Physiological and Biochemical Responses to Short-Term Cold Stimulation of Pak Choi Under Heat Stress

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Abstract

Heat stress has become a global concern which seriously affects the growth and production of crop as global warming progresses. Current cultivation techniques of protected horticulture can alleviate this problem, but it is expensive and ineffective. This study aims to use short-term cold stimulation to alleviate the stress response of pak choi under heat stress, and analyze the physiological and biochemical responses of pak choi to short-term cold stimulation, including photosynthetic system, plant antioxidant system, gas exchange capacity, Organic matter content, etc. This method can be used as an auxiliary measure to contribute to the development of protected horticulture in the future.

1. Introduction

Global warming and extreme high temperature restricts the growth, development, and geographic distribution of crops (Lobell,et al, 2003). In summer, the temperature in the facility often exceeds 40 °C. Under high temperature stress, plants will have many symptoms of heat damage, such as leaf rewinding, chlorosis, damaged organs, weakened photosynthesis and even cause death. The growth period is shortened, the quality of the product is reduced, agricultural production faces severe challenges.

Protected horticulture is a modern agricultural production mode which adopts engineering technology to produce crops efficiently under the condition of relatively controllable environment. It has the advantages of high land use rate, relatively controllable environment and convenient for mechanized operation, which has been paid attention to all over the world. In the past decades, the proportion of protected horticulture in agricultural production has constantly increased. In summer, plastic greenhouses and other horticultural facilities will aggravate heat stress, which is one of the important reasons restricting the development of the vegetable industry. Currently, agricultural production mainly adopts environmental control measures to deal with extreme weather. Among them, ventilation and cooling facilities are the most widely used. External shading, wet curtains, side window openers, film roller shutters, etc, joint with the automatic control system to adjust the temperature to facilitate the growth of crops. However, The current environmental control facilities are ineffective and costly, so some environmentally friendly and convenient auxiliary measures are necessary to alleviate the stagnant growth of crops under heat stress.

Pak choi (Brassica rapa ssp. Chinensis) is a Brassica vegetable which is widely grown in Asia (Liu et al, 2020). Because of the high nutritional value and good taste, it has high commercial value. In order to be able to supply Pak-choi all year round, we

use greenhouses to produce cabbage in large quantities. At present, the extreme high temperature in summer caused by the global warming often inhibits its growth and development. We use air conditioning and other facilities to provide a suitable growth environment for Pak choi, but it has the disadvantages of high cost and low income. Thus, an effective method is needed to assist the current horticultural facilities to help crops in survive extremely hot weather. We suppose that low temperature water can instantaneously reduce plant body temperature and soil temperature, and mitigate the damage caused by high temperature. In the present study, we aimed to characterize the photosynthetic activity and antioxidant capacity of Pak-choi to low temperature water treatment. We found that low temperature treatment affected the photosynthetic electron transport, gas exchange capacity and antioxidant enzyme activity, which contributes to the growth of Pak-choi.

2. Materials And Methods

Plant material and cold water treatment

The Pak choi plants was grown in chamber under a 16-h light (L, 35°C) / 8-h dark (D, 25°C) cycle at 300μmol photons m^{-2}•s^{-1} and 60% relative humidity (RH). Treatment seedlings were grown in plate with 120 pots containing filled mixture of soil and vermiculite (3:1). At the three-leaf stage, plants were irrigated every day with 800 mL water (15 and 20 °C). The control plants were irrigated with 800 mL room temperature water every day. The treatment was done for 30 days. Measurements were performed on the third fully expanded true leaves from different individuals.

Growth traits measurement

Plant height, stem diameter were determined by ruler and Vernier caliper respectively. Fresh and dry weight were measured by electronic balance. The total leaf area and leaf number were calculated by Image J 1.8.0. The length, root surface area and root tip number were were scanned by EPSON SCAN and measured by WinRHIZO (2007d). A total of 0.2 g of fresh sample from the third leaf was taken and placed into a 20 mL tube with 10 mL anhydrous ethanol for 24 h under dark conditions. The absorbance at wavelengths of 665, 649 and 470nm was measured by an enzyme-labeled instrument (Thermo, USA). Their contents with three treatments were calculated in 20mg/L according to the equations (Daudi and Brien et al, 2012; Kumar et al, 2014). The chlorophyll content was quantitated as previously described (Kwan et al, 2016). The content of soluble protein (Bradford et al., 1976) and soluble sugar (Zheng et al, 2008) was measured as described in the previous study. Four technical replicates and four biological replicates were performed in all experiment. MDA content was determined using the reported method (Siripornadulsil etal, 2002).

Measurement of gas exchange and chlorophyll fluorescence parameters

GFS-3000 measuring systems (Heinz Walz, Effeltrich, Germany) was used to obtain the gas exchange parameters. The third leaf from each plant was used in this measurement. The net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), intercellular CO_2 concentration (Ci) were determined at a concentration of ambient CO_2 (300 μmol•mol^{-1}) and a photosynthetic photon flux density (PPFD) of 600 μmol•m^{-2}•s^{-1}. Fluorescence induction kinetics and then the rapid light curves (RLCs) were recorded with Dual-PAM-100 (Heinz Walz Effeltrich, Germany) according to the method described previously (Zhang et al., 2014). The light intensity gradient of the RLC was 2, 13, 40, 80, 114, 157, 213, 279, 358, 592, 956, 1207, 1537μmol•m^{-2}•s^{-1} .

Measurement of P700 redox kinetics and PQ pools

The redox state of P700 was determined *in vivo* with the Dual-PAM software using a described method (Schrei etal, 2008). The P700 signal was caught during a single turnover flashes (ST, 50ms, PQ pools being oxidized) followed by multiple turnover flashes (MT, 50 ms, PQ pools are fully reduced) in the presence of far-red (FR) background light. The ST- and MT-areas refers to the the complementary area between the oxidation curve of P700 after ST and MT excitation and the stationary level of $P700^+$ under FR. The ST- and MT-areas was used to calculate the functional pools sizes.

Determination of antioxidant enzyme activity

Fresh leaf samples from plants were harvest after treatment which were used for enzyme extraction. The antioxidant enzyme activity assays were carried out at 4˚C. The H_2O_2 release was detected as reported previously (Christensen et al, 1997). The CAT activity was calculated by measuring the decrease in the absorbance of H_2O_2 at 240 nm (Aebi et al, 1984). The POD activity was determined according to the protocol as described previously (Trevisan, Scheffer and Verpoorte, 1997). The SOD activity was investigated by the method as described previously (Stewart and Bewley, 1980).

Statistical analysis

The data processing was conducted on randomly selected samples from four independent biological and technical replicates. Statistical analyses were performed by ANOVA using SPSS software (SPSS, Chicago, USA). The comparisons between the mean values were accomplished by the least significant difference.All graphs were made

using by graphpad 8.0.

3. Results

Pak choi with cold treatment shows great biomass under heat stress

Environmental stresses have become an important constraint impacting grain yields in crops. Perception mechanism of plant involved in heat stress is very complicated, which has not been fully understood yet. Plasma membranes sense heat stress and cause the elevated level of Ca^{2+}, which can act as a second messenger to induce the expression of heat stress response genes (Ma et al, 2020). We assumed that cooling can instantly reduce the plant surface temperature in order to reduce the damage of high temperature to plant plasma membrane. To investigate this possibility, we set the temperature of water as 15, 20 and 25 °C, which were applied to the seedlings divided into T1, T2 and T3 groups respectively. The morphological indexes of three treatments of pak choi under heat stress were examined (Table 1). The phenotypes of the three groups of seedlings are shown in Figure 1. The T2 seedlings shows greater growth vigor than T1 and T3. the T3 seedlings showed slower growth rate and smaller leaves, which can be explained that the low temperature water alleviates the damage of heat stress to the growth and metabolism of pak choi to a certain extent.

Table 1
The growth index of pakchoi seedlings on water of varying temperatue.

water temperature(°C)	dry weight(g)	fresh weight(g)	leaf area(cm^2)	leaf number	Stem diameter (mm)
15	0.2767±0.0723a	3.23±0.9522b	72.1077±10.6058b	8±0.0000b	2.5700±0.6128a
20	0.2433±0.0513a	2.5967±1.1926b	93.5547±16.7950a	8.3333±0.5773b	2.98±0.6605a
25	0.3167±0.0450a	4.86±0.1852a	82.6599±7.0193b	9±0.000a	2.2767±0.2103b

water temperature(°C)	Total root length	Root surface area	root tip number	Plant height (cm)
15	31.8757±0.949a	8.648±0.4825a	378±2.6457b	13.3667±1.8448a
20	26.2±1.5488b	8.6223±0.2100a	298±12.49a	13.1673±1.1676a
25	28.3433±0.9342b	7.1828±0.4015b	241.333±6.429c	10.0652±1.1471b

Leaf is responsible for photosynthesis of the crop, and the leaf area affects light absorption directly. We counted the leaf number and area of pak choi within the three groups. The result suggested that there is no significant difference in the leaf number among the three groups of pak choi. The leaf area of the T2 group has increased by 13.18% compared to the T1 group, and has increased by 29.74% compared with the T3 group (Table 1). Larger leaf area is conducive to the accumulation of crop assimilation. Root is an important plant organ which has multiple functions, including acquisition and fixation of water and nutrient, perception of environment changes in soil, and synthesization of phytohormone (Ma et al, 2018). Thus, root branching and root surface are important aspect of root system architecture which directly affect the absorption of water and nutrients. We then measured the total root surface area, root length, as well as root tip number. The cold water treatment has no obvious effect on the root surface area, but the root length and root tip number are significantly increased. Compared with the control, the root length of T1 and T2 increased by 7.56% and 12.46%

respectively. Concerning root tip number, T1 and T2 increased by 19.01% and 26.84% compared to control, respectively (Table 1). We guess that longer root length and more root tips will help plants absorb more water and nutrients.

Chlorophyll is the main pigment for photosynthesis of plants, which functions in harvesting light energy and driving electron transfer (Wang et al, 2018). Accumulating evidence suggested that chlorophylls were degradated and the photosynthetic machinery was dismantled in plants under heat stress (Wang et al, 2018). The chlorophyll breakdown into nonphototoxic pigments in combination with carotenoid retention or anthocyanin accumulation (Hörtensteiner, 2009; Hörtensteiner, 2006; Stefan and Urs, 2002; Ginsburg and Matile, 1997). We next analyzed the chlorophyll content in treated seedlings. In light of our results, the content of chlorophyll a, chlorophyll b and carotenoid in pak choi treated with low temperature water were significantly higher than control. The level of Chl a in T1 and T2 seedlings increased by 11.03% and 38.19% compared to control respectively. Moreover, the ratio of Chl a/ Chl b in T2 was significantly higher than control. Meanwhile, a decrease in Carotenoid level was observed in T2 compared to control (Figure 2).

To confirm the greater biomass accumulation in pak choi treated with low temperature water, we measured the the content of soluble sugar, protein and Vitamin C (Figure 2), which affects the osmotic pressure in plant cells. In the experimental results presented in Figure 2, T2 plant leaves accumulated about 1.5 fold increase in soluble sugar and about 1.22 fold increase in soluble protein than control. It suggests that more soluble sugar and soluble protein are synthesized when treated with cold water. The level of Vitamin C was also enhanced under cold water treatment. On the other hand, The altered level of soluble sugars and proteins are is advantageous to regulate osmotic pressure within the cell.These data suggested that the nutritional content of pak choi has been improved under cold water treatment.

Cold treatment enhances the activities of ROS-scavenging enzymes

In plants, ROS are generated in the redox reactions, like respiration and photosynthesis. ROS participate in all aspects of growth and development, such as cell proliferation and differentiation, gravitropism, programmed cell death, seed germination, root hair growth and pollen tube development, senescence (Singh et al, 2016). In many cases, heat stress contribute to various metabolic changes, known as elevated ROS levels which generates mainly in PS II and PSI (Asada et al, 2006). To enhance the heat tolerance and detoxify ROS under environmental stress, plants recruit the antioxidant enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT). To confirm whether alternative detoxification pathways were activated under low temperature water treatment, we measured the activity of ROS-scavenging enzymes. We found that the activity of SOD, APX, CAT in T2 seedlings were highest than T1 and T3 seedlings (Figure 3), which is line with previous findings (Ara et al, 2013). On top of this, we determined the release of H_2O_2 and O^{2-} from the three group of pak choi. In our study, these data suggest that H_2O_2 production decreased 32.32% in T2 compared to control (Figure 4). It showed that plants under low temperature treatments had the stronger ability to remove H_2O_2 and O^{2-} compared to control. In order to detect the level of membrane lipid peroxidation in plants, we also tested the differences in plant malondialdehyde (MDA) content and relative electrical conductivity (REC) between treatments. We found that plants have lower MDA content and lower relative electrical conductivity under low temperature treatment (Figure 4). MDA is a substance produced by membrane lipids under the action of reactive oxygen species(Djanaguiraman et al, 2010). Its content reflected the degree of plasma membrane damage. It is proposed that the REC of the leaves can be measured to determine the damage of the high temperature to the leaves. In accordance with these above findings, our study suggested that low temperature water treatment on the one hand reduces the damage of high temperature to leaf cell membrane lipids, on the other hand, it improves the activity of various enzymes to scavenge free radicals to avoid damage to plants.

Enhanced photosynthetic capacity in pak choi with cold water treatment under heat stress

Multi environmental stresses can alter the activity of the photosynthetic electron transport chain, which can reduce photochemical reaction efficiency, create excess absorption of light energy, and induce aggravated photoinhibition (Xalxo et al, 2020). Recent research showed that elevated ambient temperature have profound effects on photosynthetic capacity of plants (Wahid et al, 2007; Allakhverdiev et al, 2008; JA et al, 1980). It affects the chlorophyll content, photosynthetic enzyme activity, stomatal opening as well as hormone secretion of plant. High temperature seriously impede photosynthetic efficiency, diminishes productivity and shortens the plant life cycle (Xalxo et al, 2020). To get detailed information about gas exchange parameters, we measured the net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E) and intercellular CO_2 concentration (Ci) [Figure 5]. The Pn of pak choi under low temperature water treatment was significantly higher than the control. the Pn of pak choi was 12.61 μmol $m^{-2}•s^{-1}$ (T1), 13.75$m^{-2}•s^{-1}$(T2), 11.33$m^{-2}•s^{-1}$(T3) 6h later. E and gs of T1 and T2 was significant higher than control while Ci of T1 and T2 is significantly lower than control. The above findings suggested that low temperature water treatments can enhance the gas exchange capacity of pak choi under heat stress.

Fv/Fm and Pm of pakchoi under cold water treatment

Fv/Fm is an important indicator for studying the effects of photoinhibition or various environmental stresses on photosynthesis. As the strong evidence supports that the Fv/Fm value of plants will decrease under various environmental stresses. Thus we measured the maximum photochemical efficiency of PSII (Fv/Fm) of pakchoi leaves with three treatments.

We found that the Fv/Fm of pak choi under low temperature water treatment was higher than that of control. It is worth noting that the Fv/Fm of T1 is lower than the T2 (Figure 6). This may be explained that extremely low temperature water will inhibit plant photosynthetic activity. Meanwhile, the maximum oxidation state of PSI (Pm) of three treatment was measured (Figure 6). The Pm of the pakchoi under low temperature water treatment is approximately 1.45 time that of control. These results indicated that low temperature treatment counteract the inhibition of redox state of PSI and reduced the damage induced by heat stress. Thus we proposed that short time heat adjustment improved the maximum light energy conversion efficiency of PS II in pak choi leaves.

In order to clarify the potential photosynthetic activity of pak choi under varying temperature of water treatment, we measured the rapid light curves of plants under different ambient light intensity. photochemical quenching coefficient (qP) of pak choi leaves show a rapid elevation at all illumination conditions (Figure 7). Many studies showed that qP reflects the photosynthetic activity of plants. The result suggested that the activity of PSII photochemical reactions under low temperature water treatment was higher than that under room water treatment.

Effect of cold water treatment on the PSII and PSI Activity of pak choi Leaves under heat stress

Previous reports demonstrated that heat stress damaged thylakoid membranes, reduced the activities of membrane associated electron carriers and enzymes (Zhao et al, 2020; Hasanuzzaman et al, 2013). Under heat stress, the Fv/Fm and PSII electron transfer efficiency in plants was decreased, and the formation of NADPH and ATP was prevented which further resulted in the reduced photosynthetic rate. Since PSII is the most heat-sensitive complex within the chloroplast thylakoid membrane protein complexes (Hasanuzzaman et al, 2013; Szymańska etal, 2017), we measured the photosynthetic quantum yields for PSI and PSII (Figure 8). We observed that the effective quantum yield of PSII gradually decreased with increasing light intensity in three treatment, while the Y(II) was significantly higher under the cold water treatments compared to control (Figure 8). Our results showed cold water treatment caused a modest elevation in the quantum yield of non-regulated energy dissipation in PSII [Y(NO)](Figure 1). The higher Y(NO) of control compared to T1 and T2 indicated that control lost the ability to protect themselves in excess light. Y (NPQ) represents the energy dissipated into heat through the regulatory photoprotection mechanism. It has been proposed that plants can avoid ROS damage caused by excessive light excitation through antioxidant system and NPQ mechanism (Zhang et al, 2014). In our research, the quantum yield of regulated energy dissipation in PSII [Y(NPQ)] was highest in T2 seedlings. The NPQ was increased at almost all light intensities in three treatments. It indicated that the low temperature water treatment enhanced the capacity to induce photoprotection of PSII. Additionally, The quantum yield of PSI [Y(I)] increase first and then decrease rapidly with the increasing light intensity. The Y(I) of T2 was higher than T1 and T3 especially under high light intensities. It suggested the low temperature water treatment increase the the quantum yield of PSI. In addition, we observed that cold water treatment significantly reduced the quantum yield of non photochemical energy dissipation caused by donor side restriction (ND) while the quantum yield of PSI non-photochemical energy by the receptor side restriction (NA) has no significant difference between treatments (Figure 7). All of these observations strengthen that the cold water treatment.

Effect of cold water treatment on the Electron Transport Rate of pak choi Leaves under heat stress

The electron transfer rate of PSII [ETR (II)] and PSI [ETR (I)] were measured to evaluate the photosynthetic performance. As shown in Figure 8, electron transport rates of both PSII and PSI, ETR (I) were significantly higher with CWT compared to those of control plants under high light level. The ETRI and ETRII of pak choi leaves rapidly increased with increasing light intensity in all treatments. We also found the low temperature water treatment significantly improve the cyclic electron flow around PSI (CEF) (Figure 1). Previous studies demonstrated that CEF compel the excess electron flow to NADPH and O_2, avoiding the generation of ROS. Besides, CEF deplete the excess reducing power of NADPH via NADPH dehydrogenase-dependent pathway. The stimulated CEF contributed the high level of Y (NPQ). Thus we induced that the CWT can stimulate the CEF to protect the PSI from photo-inhibition.

Redox state of PSI and PQ pool size

The response of P700 redox kinetics to diverse water temperature was measured with applications of ST or MT flashes on an FR light background. In the experimental results presented in Figure 6, The plastoquinone (PQ) pool size in the pak choi leaves under cold water treatments exhibited a 1.48 fold larger functional PQ pool in comparison with control. We supposed that the reduction of PQ is responsible for the blocking of the electron transport. As Figure 1 shown, Turn on the far red light, the P700 signal rises, turn off the far red light after it is stable, and $P700^+$ quickly restores until the signal is stable. Illumination of the sample with FR light contributes to the change in A820 which reflects the dynamic changes of P700 to $P700^+$. Our result showed that there was no significant difference in the rate of dark reduction between treatments according to the initial slope method.

4. Discussion

Under the condition of global warming, plants will be faced with greater frequencies of high-temperature events, which poses a threat to crop production and food supply (Rowhani et al, 2016; Lobell et al, 2011). Although the mechanism of plant response to heat stress have been outlined in model plant, the regulatory work involved in pak choi has remain elusive. The optimum growth temperature of pak choi is 15-20 ℃, and the ambient temperature in summer is much higher than required growth temperature of pak choi. Under heat stress pak choi often shows slow growth rate, yellow leaves, serious diseases and dysplasia. Over the last decade, the protected horticulture industry has developed rapidly, which enable plants grow well in extreme weather through precise temperature control in greenhouse. However, this method has high cost, but low income and is not suitable for large-scale utilization. Therefore, some auxiliary means should be adopted to help plants adapt to heat stress.

when exposed to high temperature plants showed dysfunction of plant cell membrane and production of ROS (Wang et al, 2018). Ambient temperature also affects pigment synthesis in plants. On top of this, heat stress injure plant membrane system and reduce PSII electron transfer efficiency, and prevented the formation of NADPH and ATP, which impair the CO_2 fixation, Organic metabolism, and reduce the yield and quality of plants. In this paper, we investigated the effect of short-term cold water treatment on pak choi under high temperature at the physiological level. We set the temperature of irrigation water to three gradients (15, 20, 25℃). We found the seedlings irrigated with water (20℃) grow best among the three groups. Cold water treatment can effectively inhibit the reduction of soluble sugar, soluble protein vitamin C and pigment. In order to detect the level of membrane lipid peroxidation in plants, we measured the MDA content and REC in pak choi. The results showed that cold water treatment effectively Damage of high temperature to plant cell membrane system. we also detected activity of several ROS scavenging enzymes. The result showed that low temperature water treatment can improve the activity of plant protective enzymes, reduce the production of free radicals and reduce the damage to the structure and function of plant cell membrane. Since Photosynthesis, respiration and transpiration are light sensitive processes, gas exchange capacity of plants were greatly limited by ambient temperature. We measured the gas exchange parameters of pak choi under different treatments. The result suggested that cold water treatment could effectively mitigate the decline of photosynthetic rate and increase carbon dioxide fixation. we surveyed the electron transfer efficiency, light energy distribution, electron transport rate and redox state of PSI. We found that irrigation water temperature influenced the activity of the photosynthetic electron transport. Based on these observations, we suggested that the low temperature water treatment contributed improved carbon fixation and increased CEF which further induced NPQ mechanism to provide photoprotection. However, the underling molecular mechanism of water temperature regulating plant growth remained unknown and more research is needed to provide insight into the regulatory network regarding the irrigation water temperature promoting crop growth.

Conclusion

Therefore, a fundamental understanding of the response of photosynthetic physiology to low temperature water treatment is helpful for crops to survives under heat stress. Indeed, our results provide insights into the effects of cold water treatment on the photoprotection mechanisms which protect crops from photo-inhibition.

Declarations

Author contributions

Qingliang Niu conceived and designed research. Jing Yu conducted experiments and wrote the manuscript, Jinyang Weng, Pengli Li analyzed data, Jinyang Huang and Liying Chang modified the paper. All authors read and approved the final version for publication.

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Figures

Figure 1

(A). Rapid light curve of the cyclic electron flow around PSI (CEF) and response of far-red light induced P700 redox kinetics to different water temperature treatments; (B) Rapid light curve (RLC) of the quantum yield of non-regulated energy dissipation in PSII [Y(NO)]; (C) Phenotypes of pak choi plants under different water temperature treatments.

Figure 2

The soluble sugar, soluble protein, vitamin C and pigment content of pak choi under different water temperature treatments. Letters above the bars indicate significant differences at $P < 0.05$ (Student's t-test).

Figure 3

Antioxidant enzyme activities of pak choi under different water temperature treatments.

Figure 4

(A) The release amount of H_2O_2 and O^{2-} of pak choi under different water temperature treatments. (B) MDA content and relative conductivity of pak choi under different water temperature treatments.

Figure 5

Response of gas exchange parameters of pak choi leaves to different water temperature treatments. (A) Net photosynthetic rate (Pn); (B) Transpiration rate (E); (C) Stomatal conductance (gs); (D) Intercellular CO_2 concentration (Ci).

Figure 6

(A) Response of far-red light induced P700 redox kinetics to different water temperature treatments; (B) Response of functional PQ pools to different water temperature treatments;(C) Effects of different water temperature treatments on the maximum oxidation state of PSI (Pm) of pak choi leaves; (D) Effects of low temperature water treatment on the maximum photochemical efficiency of PSI (Fv/Fm) of pak choi leaves;

Figure 7

Effects of different water temperature treatments on the rapid light curves of photosynthetic quantum yields of PSI and PSII. (A) RLC of the quantum yield of regulated energy dissipation in PSII [Y(NPQ)]; (B) RLC of the quantum yield of PSI non-photochemical energy dissipation due to the donor-side limitation [Y(ND)]; (C) RLC of the quantum yield of PSI non photochemical energy due to the acceptor-side limitation [Y(NA)]. (D) photochemical quenching coefficient (qP). Values are means of four replicates±SD. Statistically significant differences of every sample compared to the control are marked by asterisks (*: $p < 0.05$, **: $p < 0.01$)

Figure 8

Effects of low temperature water treatment on photosynthetic electron transport in pak choi. (A) Rapid light curve (RLC) of the photosynthetic electron transport rate of PSII (ETRII) of pak choi; (B) RLC of the photosynthetic electron transport rate of PSI (ETRI); (A) RLC of the effective quantum yield of PSII photochemistry [Y(II)]; (B) RLC of the quantum yield of PSI photochemistry [Y(I)];