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Effects of High Temperature and Waterlogging Stress on Cellulase Activity of Non-heading Chinese Cabbage

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Abstract Two non-heading Chinese cabbage varieties 'Heiyoudong' and 'Suzhouqing' were used as materials to study the effects of high temperature and waterlogging stress on the activities of enzymes related to cellulose synthesis in non-heading Chinese cabbage. The two varieties had different resistance to high temperature and waterlogging. Three temperature gradients of 24℃, 35℃ and 40℃ were set, and each temperature was set two treatments, waterlogging and non-waterlogging. The results showed that under high temperature stress, the CesA, SS, and KOR enzyme activities of 'Heiyoudong' and 'Suzhouqing' showed a trend of first increasing and then decreasing, while the trend of CE enzyme activity was opposite. Under the combined stress of high temperature and waterlogging, the changed trend of the enzyme activities related to cellulose synthesis in 'Heiyoudong' and 'Suzhouqing' is similar to that of a single high temperature stress, but the degree of impact is greater than that of a single high temperature stress. In addition, regardless of high temperature stress, high temperature and waterlogging combined stress, the increase in CesA, SS, and KOR activities in 'Heiyoudong' was greater than that of 'Suzhouqing', but the decrease was less than that of 'Suzhouqing'; The increase in CE activity of 'Suzhouqing' is less than that of 'Suzhouqing', but the decrease rate is greater than that of 'Suzhouqing'.

Keywords Non-heading Chinese cabbage; High temperature; Waterlogging; Cellulase enzyme

Cellulose is the most abundant biomacromolecule and an important renewable resource on Earth, and is an important component of the primary and secondary cell walls of plants (Delmer, 1999). Cellulose cannot be digested by enzymes in the human gastrointestinal tract and is an insoluble dietary fiber, but it is also beneficial to human health (Zhao et al., 2014). The content of cellulose was slightly different among non-heading Chinese cabbage varieties and different parts of the same variety, and its content was closely related to the edible taste, nutritional quality and cultivation method of vegetables (Cui et al., 2008). In recent years, with the rapid development of biotechnology, more and more researchers have paid attention to the cellulose synthase gene, and the structure and function of the gene have been continuously improved (Sun, 2018). Sucrose synthase (SS) catalyzed the binding of UDPG from sucrose and UDP to cellulose synthase complex to synthesize β-1, 4-glucoside chain. Then, the intra-molecular and intermolecular hydrogen bonds and van der Waals forces made the 36 β-1, 4-glucoside chains formed by glucose residues to converge into glucan chains, and finally, with the regulation and assistance of KORRIGAN (KOR), further polymerized into cellulose structural unit microfibrils (Liu, 2010). Therefore, the main function of cellulose synthase (CesA) is to produce β-1, 4 glucose chains, and the initiation, extension and termination of β-1, 4 glucose chains is the process of cellulose biosynthesis in plants. This process requires the co-participation of multiple cellulose synthase genes, such as cellulose synthase, sucrose synthase, cellulase enzyme (CE) and KOR (Li et al., 2005).

Non-heading Chinese cabbage (*Brassica campestris* L. *ssp. chinensis* Makino) are a subspecies of Yuntaishu (*Brassica*) in Shizi Huake (*Brassicaceae*). It is also known as Qingcai (*Brassica rapa* var. *chinensis* (Linnaeus) Kitamura) and Xiaobaicai in Chinese. It originated in China and is widely planted in the middle and lower reaches of the Yangtze River. It is a popular leafy vegetable (Lu et al., 2018) with a short growth cycle and rich nutrition, containing a large amount of vitamins, dietary fiber and minerals. Non-heading Chinese cabbages like cold climate, shallow root system. Therefore, it often suffers from high temperature and waterlogging stress, slows down its growth, has low yield in summer cultivation, bitter taste, increases cellulose content and decreases quality (Liu et al., 2005). Therefore, it has become an urgent problem to breed non-heading Chinese cabbage varieties with high temperature and waterlogging resistance, so as to maintain good taste and improve nutritional value. In this study, the effects of high temperature and waterlogging stress on cellulose synthesis related enzyme activities of two non-heading Chinese cabbage varieties ‘Heiyoudong’ and ‘Suzhouqing’ with different waterlogging and high temperature tolerance were studied. This paper provides an effective way and theoretical basis for the application of non-heading Chinese cabbages in the efficient production and quality improvement of vegetables.

1 Results and Analysis

1.1 Effects of high temperature stress on enzymatic activities related to cellulose synthesis in non-heading Chinese cabbage

1.1.1 Effect of high temperature stress on CesA activity of non-heading Chinese cabbage

Under high temperature stress, the CesA activity of non-heading Chinese cabbage increased first and then decreased, and the range of change increased with the increase of stress temperature. Under T2 stress for 24 h, the CesA activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 26.25% and 14.29% compared with that of T1, respectively. Under T3 stress for 24 h, CesA activity increased by 52.50% and 39.84% compared with T1, respectively. After 48 h of high temperature stress, CesA activity decreased compared with that of 24 h, but was still significantly higher than that of T1. Under the same high temperature stress, the decrease of CesA activity of ‘Heiyoudong’ was smaller than that of ‘Suzhouqing’. After 5 days of recovery, the CesA activity of ‘Heiyoudong’ had no significant change, but was still significantly higher than that of T1, and the CesA activity of ‘Suzhouqing’ recovered to the level of T1 (Figure 1).

The effects of high temperature stress on the activity of CesA in different varieties were slightly different. Under the same treatment, the increase amplitude of CesA activity of ‘Heiyoudong’ was greater than that of ‘Suzhouqing’, but the decrease amplitude was smaller than that of ‘Suzhouqing’.

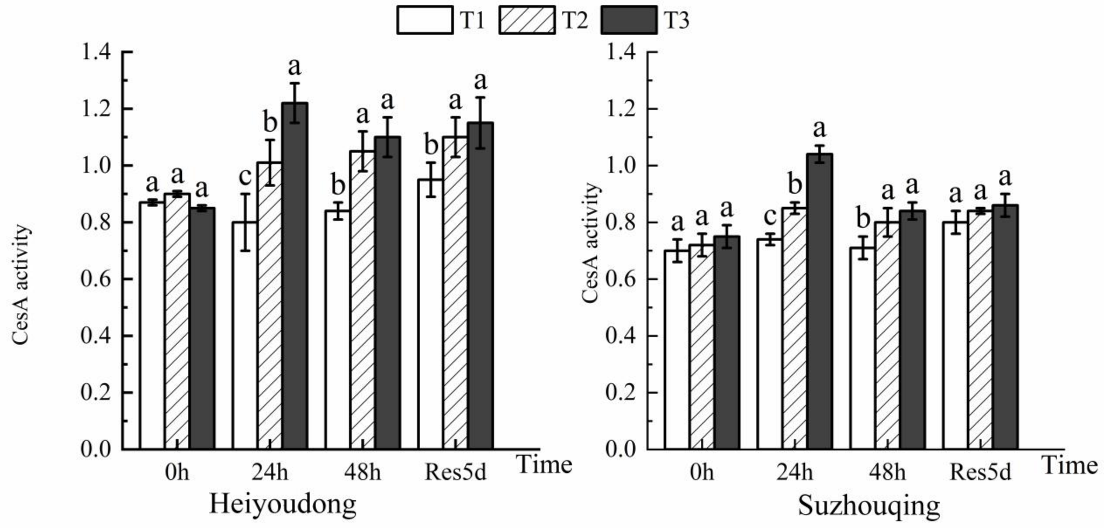

Figure 1 Effect of high temperature stress on CesA activity in non-heading Chinese cabbage

1.1.2 Effect of high temperature stress on SS activity of non-heading Chinese cabbage

Under high temperature stress, the SS activity of non-heading Chinese cabbage increased first and then decreased, and the change range increased with the increase of stress temperature. Under T2 stress for 24 h, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased 40.32% and 23.59% compared with T1, respectively. Under T3 stress for 24 h, the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 72.15% and 45.63% higher than that of T1, respectively. After 48 h of high temperature stress, the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased compared with 24 h, but was still significantly higher than that of T1. Under T2 stress for 48 h, the SS activity of

‘Heiyoudong’ and ‘Suzhouqing’ was 37.36% and 17.51% higher than that of T1, respectively. Under T3 stress for 48 h, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 47.64% and 30.32% compared with T1, respectively. After 5 days of recovery, the SS activity of ‘Heiyoudong’ had no significant change, but was still significantly higher than that of T1, and the SS activity of ‘Suzhouqing’ recovered to the level of T1 (Figure 2).

High temperature stress affected the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ to different degrees. Under the same treatment, the SS activity of ‘Heiyoudong’ increased more than ‘Suzhouqing’, but decreased less than ‘Suzhouqing’.

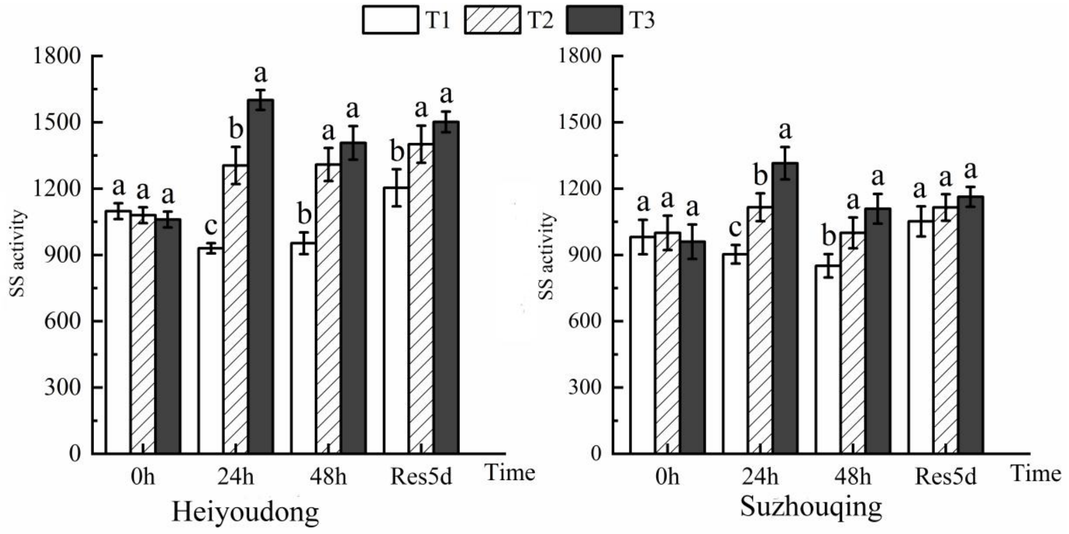

Figure 2 Effect of high temperature stress on SS activity in non-heading Chinese cabbage

1.1.3 Effect of high temperature stress on KOR activity of non-heading Chinese cabbage

Under high temperature stress, the KOR activity of non-heading Chinese cabbage increased first and then decreased, and the range of change increased with the increase of stress temperature. Under T2 stress for 24 h, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 30.77% and 18.11% compared with T1, respectively. Under T3 stress for 24 h, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 53.85% and 33.86% higher than that of T1. After 48 h of high temperature stress, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased compared with 24 h, but was still significantly higher than that of T1. Under T2 stress for 48 h, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 15.33% and 10.91% higher than that of T1, respectively. Under T3 stress for 48 h, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 23.33% and 19.09%, respectively, compared with that of T1. After 5 days of recovery, the KOR activity of ‘Heiyoudong’ did not change significantly, but was still significantly higher than that of T1. The KOR activity of ‘Suzhouqing’ recovered to the level of T1 (Figure 3).

The response degree of KOR activity to high temperature stress was different among different varieties. Under the same treatment, the KOR activity of ‘Heiyoudong’ increased more than that of ‘Suzhouqing’, but decreased less than that of ‘Suzhouqing’.

1.1.4 Effect of high temperature stress on CE activity of non-heading Chinese cabbage

Under high temperature stress, the CE activity of non-heading Chinese cabbage decreased first and then increased, and the change range increased with the increase of stress temperature. Under T2 stress for 24 h, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased by 33.03% and 20.77% compared with T1, respectively. Under T3 stress for 24 h, CE activity was 50.50% and 33.43% lower than that of T1. After 48 h of high temperature stress, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ was increased compared with 24 h, but significantly lower than that of T1. Under T2 stress for 48 h, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 23.10% and 12.42% lower than that of T1, respectively. Under T3 stress for 48 h, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased by 29.07% and 16.16% compared with T1, respectively. After 5 days of recovery, the CE activity of

‘Heiyoudong’ had no significant change and was lower than T1. The CE activity of ‘Suzhouqing’ had no significant change, and all of them returned to T1 level (Figure 4).

CE activity of different varieties was different in response to high temperature stress. Under the same treatment, the CE activity of ‘Heiyoudong’ increased less than that of ‘Suzhouqing’, but decreased more than that of ‘Suzhouqing’.

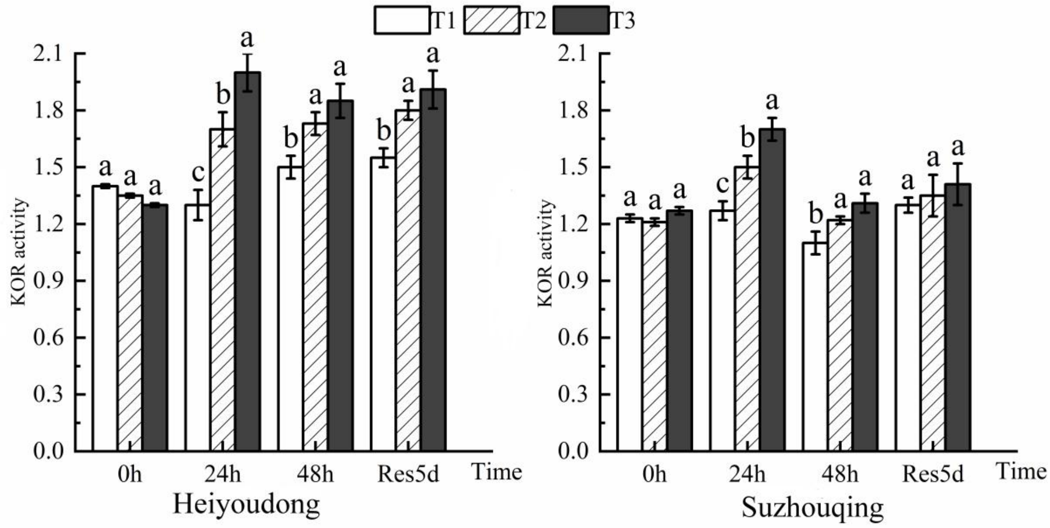

Figure 3 Effect of high temperature stress on KOR activity in non-heading Chinese cabbage

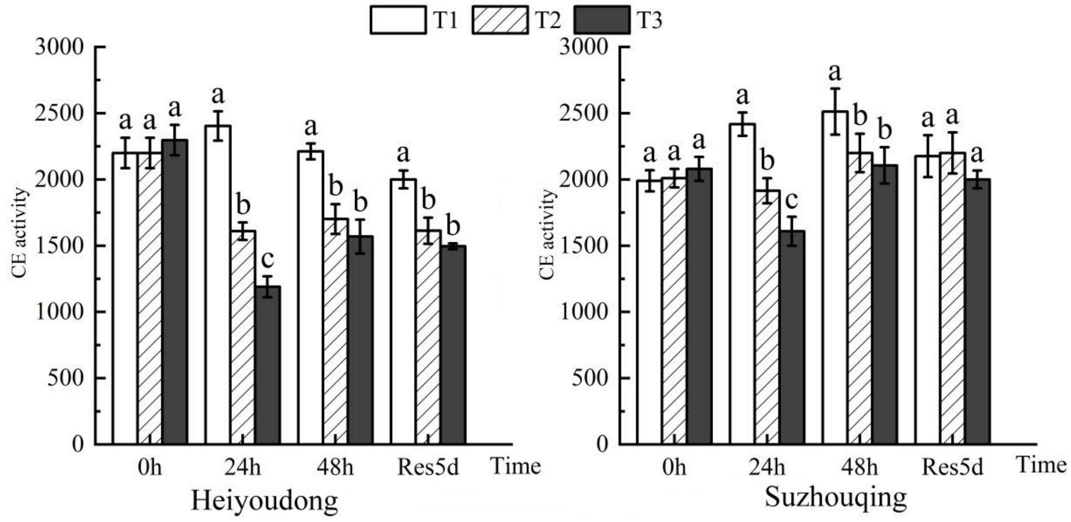

Figure 4 Effect of high temperature stress on CE activity in non-heading Chinese cabbage

1.2 Effects of high temperature and waterlogging stress on enzymatic activities related to cellulose synthesis in non-heading Chinese cabbage

1.2.1 Effects of high temperature and waterlogging stress on CesA activity of non-heading Chinese cabbage

Under high temperature and waterlogging stress, the CesA activity of non-heading Chinese cabbage increased first and then decreased, and the range of change increased with the increase of stress temperature. Under W2 stress for 24 h, the CesA activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased significantly by 20.00% and 10.67% compared with W1, respectively. Under W3 stress for 24 h, Ces activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 34.44% and 22.67% higher than that of W1, respectively. After 48 h of high temperature and waterlogging stress, the CesA activity of ‘Heiyoudong’ and ‘Suzhouqing'' showed a downward trend and was significantly lower than that of W1. Under W2 stress for 48 h, the CesA activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 19.10% and 29.85% lower than that of W1. Under W3 stress for 48 h, the CesA activity of ‘Heiyoudong’ and ‘Suzhouqing'' decreased by 32.58% and 44.89% compared with W1, respectively. After 5 days of recovery, the CesA activity of

‘Heiyoudong’ and ‘Suzhouqing’ was still not improved, and was significantly lower than that of W1 (Figure 5).

The effects of high temperature and waterlogging stress on the activity of CesA in different varieties were slightly different. Under the same treatment, the increase amplitude of CesA activity of ‘Heiyoudong’ was greater than that of ‘Suzhouqing’, but the decrease amplitude was smaller than that of ‘Suzhouqing’.

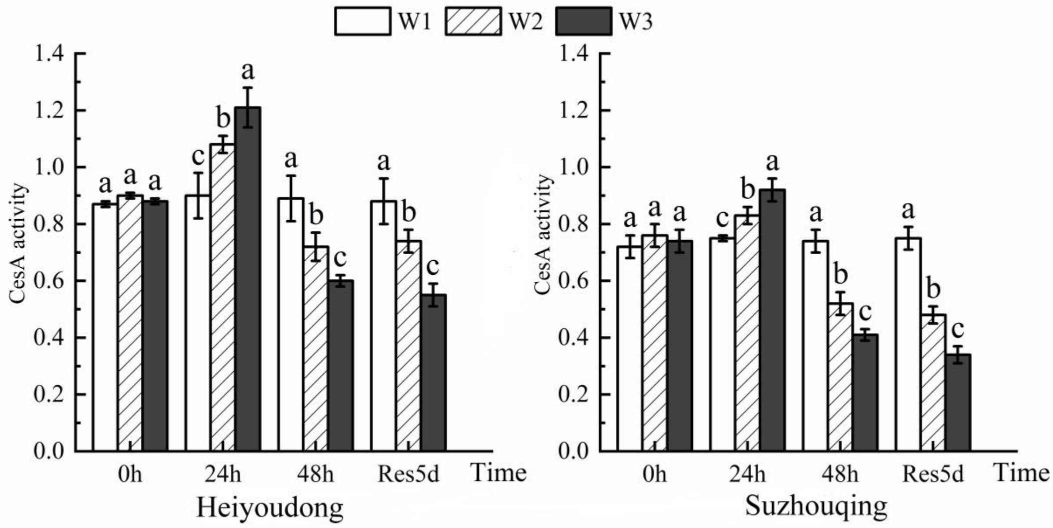

Figure 5 Effect of high temperature and waterlogging stress on CesA activity in non-heading Chinese cabbage

1.2.2 Effects of high temperature and waterlogging stress on SS activity of non-heading Chinese cabbage

Under high temperature and waterlogging stress, the SS activity of non-heading Chinese cabbage increased first and then decreased, and the change range increased with the increase of stress temperature. Under W2 stress for 24 h, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ was significantly increased by 26.50% and 18.86% compared with W1, respectively. Under W3 stress for 24 h, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 44.20% and 34.59% higher than that of W1, respectively. After 48 h of high temperature and waterlogging stress, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ showed a decreasing trend and was significantly lower than that of W1. Under W2 stress for 48h, the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 16.00% and 35.11% lower than that of W1, respectively. Under W3 stress for 48 h, SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased by 33.00% and 50.30% compared with W1, respectively. After 5 days of recovery, the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ was still significantly lower than that of W1 (Figure 6).

High temperature and waterlogging stress affected the SS activity of ‘Heiyoudong’ and ‘Suzhouqing’ to different degrees. Under the same treatment, the SS activity of ‘Heiyoudong’ increased more than ‘Suzhouqing’, but decreased less than ‘Suzhouqing’.

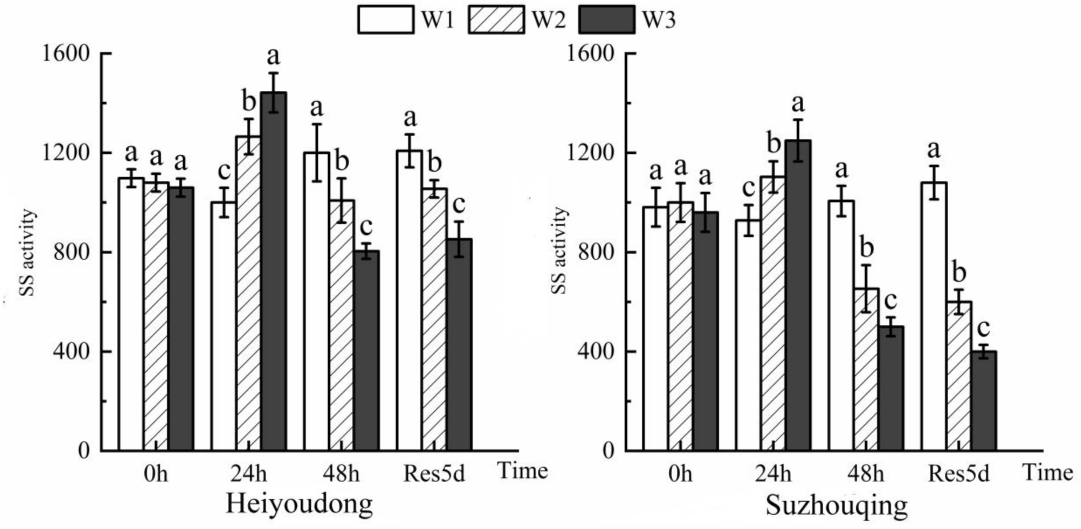

Figure 6 Effect of high temperature and waterlogging stress on SS activity in non-heading Chinese cabbage

1.2.3 Effect of high temperature and waterlogging stress on KOR activity of non-heading Chinese cabbage

Under high temperature and waterlogging stress, the KOR activity of non-heading Chinese cabbage increased first and then decreased, and the range of change increased with the increase of stress temperature. Under W2 stress for 24 h, KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 17.24% and 12.00% compared with W1, respectively. Under W3 stress for 24 h, KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ increased by 37.93% and 28.00%, respectively. After 48 h of high temperature and waterlogging stress, KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ showed a decreasing trend and was significantly lower than that of W1. Under W2 stress for 48 h, KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ was 14.38% and 32.67% lower than that of W1, respectively. Under W3 stress for 48 h, the KOR of ‘Heiyoudong’ and ‘Suzhouqing’ decreased by 30.70% and 50.00% respectively. After 5 days of recovery, the KOR activity of ‘Heiyoudong’ and ‘Suzhouqing’ was still not improved, and was significantly lower than that of W1 (Figure 7).

The KOR activity of different varieties was different in response to high temperature and waterlogging stress. Under the same treatment, the KOR activity of ‘Heiyoudong’ increased more than that of ‘Suzhouqing’, but decreased less than that of ‘Suzhouqing’.

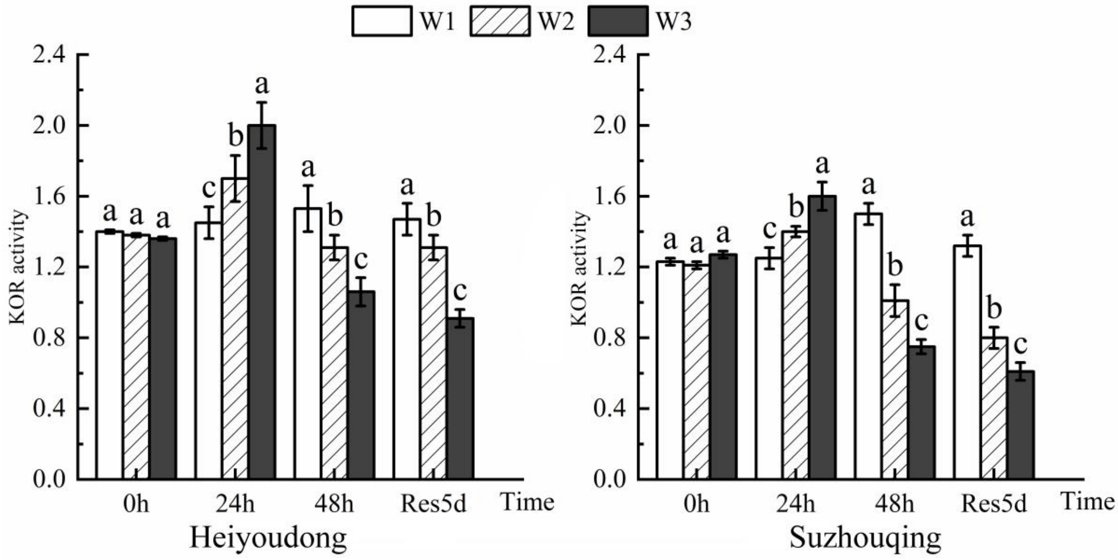

Figure 7 Effect of high temperature and waterlogging stress on KOR activity in non-heading Chinese cabbage

1.2.4 Effects of high temperature and waterlogging stress on CE activity of non-heading Chinese cabbage

Under high temperature and waterlogging stress, the CE activity of non-heading Chinese cabbage decreased first and then increased, and the change range increased with the increase of stress temperature. Under W2 stress for 24 h, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ decreased by 14.84% and 9.52% compared with that of W1, respectively. Under W3 stress for 24 h, CE activity decreased by 30.85% and 19.05%, respectively. After 48 h of high temperature waterlogging stress, the CE activity of varieties ‘Heiyoudong’ and ‘Suzhouqing’ was increased compared with 24 h, and was significantly higher than the control level. Under W2 stress for 48 h, CE activity was 18.16% and 28.91% higher than that of W1, respectively. Under W3 stress for 48 h, CE activity increased by 32.07% and 54.75%, respectively. After 5 days of recovery, the CE activity of ‘Heiyoudong’ and ‘Suzhouqing’ under high temperature waterlogging treatment was still significantly higher than that of W1 (Figure 8).

CE activity of different varieties was different in response to high temperature and waterlogging stress. Under the same treatment, the CE activity of ‘Heiyoudong’ increased less than that of ‘Suzhouqing’, but decreased more than that of ‘Suzhouqing’.

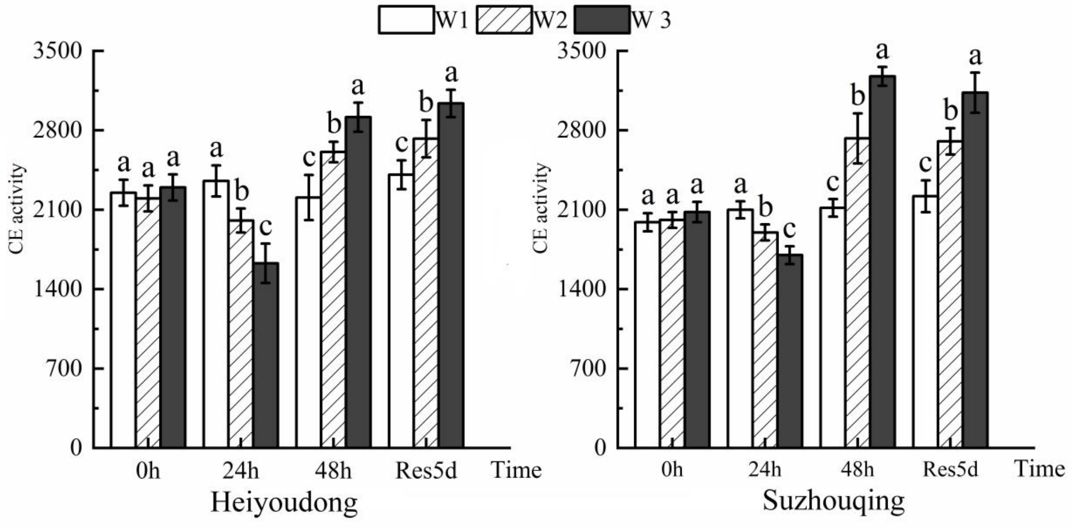

Figure 8 Effect of high temperature and waterlogging stress on CE activity in non-heading Chinese cabbage

2 Discussion

Cellulose is the most abundant biological macromolecule and an important renewable resource on earth. It is also the main component of the primary and secondary cell walls of plants. The content of cellulose is closely related to the taste, quality and resistance of vegetables. The synthesis of plant cellulose is regulated by temperature. Jiang (2005) proposed that the optimal temperature for cotton fiber development is about 24℃, but when the temperature is lower than 15℃, the activity of sucrose synthase decreases, the activity of β-1, 3-glucanase increases, the conversion efficiency of soluble sugar to cellulose decreases, the content of cellulose decreases, and the content of soluble sugar increases relatively. It has adverse effect on cotton yield and fiber quality. Cellulose synthesis requires the participation of multiple *CesA* genes, the main function of CesA is the production of β-1, 4 glucose chains. In addition to CesA, the activity and gene expression of SS also directly affect the synthesis and accumulation rate of cellulose, which is also a key enzyme in the process of plant cellulose synthesis (Shimizu et al., 1997). Berenice et al. (1991) found that SS mainly converts excess carbohydrates into cell wall polysaccharides under hypoxic conditions in potato, which can participate in the glycolytic metabolism process in plants after ventilation recovery. Thus, ATP growth and NAD^+ regeneration were maintained and plant tolerance to waterlogging stress was improved. Liu et al. (2016) found that in the process of waterlogging treatment, the activities of SS, SPS, AI and aldolase first increased and then decreased. This may be the response of leaves to waterlogging stress. In this study, SS activity increased under the combined stress of high temperature and waterlogging for 24 h. This was consistent with the results of previous research (Berenice et al., 1991; Liu et al., 2016). However, with the extension of time, the SS activity was lower than the normal temperature level, and the SS activity showed an increasing trend under the single stress of high temperature. CE is a complex enzyme of hydrolyzed cellulose and can decompose cellulose into cellobiose and glucose. Some studies have shown that under waterlogging stress, plants produce a large amount of ethylene, which promotes the expression of related genes and enhances the activity of CE, thus expanding the intercellular space and generating aerenchyma to adapt to the anoxic environment caused by waterlogging, thus reducing the damage to plants caused by waterlogging adversity (Zhao, 2003). The results of this study showed that single high temperature stress did not increase the CE activity of ‘Heiyoudong’, but decreased, while ‘Suzhouqing’ increased slightly. Under the combined stress of high temperature and waterlogging, ‘Heiyoudong’ and ‘Suzhouqing’ showed a trend of decreasing first and then increasing with the increase of time, which was partially different from the study of Zhao (2003). KOR is a membrane-associated endoglucanase that edits and monitors the transformation of glucan chains into microfilaments, cuts into defective glucan chains, and is associated with cellulose synthesis on cell walls (Delmer and Haigler, 2002; Mølhøj et al., 2002). Therefore, the biosynthesis of plant cellulose is regulated by CesA, SS, KOR, CE and other enzymes.

This study showed that under high temperature stress, the CesA, SS and KOR enzyme activities of ‘Heiyoudong’ and ‘Suzhouqing’ all increased first and then decreased, while the CE enzyme activities showed the opposite pattern. The increase of CesA, SS and KOR enzyme activities and the decrease of CE enzyme activities were conducive to the accumulation of cellulose, which enhanced the mechanical support ability and rigidity of the cell wall to adapt to high temperature stress. However, with the extension of stress time, the activities of CesA, SS and KOR decreased and the activities of CE increased, which promoted the degradation of cellulose. On the one hand, it may be that high temperature stress inhibited the activity of enzymes related to cellulose synthesis; on the other hand, high temperature stress may hinder the conversion of soluble sugar to cellulose. It may also be that callose is synthesized in large quantities in plants, and that callose competes with cellulose for the same substrate UDPG. This reduces the substrate for cellulose synthesis, thus reducing the synthesis rate and inhibiting the activity of related enzymes. In addition, under the condition of drought and water shortage, the expression of genes related to cellulose synthesis was blocked, leading to the decrease of cellulose content (Yue et al., 2010). Tang et al. (2017) found that the nutrient accumulation of cotton in full flowering stage decreased the most after 10 days of flooding. Compared with the control, the quality of cotton was significantly reduced and the yield was seriously reduced. The above studies show that water also has an effect on cellulose synthesis. This study showed that under high temperature stress, the decrease amplitude of CesA and CE enzyme activities in ‘Heiyoudong’ was lower than that in ‘Suzhouqing’, while the increase amplitude of SS and KOR was higher than that in ‘Suzhouqing’. Under the combined stress of high temperature and waterlogging, the increasing trend of CesA enzyme activities in ‘Heiyoudong’ was higher than that in ‘Suzhouqing’, while the decreasing trend of SS, KOR and CE enzyme activities was lower than that in ‘Suzhouqing’. This result may be related to the different degrees of high temperature and waterlogging tolerance of the two varieties. The influence of enzyme activities of CesA, SS, KOR and CE in ‘Suzhouqing’ was more severe than that under single high temperature stress, which may be a stress response of ‘Heiyoudong’ and ‘Suzhouqing’ to cope with high temperature flooding adversity.

3 Materials and Methods

3.1 Materials of test

In this experiment, ‘Heiyoudong’ with strong waterlogging resistance and high temperature resistance and ‘Suzhouqing’ with weak waterlogging resistance and low temperature resistance were used as materials. These two varieties, with dark green leaves, good upright and excellent commodity traits, were selected and bred independently by the Green Vegetable Research Group of Horticulture Research Institute of Shanghai Academy of Agricultural Sciences.

3.2 Methods of test

The experiment was conducted at the Horticulture Research Institute, Shanghai Academy of Agricultural Sciences from May 2018 to January 2019. The seeds were seeded in a plastic nutrient bowl with an upper diameter of 7 cm, a lower diameter of 5 cm and a height of 7 cm. Earthworm soil was selected to grow the seedlings, and the seedlings were placed in a plastic greenhouse for conventional cultivation management. When the seedlings grow to four leaves and one heart, the plants with good growth and uniformity were randomly grouped and transferred to the artificial climate chamber for experimental treatment. The temperature of the three artificial climate chambers was set as 24°C/19°C (d/n) (CK), 35°C/25°C (d/n), 40°C/30°C (d/n), the humidity was constant 75%, and the light was 10 h/14 h (d/n). Two varieties, ‘Heiyoudong’ and ‘Suzhouqing’, are placed in each artificial climate box. Each variety is treated with waterlogged and non-waterlogged respectively, with a total of 12 horizontal treatments (Table 1). Waterlogging treatment means that the water surface is about 1 cm above the soil layer surface, and the lost water is timely replenished every day to ensure the waterlogging height, while non-waterlogging treatment carries out conventional water management. After 48 h treatment in the artificial climate box, the seedlings were moved to the plastic greenhouse to recover for 5 days. Three replicates were set for each treatment, and three plants were selected for each replicate. The third functional leaf of each treated plant was rapidly frozen in liquid nitrogen at 0, 24 and 48 h after experimental treatment and on the 5th day of recovery after stress relief (Res 5 d), and was stored in the refrigerator at -80°C for the determination of related physiological and biochemical indexes.

Table 1 Processing level of non-heading Chinese cabbage

Treatment level	Temperature		
	24 °C	35 °C	40 °C
Waterlogging	W1	W2	W3
Non-waterlogging	T1	T2	T3

3.3 Determination of enzyme activity related to cellulose synthesis

Determination methods: The activities of cellulose synthase (CesA), sucrose synthase (SS), cellulase enzyme (CE) and KOR in the samples were determined by double antibody sandwich method.

The antibody was captured by purified plant cellulose synthetase and coated on the microplate to prepare solid phase antibody. Plant cellulose synthase was added into the coated micropores successively, and then combined with HRP labeled detection antibody to form antibody-antibody-enzyme-labeled antibody complex. After thorough washing, substrate TMB was added for color development. TMB turns blue under the catalysis of HRP enzyme, and finally turns yellow under the action of acid. The shade of the color was positively correlated with the plant cellulose synthase in the sample. The absorbance (OD value) of the sample was measured at 450 nm wavelength with the enzyme label instrument, and the content of plant cellulose synthase in the sample was calculated by the standard curve (kit from Shanghai Enzyme-linked Biotechnology Co., Ltd.). The activities of sucrose synthase (SS), cellulase enzyme (CE) and KOR were measured by this method.

3.4 Processing of data

Microsoft Office Excel 2003 software was used for data entry and SPSS19.0 statistical software was used for variance analysis of the data. Duncan's new complex range method was used to make multiple comparisons, and origin 8.0 software was used to make charts.

Authors’ contributions

ZHF was the experimental designer and executor of this study. GL and HXY completed data analysis and the writing of the first draft of the paper. LXF and XDD participated in the experimental design and analyzed the experimental results. ZYY was the proposer and leader of the project, directing experimental design, data analysis, paper writing and modification. All authors read and approved the final manuscript.

Acknowledgments

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