivy (Table 2) as the comprehensive evaluation index for the heat resistance of ivy. The results show (Table 3) that the comprehensive heat resistance of the four types of ivy is ‘Sark’> ‘Ingelise’> ‘Wonder’> ‘Golden Ivalace’. ‘Sark’ is the most heat-resistant variety, ‘Ingelis’ is a moderately heat-resistant variety, ‘Golden Ivalace’ is a thermosensitive variety, and Wonder also has poor heat resistance, but slightly stronger than ‘Golden Ivalace’.

3 Discussion

Plants adopt multiple mechanisms to defend against various forms of biotic or abiotic stress they face in their living environment. The enhanced expression of various heat shock proteins and other stress-related proteins in plants, as well as the production of reactive oxygen species, constitute the main response of plants to heat stress. To cope with heat response, plants adopt many mechanisms for defense, such as maintaining membrane stability, producing antioxidants, clearing ROS, accumulating and regulating compatible solutes. A deeper understanding of the physiological and biochemical reactions and heat tolerance mechanisms of plants to high temperatures provides the possibility for improving plant heat tolerance.

Table 2 The subordinate function values of physiological Indexes of four ivy varieties

Measurement indicators	D1				D3				D5				D7			
	Sark	Ingelise	Wonder	Golden Ivalace	Sark	Ingelise	Wonder	Golden Ivalace	Sark	Ingelise	Wonder	Golden Ivalace	Sark	Ingelise	Wonder	Golden Ivalace
Artificial high temperature heat damage index	0.01	0.86	0.80	0.69	0.82	0.69	0.68	0.57	0.73	0.64	0.59	0.38	0.60	0.27	0.15	0.08
Total chlorophyll content	0.86	0.36	0.70	0.17	0.58	0.27	0.39	0.25	0.52	0.23	0.35	0.14	0.34	0.12	0.16	0.00
SOD	0.62	0.36	0.17	0.22	0.83	0.81	0.71	0.41	0.78	0.68	0.44	0.32	0.40	0.18	0.03	0.06
MDA	0.85	0.92	0.79	0.79	0.80	0.78	0.60	0.54	0.69	0.64	0.46	0.36	0.52	0.50	0.23	0.11
Pro	0.28	0.30	0.08	0.18	0.69	0.74	0.47	0.51	0.98	0.82	0.45	0.43	0.70	0.53	0.31	0.32
CAT	0.61	0.46	0.22	0.17	0.85	0.81	0.62	0.30	0.73	0.59	0.38	0.16	0.30	0.12	0.04	0.02
Fv/Fm	0.93	0.80	0.69	0.53	0.78	0.58	0.55	0.37	0.51	0.38	0.24	0.21	0.27	0.15	0.09	0.04
ETR	0.80	0.82	0.85	0.73	0.82	0.80	0.25	0.27	0.61	0.61	0.26	0.12	0.61	0.45	0.03	0.23
Average membership function value	0.62	0.61	0.54	0.43	0.77	0.68	0.53	0.40	0.69	0.57	0.40	0.26	0.47	0.29	0.13	0.11

Table 3 Comprehensive evaluation of heat resistance of four ivy varieties

Variety	Comprehensive membership function value	Ranking
Sark	0.639	1
Ingelise	0.539	2
Wonder	0.399	3
Golden Ivalace	0.302	4

High temperature can induce the degradation of chlorophyll, and heat-resistant varieties have higher and relatively stable chlorophyll content. Studies have shown that the reduction of chlorophyll content by high temperatures is mainly achieved by inhibiting the activity of enzymes related to chlorophyll synthesis, such as the first enzyme in the pyrrole biosynthesis pathway, 5-aminolevulinate dehydratase (ALAD), whose activity significantly decreases at high temperatures (Mohanty et al., 2006). Another study suggests that the decrease in chlorophyll caused by high temperatures may be due to the occurrence of peroxidation of chloroplasts and thylakoid membrane liposomes (Kaushal et al., 2016). In the entire high temperature stress of this experiment, the chlorophyll a and total chlorophyll content of the heat-resistant varieties ‘Sark’ and ‘Ingelis’ were higher than those of the thermosensitive variety ‘Golden Ivalace’.

The relationship between the changes in chlorophyll a and total chlorophyll content of ‘Wonder’ and its heat resistance is relatively more complex. On the third day of heat treatment, it is higher than the medium resistance ‘Ingelise’, but on the seventh day, it is lower than ‘Ingelise’. It can be seen that the use of chlorophyll content to evaluate plant stress resistance varies among different plants, varieties, and time periods of high temperature stress. There have been similar reports by previous researchers, such as Shen and Zhao (2018) who found that most Rhododendron varieties experienced varying degrees of decrease in chlorophyll a, chlorophyll b, and total chlorophyll content after high temperature stress, but some varieties experienced an increase in chlorophyll b after high temperature stress. Throughout the entire high-temperature stress process in this experiment, the trend of total chlorophyll content in different varieties was similar to that of chlorophyll a content, showing an increase on the first day of stress followed by a continuous decrease, while chlorophyll b showed no significant change pattern.

The effects of high temperature stress on plant photosynthesis mainly involve photosystem II (PSII), ribulose-1,5-diphosphate carboxylase/oxygenase (Rubisco), cytochrome B559 (Cytb559), and plastiquinone (PQ) (Mathur et al., 2014), among which PSII is the most sensitive component to high temperature stress in photosynthesis (Hasanuzzaman et al., 2013). Fv/Fm reflects the maximum photoelectrochemical efficiency when the PSII center is fully open, while ETR reflects the efficiency of electron transfer and the apparent photosynthetic electron transfer rate. When not under stress, Fv/Fm is relatively stable, but under high temperature stress, Fv/Fm significantly decreases (Tu et al., 2013; Song et al., 2014). Four types of ivy grew differently after high temperature stress, and the Fv/Fm values of all tested ivy decreased to varying degrees compared to the control group. The Fv/Fm value of the thermosensitive ‘Golden Ivalace’ decreases first. At different time points of high-temperature treatment, the decrease in Fv/Fm values of heat-resistant varieties ‘Sark’ and ‘Ingelise’ is smaller than that of thermosensitive varieties ‘Golden Ivalace’ and ‘Wonder’, which also confirms that the more Fv/Fm decreases, the greater the damage to PSII, and the weaker the high-temperature resistance. The amplitude of Fv/Fm changes reflects the plant's ability to respond to stress and is related to the plant's stress resistance.

When plants are subjected to high temperature stress, if the intracellular antioxidant enzymes still cannot fully respond to the oxidative stress response induced by high temperature stress, a large amount of ROS will accumulate in the cell, leading to lipid peroxidation, and MDA is a marker of lipid peroxidation (Tu et al., 2013). The more MDA accumulates, the greater the degree of damage. Song et al. (2014) found that the content of MDA in poplar trees did not change significantly after 3 and 4 hours of heat treatment, but increased significantly after 24 hours. Generally speaking, the content of MDA is negatively correlated with plant stress resistance, and the MDA content of heat-resistant materials is significantly lower than that of non heat-resistant materials under high temperature stress (Wilson et al., 2014). We found that MDA accumulated in all four ivy varieties under high temperature stress, but the accumulation of heat resistant ‘Sark’ and moderately heat resistant ‘Ingelise’ was significantly lower than that of heat sensitive ‘Golden Ivalace’ and ‘Wonder’ throughout the entire high temperature stress process (Figure 4A).

Plants under environmental stress also accumulate large amounts of metabolites, especially amino acids. There is a large amount of data indicating that proline can accumulate as a cell osmoprotectant when plants are under stress, to maintain cell expansion or osmotic balance, enhance plant stress resistance, stabilize cell membranes to prevent electrolyte leakage, and coordinate the balance of ROS in the body (Tu et al., 2013; Gosavi et al., 2014). In addition, when plants respond to various stress responses, proline can also play a beneficial role as a metal chelating agent, antioxidant defense molecule, and signaling molecule. When low concentrations of proline are applied in vitro, it can enhance plant tolerance (Hayat et al., 2012).

Studies have shown a positive correlation between proline content and plant stress resistance (Kumar et al., 2012), and similar results were also found in this study. When ivy is subjected to high temperature stress, proline accumulates to varying degrees in different varieties. On the third or fifth day of heat treatment, proline accumulation reaches its peak and then begins to decline. During the entire process of high temperature stress, the accumulation of proline in heat-resistant ‘Sark’ and moderately heat-resistant ‘Ingelise’ was higher than that in thermosensitive ‘Golden Ivalace’ and ‘Wonder’, and there was a significant difference ($P<0.05$) (Figure 4B).

High temperature can generate ROS accumulation, thereby triggering oxidative stress response in plants. High temperature can disrupt the balance between the generation and elimination of ROS within cells, leading to a significant accumulation of reactive oxygen species and affecting plant growth and development. Usually, plants induce an increase in antioxidant enzymes in response to high temperature stress, thereby initiating antioxidant protection mechanisms to maintain their own redox balance (Hameed et al., 2012). In the experiment, the activities of SOD and CAT in ivy under high temperature stress were analyzed. The results showed that the heat-resistant variety ‘Sark’ reacted the most rapidly and the activities of SOD and CAT increased the fastest when ivy was subjected to high temperature stress.

There was a significant increase on the first day of high temperature treatment, followed by the moderately heat-resistant variety ‘Ingelise’, with a smaller increase. On the third day of heat treatment, all tested ivy SOD and CAT enzyme activities showed significant improvement compared to the control group. Afterwards, the SOD and CAT activities of heat resistant ‘Sark’ and moderate heat resistant ‘Ingelis’ decreased slowly, while those of heat sensitive ‘Golden Ivalace’ and ‘Wonder’ decreased rapidly. This indicates that high temperatures can stimulate the antioxidant enzyme activity of ivy. Compared with thermosensitive ivy, heat-resistant materials have a faster increase in SOD and CAT enzymes and a slower decrease. It is speculated that thermosensitive ivy may have a stronger ability to counteract the harmful effects of ROS and achieve the goal of heat resistance.

This experiment conducted a comprehensive analysis of the heat damage index, chlorophyll content, Fv/Fm, MAD, proline, SOD, and CAT activities of four ivy varieties under artificial high temperature stress using membership functions to determine their heat tolerance: ‘Sark’> ‘Ingelis’> ‘Wonder’> ‘Golden Ivalace’. Artificial high temperature stress seriously affects the structure and function of the light system in ivy leaves, resulting in varying degrees of decrease in chlorophyll content, Fv/Fm values, ETR, and increase in MDA content in four ivy species.

Proline content, SOD and CAT activities show an initial increase followed by a decrease with increasing stress duration. After high temperature stress, the Fv/Fm values, Proline content, SOD and CAT activities of heat-resistant varieties ‘Sark’ and ‘Ingelise’ were higher than those of thermosensitive varieties ‘Golden Ivalace’ and ‘Wonder’, while the MDA content was lower than that of thermosensitive varieties. This study analyzed the morphological, physiological, and biochemical indicators of ‘Sark’, ‘Ingelise’, ‘Golden Ivalace’, and ‘Wonder’ ivy under artificial high temperature stress, which will contribute to the screening or cultivation of heat-resistant ivy in the future.

4 Materials and Methods

4.1 Experimental materials

The four types of trial ivy are *Hedera hibernica* ‘Sark’, *Hedera helix* ‘Ingelise’, *Hedera helix* ‘Golden Ivalace’, and *Hedera helix* ‘Wonder’, all provided by Shanghai Botanical Garden. These materials are all two-year-old potted seedlings with branch cuttings and roots. The potted soil is perlite: peat: coarse sand=2:2:1, and unified cultivation and management measures are adopted for maintenance. The experiment was conducted in September 2020 at the research base and laboratory of Shanghai Botanical Garden, as well as the laboratory of the School of Life Sciences at Shanghai University.

4.2 High temperature stress

Select plants with consistent growth for each variety and cultivate them in an artificial climate incubator (20 ℃, 16 hours of light/8 hours of darkness, relative humidity of 70%, light intensity of 8000 lx) for two weeks. Then, treat them continuously at 40 ℃ for 7 days. During high temperature treatment, in order to avoid drought stress, it is necessary to moisturize and hydrate during this period. After high temperature stress, the morphological changes were observed and photographed at 0, 1, 3, 5, and 7 days, and chlorophyll fluorescence was measured on the 3rd to 5th leaves from top to bottom of each plant. After measurement, remove the leaves and freeze them with liquid nitrogen, then place them in a -80 ℃ ultra-low temperature freezer for later use.

4.3 Observation of heat damage index

Observe and record the growth status of various ivy plants during high temperature stress, and classify the degree of heat damage. Referring to the method proposed by Tian et al. (2021) and combined with the research object, the heat damage index is divided into four levels: level 0 indicates no damage and the leaves are completely green; Grade 1 refers to mild damage to the leaves, with less than one-third of the leaf edge or tip showing yellowing; Grade 2 indicates significant leaf damage, with 1/3 to 2/3 of the leaf area showing yellowing. Grade 3 indicates severe leaf damage, with over 2/3 of the leaf area showing yellowing, and even the entire leaf wilting and dying. The calculation of heat damage index is as follows: Heat damage index=∑ (number of leaves at this level of heat damage index multiplied by this level of heat damage)/(total number of surveyed leaves multiplied by the highest level value) * 100%.

4.4 Determination of physiological indicators

The determination of chlorophyll content was carried out using the ethanol acetone method. Anhydrous ethanol and acetone were thoroughly mixed in a ratio of 1:2 (v: v) as the extraction solution. Chlorophyll fluorescence parameters were measured using FluorCam. The content of malondialdehyde (MDA) was determined using the thiobarbituric acid method. The content of proline was determined using acidic indene. The activity of superoxide dismutase (SOD) was determined using the nitrogen blue tetrazole reduction method. Measure the activity of catalase (CAT) using ultraviolet absorption method.

4.5 Heat resistance evaluation method

Under adversity stress, plant physiological changes are extremely complex, and a single indicator cannot truly reflect the strength of plant stress resistance. This experiment uses the membership function method to comprehensively evaluate the heat resistance of four ivy varieties. If the index is positively correlated with heat resistance: U (Xij)=(Xij Xjmin)/(Xjmax Xjmin). U (Xij) is the membership function value, Xij is the measured value of the j index for plant i under high temperature stress, Xjmin is the minimum measured value of the j index for all test materials under high temperature stress, and Xjmax is the maximum measured value of the j index for all test materials under high temperature stress. If the index is negatively correlated with heat resistance: U (Xij)=1- (Xij Xjmin)/(Xjmax Xjmin).

Calculate the average membership function values of each evaluation index for four types of ivy under different degrees of high temperature stress, and then calculate the sum of the average membership function values to obtain the comprehensive membership function values for different ivy varieties. The stronger the heat resistance of the test material, the larger the comprehensive membership function value.

4.6 Data analysis

Excel 2016 was used to statistically organize the data and create charts. SPSS 17.0 was used to analyze the significance of differences between samples.

Authors' Contributions

ZXL was the primary author of this study, responsible for data collection, analysis, and drafting the manuscript. FCZ was contributed to the study design. ZXH, XS, TZX, ZY, and WHY participated in revising the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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