Influence of High Temperature on Photosynthesis, Antioxidative Capacity of Chloroplast, and Carbon Assimilation among Heat-tolerant and Heat-susceptible Genotypes of Nonheading Chinese Cabbage

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Abstract. High temperature (HT) is a major environmental stress limiting oversummer production of nonheading Chinese cabbage (NHCC, Brassica campestris ssp. chinensis Makino). In the present study, the effects of HT on photosynthetic capacity, including light reaction and carbon assimilation, were completely investigated in two NHCC, ‘xd’ (heat-tolerant), and ‘sym’ (heat-susceptible). The two genotypes showed significant differences in plant morphology, photosynthetic capacity, and photosynthetic metabolism (carbohydrate assimilation). HT caused a decrease in photosynthesis, chlorophyll contents, and photochemical activity in NHCC. However, these main photosynthetic-related parameters, including net photosynthetic rate ($P_N$), maximal photochemical efficiency of PSII ($F_v/F_m$), and total chlorophyll content in ‘xd’, were significantly higher than those of ‘sym’ plants. The antioxidant contents and antioxidative enzyme activities of ascorbic acid-reduced glutathione cycle in the chloroplast of ‘xd’ were significantly higher than those of ‘sym’. Microscopic analyses revealed that HT affected the structure of photosynthetic apparatus and membrane integrity to a different extent, whereas ‘xd’ could maintain a better integrated chloroplast shape and thylakoid. Inhibited light reaction also hampered carbon assimilation, resulting in a decline of carboxylation efficiency and imbalance of carbohydrate metabolism. However, larger declined extents in these data were presented in ‘sym’ (heat-susceptible) than ‘xd’ (heat-tolerant). The heat-tolerant genotype ‘xd’ had a better capacity for self-protection by improved light reaction and carbon assimilation responding to HT stress.

Climate model predicts that the average global temperature will increase 1.8 to 4 °C before the end of the 21st century. The additional warming above 2 °C could result in losses of more than 25% to crop yield (IPCC, 2014). Global-scale assessment by IPCC (2013) revealed that the frequency of extreme high temperature (HT) has increased in large parts of Asia, Australia, and Europe. HT could hamper plants growth or cause considerable pre- and postharvest losses because of a shortened life cycle and accelerated senescence. The photosynthesis in C3 plant is more sensitive to HT, especially the photosynthetic apparatus (Wahid and Rasul, 2005). HT differently affects the stability of many proteins and membrane system and alters the efficiency of enzymatic reactions in the cell (Hemantaranjan et al., 2014). Diminished photosynthetic capacity is a primary damage effect by HT. Therefore, research on photosynthetic capacity in vegetable plants has become a crucial approach to evaluate the heat tolerance of vegetable genotypes.

NHCC is one of the most important vegetables in China and is now cultivated around the world. It is very popular in the world and has a great market demand. NHCC is sensitive to heat stress and it could cause inevitable injury. To achieve oversummer production and meet the needs of year-round market, it is important to breed heat-tolerant NHCC genotype by exploring the heat tolerance mechanisms in various genotypes. The present study was carried out with two genotypes NHCC (heat-tolerant and heat-susceptible) to investigate the photosynthetic capacity, including photosynthesis, antioxidative system, and ultrastructure of chloroplast, and photosynthetic metabolism responding to HT. Determination of responses and possible strategies to improve HT tolerance is significant to NHCC to select and breed heat-tolerant genotype.

Materials and Methods

Plants materials and experimental design. Seeds of NHCC cultivars were germinated in pots in a phytotron at Anhui Agricultural University. Germinated seedlings were grown under controlled conditions (day/night 20 °C/15 °C), PPF density (PPFD) of 600 µmol·m−2·s−1, relative humidity of 70%, and a photo period of 14/10 h (light/dark). The seedlings were cultivated under normal growth conditions.

Plants with the six-leaf stage were divided into two groups, respectively. The control group was kept at 20 °C/15 °C. Based on the previous experiment, the treatment groups were subjected to HT at 37 °C/27 °C. The control group of growth condition was similar to the one previously described. After 6 d, the plants were sampled for physiological and biochemical analyses. The seedlings were organized in a complete randomized block design.

Growth parameters. Growth parameters were obtained from 15 seedlings in each replication. Plant height, leaf length, leaf width, petiole length, and petiole width were measured by a ruler. Stem diameter was measured by a vernier caliper. The shoot fresh weight was directly weighted, then the seedlings were dried in an oven at 75 °C for 48 h to gain the shoot dry weight.

Chlorophyll (Chl) contents. The Chl content was determined as the method of Strain and Svec (1966). The pigments were extracted from the forth fully expanded leaf with a mixed solution containing acetone, ethanol, and water (the volume ratio, 4:5:4:5:1). Photosynthetic parameters and CO2 exchange. Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), intercellular CO2 concentration ($C_i$), and transpiration rate ($T_i$) were measured using a portable photosynthetic system Li-6400 (LI-COR, Inc., USA) on the forth fully expanded leaf, which is measured from the center of the plant. Carboxylation efficiency (CE) was calculated using the following relationship: CE (%) = ($P_N/C_i$) × 100.

Simultaneously, the CO2 assimilation vs. CO2 concentration curve was measured at a PPF of 700 µmol·m−2·s−1 with Li-6400. The maximum carboxylation rate of Rubisco ($V_{c,max}$) and maximum rates of RuBP regeneration ($J_{max}$) were estimated by fitting a maximum likelihood regression below and above the inflexion of the A/CO2 response curve according to McMurtrie and Wang (1993).

Chl fluorescence. The Chl fluorescence was measured using a portable fluorometer (PAM 2100; Walz, Effeltrich, Germany) as per Kooten and Snel (1990). The fully expanded forth leaves were dark-adapted for 30 min before measurement. Fo and Fm are the minimal and maximal fluorescence yield.
of leaves after dark adaptation, respectively; Fo’, Fs, and Fm’ are the minimal, steady-state, and maximal fluorescence level in illuminated leaves, respectively. These fluorescence parameters were used to calculate the following: 1) the actual photochemical efficiency of PSII, ΦPSII = (Fm’ – Fs)/Fm’; 2) the photochemical fluorescence quenching coefficient, qP = (Fm’ – Fs)/(Fm’ – Fo’); 3) nonphotochemical quenching coefficient, qN = (Fm – Fm’)/(Fm’ – Fo’); and 4) the maximal photochemical efficiency of PSII, ΦV/Fm = (Fm – Fo)/Fm’.

Isolation of chloroplast. Chloroplast isolation was carried out in accordance with the method of Joly and Carpenter (2011) with slight modifications. Forty gram of leaves were grinded in about 150 mL of buffer A (50 mM Hepes NaOH, 330 mM sorbitol, 2 mM EDTA, 1 mM MgCl2 and 1 mM MnCl2), and the homogenate was filtered two times on two layers of Miracloth tissue. The filtrate was centrifuged at 750 × g for 60 s. The supernatant was discarded, and the pellet was resuspended in about 30 mL of buffer B (50 mM Hepes–NaOH, 330 mM sorbitol, 2 mM EDTA, 1 mM MgCl2, 1 mM MnCl2, 10 mM KCl and 1 mM NaCl). Then, the mixture was centrifuged again, and the supernatant was discarded. Finally, the pellet was resuspended in a small volume of buffer B (1.5 mL). All procedures were carried out at 4 °C.

Malondialdehyde (MDA) and hydrogen peroxide (H2O2) content in chloroplast. The level of lipid peroxidation was measured by estimating MDA content (a product of lipid peroxidation) using thiobarbituric acid (TBA) according to Xu et al. (2008).

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Table 1. Effects of high temperature on plant morphology in two nonheading Chinese cabbage cultivars.

| Treatment | Plant ht (cm) | Stem diam (mm) | Shoot fresh wt (g) | Shoot dry wt (g) |
|-----------|--------------|----------------|-------------------|-----------------|
| Cont-sym  | 9.07 ± 0.93 b| 3.99 ± 0.20 b  | 5.16 ± 0.37 b     | 0.39 ± 0.03 b   |
| Cont-xd   | 9.33 ± 0.61 a| 4.52 ± 0.21 a  | 5.66 ± 0.27 a     | 0.47 ± 0.02 a   |
| HT-sym    | 10.87 ± 0.21 a| 2.71 ± 0.18 d | 4.17 ± 0.20 d     | 0.30 ± 0.03 c   |
| HT-xd     | 8.97 ± 0.93 | 3.64 ± 0.05 c  | 4.89 ± 0.12 c     | 0.41 ± 0.04 b   |

Values are represented as the mean ± se (n = 15). Different letters in the same column indicate significant differences at P < 0.05 according to Duncan’s multiple range tests.

Results

Morphology parameters. Compared with control, stem diameter, fresh, and dry weights of shoot in heat-susceptible genotype ‘sym’ (HT-sym) were significantly decreased by 32.08%, 19.19%, and 23.08%, respectively, whereas an increase in plant height was by 19.85% (Table 1). In heat-tolerant genotype ‘xd’ (HT-xd), the stem diameter, fresh, and dry weights of shoot were all increased by 19.47%, 18.90%, and 12.77%, respectively, and the plant height was unaffected under HT. The stem diameter and shoot dry weight of ‘xd’ were more than that of ‘sym’ exposed to HT.

Compared with control, the petiole length and width of HT-sym treatment were obviously decreased by 10.19% and 21.92%, respectively, whereas the leaf length was increased by 18.89%. HT caused a decrease in the leaf length of ‘xd’, whereas the other indexes of leaf morphology had no obvious changes. Under HT, the leaf width and petiole length of ‘sym’ were more than that of ‘xd’, and the petiole width of ‘sym’ was lower than ‘xd’.

Under control condition, the plant height, leaf length, leaf width, and petiole length in ‘sym’ were more than that of ‘xd’, and the stem diameter, shoot biomass, and petiole width of ‘sym’ were lower than that of ‘xd’. The dry weights of shoot were also decreased by 18.27%, 24.41%, and 20.31% and those of HT-xd were 9.03%, 7.52%, and 14.69%, respectively (Table 2). Under normal condition, Chl a and a + b content of ‘xd’ were obviously higher than that of ‘sym’.

Photosynthetic parameters. Under HT, Ps, gs, Ci, Tr, and CE of two genotypes were not significantly different.
NHCC showed similar declined trends (Fig. 1). Compared with respective control, \( \frac{P}{N} \) of HT-sym and HT-xd were decreased by 61.95% and 37.68%; \( g_s \) of HT-sym and HT-xd were 57.38% and 34.65%; \( T_r \) were 65.91% and 33.33%; \( C_i \) were 62.75% and 33.59%, and \( C_E \) were 59.96% and 26.74%. From the Fig. 1, the declined range of HT-sym was more than HT-xd. Under control, \( \frac{P}{N} \) and \( T_r \) of Cont-xd were higher than Cont-sym.

\[ V_c, \max \text{ and } J_m \text{ of two genotypes were significantly reduced by HT (Fig. 6). The } V_c, \max \text{ of HT-sym and HT-xd were decreased by 36.44% and 23.59%, respectively, whereas the } J_m \text{ of two genotypes were 22.74% and 20.55%. } J_m \text{ of Cont-xd was higher than Cont-sym, and } V_c, \max \text{ of two genotypes had no change under control.} \]

Fluorescence parameters. The \( \Phi_PSI II \), \( \Phi_PSI II \) and \( F_v/Fm \) of HT-sym, and HT-xd were significantly decreased compared with their respective control (Fig. 2). Compared with control, \( \Phi_PSI II \) of HT-sym and HT-xd were decreased by 39.35% and 30.15% (Fig. 2A); \( \Phi_PSI II \) of HT-sym and HT-xd were 32.43% and 27.36% (Fig. 2B); \( F_v/Fm \) were 10.48% and 3.14% (Fig. 2D). Aforementioned data showed that the declined degree of \( \Phi_PSI II \), \( \Phi_PSI II \), and \( F_v/Fm \) in HT-sym was more than HT-xd. The \( q_N \) of HT-xd reached the highest value among all treatments, which was increased by 33.93% than HT-sym (Fig. 2C).

Under normal condition, \( \Phi_PSI II \) and \( \Phi_PSI II \) of Cont-xd were higher than that of Cont-sym, whereas \( q_N \) and \( F_v/Fm \) of two genotypes had no difference.

MDA and \( \text{H}_2\text{O}_2 \) content in chloroplast. After 6 d of HT treatment, the MDA content of HT-sym and HT-xd were significantly increased by 54.59% and 29.28%, respectively, to the control (Fig. 3A). The \( \text{H}_2\text{O}_2 \) content of HT-sym showed a 1.98-fold increase to Cont-sym, and HT-xd was 1.48-fold to Cont-xd (Fig. 3B). Under normal condition, the MDA and \( \text{H}_2\text{O}_2 \) contents of two genotypes were similar.

Antioxidant content in chloroplasts. Under HT, the total AsA content of HT-sym and HT-xd were significantly declined by 15.63% and 12.16%, respectively, compared with the control (Table 3). The AsA levels of HT-sym and HT-xd were remarkably decreased by 46.04% and 25.71%, respectively. DHA level of HT-sym was higher than other treatments. Compared with control, the AsA/DHA ratios of HT-sym and HT-xd were declined by 58.22% and 32.93%, respectively. Under normal condition, levels of the total AsA, AsA, DHA, and AsA/DHA ratio had no significant difference between Cont-sym and Cont-xd.

Compared with control, the total glutathione levels of HT-sym and HT-xd were markedly increased by 12.84% and 36.21%, respectively (Table 4). The GSH of HT-sym was decreased by 40.33% to the control, whereas that of HT-xd reached a 1.40-fold increase in the control. Among all treatments, the HT-sym had the highest level of GSSG.

The GSH/GSSG ratio of HT-sym was the lowest among treatments, whereas that of HT-xd got the highest value. Under normal condition, levels of the total glutathione, GSH, GSSG, and GSH/GSSG ratio were similar between Cont-sym and Cont-xd.

Activities of antioxidative enzymes in chloroplast. HT obviously enhanced the activities of APX by 30.44% and 33.39%, respectively, in HT-sym and HT-xd (Fig. 4A). The activity of GR showed similar changes as APX; GR activities of HT-sym and HT-xd

| Table 2. Effects of high temperature on chlorophyll contents in two nonheading Chinese cabbage cultivars. |
|-----------------|-----------------|-----------------|-----------------|
| Chl a | Chl b | Chl a + b |
|-----------------|-----------------|-----------------|
| Cont-sym | 8.35 ± 0.10 b | 4.14 ± 0.06 a | 12.49 ± 0.16 b |
| Cont-xd | 9.25 ± 0.21 a | 4.11 ± 0.09 a | 13.36 ± 0.26 a |
| HT-sym | 6.83 ± 0.18 d | 3.13 ± 0.05 c | 9.95 ± 0.16 d |
| HT-xd | 7.60 ± 0.16 c | 3.80 ± 0.10 b | 11.40 ± 0.26 c |

Values are represented as the mean ± se (n = 3). Different letters in the same column indicate significant differences at \( P < 0.05 \) according to Duncan’s multiple range tests.

Fig. 1. Effects of high temperature on photosynthesis in two NHCC genotypes. Values represent the mean ± SE (n = 3). Different letters in the same column indicate significant differences at \( P < 0.05 \) according to Duncan’s multiple range tests.

Fig. 2. Effects of high temperature on chlorophyll fluorescence parameters in two NHCC genotypes. Values represent the mean ± SE (n = 3). Different letters in the same column indicate significant differences at \( P < 0.05 \) according to Duncan’s multiple range tests.
were increased by 23.15% and 59.64%, respectively (Fig. 4B). Compared with the control, significant decreases in the activities of MDAR and DHAR were observed after treatment of HT (Fig. 4C and D). MDAR activities were reduced by 30.37% and 21.68% in HT-sym and HT-xd, respectively. DHAR activity of HT-sym had a reduction by 38.08%, and HT-xd was unaffected by HT (Fig. 7C).

Under normal condition, the APX activity of Cont-xd was higher than that of Cont-sym; the activities of GR, MDAR, and DHAR had no significant difference between the two genotypes of NHCC.

**Discussion**

HT during development period is a major limiting factor on the economic yield of crop plants, especially leafy vegetable. Therefore, understanding the effects of HT on physiology and the physiological traits linked to tolerance is imperative. In the present study, the rest of the enzyme activities were similar between the two genotypes.

Table 3. Effects of high temperature on total AsA, AsA, DHA, and AsA/DHA in two nonheading Chinese cabbage cultivars.

|            | AsA + DHA | AsA | DHA | Asa/DHA |
|------------|-----------|-----|-----|---------|
| Cont-sym   | 8.96 ± 0.06 ab | 5.30 ± 0.20 a | 3.66 ± 0.15 b | 1.46 ± 0.12 ab |
| Cont-xd    | 9.54 ± 0.30 a  | 5.95 ± 0.52 a  | 3.59 ± 0.22 b  | 1.67 ± 0.17 a  |
| HT-sym     | 7.56 ± 0.40 c  | 2.86 ± 0.18 c  | 4.70 ± 0.25 a  | 0.61 ± 0.03 c  |
| HT-xd      | 8.38 ± 0.24 bc | 4.42 ± 0.27 b  | 3.96 ± 0.14 b  | 1.12 ± 0.09 b  |

AsA = ascorbic acid; DHA = dehydroascorbate.

Table 4. Effects of high temperature on total GSH, GSH, GSSG, and GSH/GSSG in two nonheading Chinese cabbage cultivars.

|            | GSH + GSSG | GSH | GSSG | GSH/GSSG |
|------------|------------|-----|------|----------|
| Cont-sym   | 17.14 ± 0.54 c | 14.01 ± 0.58 b | 3.13 ± 0.03 b | 4.48 ± 0.23 b |
| Cont-xd    | 17.87 ± 0.19 bc | 14.86 ± 0.20 b | 3.01 ± 0.15 b | 4.96 ± 0.30 b |
| HT-sym     | 19.34 ± 0.28 b | 8.36 ± 0.51 c  | 10.98 ± 0.31 a | 0.77 ± 0.07 c  |
| HT-xd      | 24.34 ± 0.82 a  | 20.77 ± 0.81 a  | 3.58 ± 0.07 b  | 5.81 ± 0.25 a  |

GSH = reduced glutathione; GSSG = oxidized glutathione.

Fig. 3. Effects of high temperature on H₂O₂ content and Malondialdehyde content in two NHCC genotypes. Values represent the mean ± se (n = 3). Different letters in the same column indicate significant differences at P < 0.05 according to Duncan’s multiple range tests.

Fig. 4. Effects of high temperature on ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) activities in two NHCC genotypes. Values represent the mean ± se (n = 3). Different letters in the same column indicate significant differences at P < 0.05 according to Duncan’s multiple range tests.
two genotypes of NHCC showed different responses to heat stress on plant morphology and photosynthetic capacity. The results indicated that heat tolerance of NHCC was genotype specific, consistent with the report of Mishra et al. (2017).

HT treatment caused an increase in the plant height, leaf, and petiole length in heat-susceptible genotype 'sym' than in that of heat-tolerant 'xd' (Table 1). Although HT markedly inhibited biomass accumulation of both genotypes, the decline degrees of shoot fresh and dry weights in heat-tolerant HT-xd were obviously less than heat-susceptible HT-sym. This result presented that NHCC might improve the heat tolerance by regulating plant architecture and leaf shape.

Photosynthesis is one of the most sensitive physiological processes, which is remarkably influenced by HT in C3 plants (Yang et al., 2006). A great reduction of PN was linked to the decreases in chlorophyll contents (Table 2) as a result of reduced antenna pigments, restraining the light harvest during HT (Camejo et al., 2006). The alterations by HT in Chl content might be due to impaired biosynthesis or accelerated pigment degradation. Although HT reduced the Chl content, the reduction extent depended on heat tolerance of NHCC against HT. Chl content of heat-susceptible 'sym' was significantly less than heat-tolerant ‘xd’ (Table 2). Similar to our previous report (Zou et al., 2017) in wucai (Brassica campestris L., one of NHCC), an accumulation of Chl might be proposed as one of the potential biochemical indicators of HT tolerant in NHCC. In addition, the result of the present study showed that the rest of the photosynthetic parameters, such as gs, Ci, and Tr in two genotypes, were also markedly inhibited compared with the control (Fig. 1). The regulation of gs is a key factor for both desiccation prevention and CO2 acquisition (showed by Ci). In our study, companied with reduced gs, Ci also presented the similar reduced trend exposed to HT in two genotypes, which indicated the efficiency reduction of mesophyll cells to use the available CO2 (Karaba et al., 2007), revealing that the decrease in PN was attributed to the stomata limitation in the early stage. In our study, accelerating Tr to cool leaf surface temperature was also contributed to the improvement of heat tolerant against HT (Sharma et al., 2014). The declined degree of each index in photosynthesis was lower in heat-tolerant genotype 'xd' than heat-sensitive 'sym', showing a higher gas exchange potential of 'xd' against HT stress.

Table 5. Effects of high temperature on sugar contents in two nonheading Chinese cabbage cultivars.

|                  | Soluble sugar | Sucrose   | Fructose | Starch    |
|------------------|---------------|-----------|----------|-----------|
| Cont-sym         | 37.43 ± 1.17  | 12.72 ± 0.62 | 12.56 ± 1.58 | 0.22 ± 0.01 |
| Cont-xd          | 35.13 ± 2.34  | 15.84 ± 0.81 | 15.31 ± 1.50 | 0.24 ± 0.01 |
| HT-sym           | 51.82 ± 3.09  | 19.03 ± 0.98 | 21.21 ± 0.71 | 0.32 ± 0.01 |
| HT-xd            | 61.74 ± 1.75  | 25.45 ± 0.96 | 27.12 ± 0.88 | 0.39 ± 0.02 |

Values are represented as the mean ± SE (n = 3). Different letters in the same column indicate significant differences at P < 0.05 according to Duncan’s multiple range tests.
with higher antioxidative enzyme activities, including APX, GR, and MDAR (Fig. 4). The higher antioxidative capacity in the chloroplast of ‘xd’ partly protected the membrane system of thylakoid from oxidative damage (declined MDA and H2O2 content).

The chloroplast is the key site for photosynthesis, in which both photochemical reactions and carbon metabolism take place. However, thylakoid membrane is highly sensitive to HT (Wang et al., 2009). In heat-susceptible ‘sym’, HT altered the structural organization of thylakoids, disrupted the GL stacking, and destroyed the double membrane structure of chloroplast, resulting in the accumulation of plastoglobulus number (Fig. 7). These alterations of chloroplast ultrastructure can negatively and directly influence the photosynthetic apparatus function and inhibit the activities of membrane-associated electron carries and enzymes, which ultimately result in a reduced rate of photosynthesis (Zhang et al., 2014). The swollen chloroplasts and disordered lamellae were more severe in heat-susceptible ‘sym’, indicating that the chloroplast and thylakoid suffered from more damage than the chloroplast in heat-tolerant ‘xd’.

Conclusions

HT considerably hampered the photosynthetic process in NHCC by altering the chlorophyll pigments content, chloroplast ultrastructure, antioxidative capacity of chloroplast, and carbohydrate metabolism homeostasis, resulting in plant growth inhibition. It was demonstrated that heat tolerance of NHCC was associated with the ability to maintain higher photosynthetic capacity in both light and dark reactions, improving the energy conversion and photosynthetic product formations to resistant heat stress damage.

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