Physiological differences in response to di-\textit{n}-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (\textit{Brassica parachinensis} L.)

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\textbf{ABSTRACT}

To increase understanding on the mechanisms of cultivar difference in contaminant accumulation in crops, this study was designed to compare the physiological responses to di-\textit{n}-butyl phthalate (DBP) exposure between low (\textit{Lvba70}) and high (\textit{Huaguan}) DBP cultivars of Chinese flowering cabbage (\textit{Brassica parachinensis} L.). Under high DBP exposure, significant differences in various physiological responses were observed between the two cultivars, which might account for the variation in DBP accumulation. Ultrastructure observation also showed different alterations or damages in the mesophyll cell structures between both cultivars, especially for the chloroplast disintegration, starch grain quantity, and plastoglobuli accumulation. Compared with \textit{Huaguan}, \textit{Lvba70} suffered greater decreases in biomass, chlorophyll content, carbon assimilation, gas exchange parameters, photosynthetic electron transport capacity, and antioxidase activities, which would have resulted in a great reduction of photosynthetic capacity. Although \textit{Lvba70} enhanced energy dissipation and activities of some antioxidant enzymes, they did not provide sufficient protection against oxidative damage caused by DBP. The result suggested that the lower DBP tolerance of \textit{Lvba70} might be associated with its poor physiological performances, which was responsible for its lower DBP accumulation to protect itself from toxicity. Additionally, \textit{Lvba70} had a significantly lower transpiration rate and stomatal conductance than \textit{Huaguan}, which might be the factors regulating DBP-accumulation variation.

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1. Introduction

Phthalic acid esters (PAEs) are commonly used as plasticizer. In recent years, PAE levels in agricultural soils have increased significantly due to the application of agricultural plastic films (Wang \textit{et al.}, 2013; Niu \textit{et al.}, 2014). Some PAE compounds and their degradation intermediates are suspected to cause cancer and disrupt the endocrine system (Niu \textit{et al.}, 2014; Mo \textit{et al.}, 2009). Di-\textit{n}-butyl phthalate (DBP), a representative PAE compound, has become one of the dominant PAEs in agricultural soil (Wang \textit{et al.}, 2013; Niu \textit{et al.}, 2014; Cai \textit{et al.}, 2008). For the general population, diet is a primary route of exposure to PAEs (Schechter \textit{et al.}, 2013). DBP can be accumulated in vegetables, which poses a potential threat to food safety and human health (Mo \textit{et al.}, 2009).

To reduce the risk of soil contaminants entering human food chain, selection of crop cultivars with low contaminant accumulation in their edible parts has been proposed as a practical solution (Wang \textit{et al.}, 2009). Chinese flowering cabbage (\textit{Brassica parachinensis} L.) is a main vegetable in southern China, which is also exported to many countries and regions, and it tends to accumulate DBP in PAE-contaminated soils (Mo \textit{et al.}, 2009). Thus, screening low-DBP cultivars and understanding their formation mechanism are very important for food safety. In our previous study, some typical low and high DBP-accumulating cultivars of Chinese flowering cabbages (\textit{B. parachinensis} L.) were screened out (Zhao \textit{et al.}, 2015).
2.1. Chemicals and materials

DBP (98.7%) was obtained from Aladdin Chemistry Co., Ltd., China, while DBP stock standard solution (1000 μg/mL in dichloromethane, 99.8%) for analysis was purchased from Sigma Chemical Co., USA. Other chemicals were obtained from Tianjin Chemical Reagent Co., China. The low- and high-DBP cultivars of B. parachinensis, L, Lvbao70 and Huan-guan, respectively, were selected for this study (Zhao et al., 2015). However, the mechanisms of cultivar differences in DBP accumulation remain unclear.

The physiological processes are the intrinsic factors regulating the uptake and accumulation of contaminants by plants (Mostofa et al., 2015; Pietrini et al., 2015). The previous studies mostly focused on the toxic effects of DBP on crop physiological processes including seed germination and growth (Liu et al., 2014; Ma et al., 2013), ultrastructure of mesophyll cells (Zhang et al., 2015a), root physiology (Zhang et al., 2015b), fruit quality (Yin et al., 2003), antioxidant activity (Ma et al., 2013, 2014; Liu et al., 2014; Zhang et al., 2015a), and protein profile (Liao et al., 2009), while very limited data are available on the effects of plant photosynthesis to DBP exposure. And, cultivar variation in physiological responses to DBP exposure was also hardly studied. It was reported that other organic contaminants could decrease photosynthesis rates and alter the photosynthetic apparatus (Ahamed et al., 2012; Li et al., 2013). Thus, the photosynthetic responses can be used as indicators to evaluate plant tolerance to pollutants (Pietrini et al., 2015).

Especially, the use of chlorophyll fluorescence analysis, in combination with simultaneous gas exchange measurements, allows the evaluation of the heterogeneity in the photosynthetic function of photosystem II (PSII) in plants subjected to environmental stress (Strasser et al., 2000; Jiang et al., 2008; Tomar and Jajoo, 2015).

Furthermore, to protect against oxidative stress induced by DBP, plant cells are equipped with intrinsic antioxidant capacity that comprises a variety of antioxidant enzymes, which can provide useful evidence to evaluate the tolerance of plants to DBP toxicity (Gill and Tuteja, 2010; Ma et al., 2014). In particular, catalase (CAT) and superoxide dismutase (SOD) are the most valuable antioxidases in the protective system of higher plants to survive oxidative stress, and their changes can affect the resistance and adaptation of plants to DBP stress (Ma et al., 2013; Zhang et al., 2015a); glutathione S-transferase (GST) is well known in detoxification reaction in plant (Kumar et al., 2013); malondialdehyde (MDA) formation characterizes the oxidative stress caused by lipid peroxidation (Ma et al., 2013). Besides, observation on cell ultrastructure can reflect directly the toxicity of DBP to plant. Although there are so many physiological responses to DBP stress, it is not clear whether DBP-induced alterations in these physiological processes are associated with DBP accumulation in plant.

In this study, greenhouse pot experiments were conducted to investigate various physiological differences (including biomass, cell ultrastructure, gas exchange, photosynthesis, and antioxidant enzyme activities) between the low- and high-DBP cultivars of Chinese flowering cabbage grown in DBP-contaminated soil. We hypothesize that there were significant differences in the physiological responses to DBP between the low and high accumulators, which were responsible for the variation in DBP accumulation.

2.2. Experimental design

The soil for pot experiments collected from an agricultural field of South China Agricultural University (Guangzhou, China) was air-dried and ground to pass through a sieve (5 mm). The soil contained 15.7 g kg⁻¹ (dry weight, DW) of organic matter, 8.22 cmol kg⁻¹ of cation exchange capacity, 1.45 g kg⁻¹ of total N, 2.01 g kg⁻¹ of total P, and 18.87 g kg⁻¹ of total K. The background concentration of DBP in this soil was 0.19 mg kg⁻¹. The pot experiment was performed in a greenhouse with natural light.

According to our previous investigation, the mean value of DBP in vegetable soils in southern China was 9.5 mg kg⁻¹ (Cai et al., 2008). Thus, the soil spiked at 10 mg kg⁻¹ was designated as low-DBP treatment. Additionally, to evaluate the differential responses of the two cultivars to higher level of DBP stress, the soil spiked at 100 mg kg⁻¹ was used as high-DBP treatment. An aliquot of soil (passed through a 2-mm sieve, 10% total quantity of soil) was spiked with a solution of DBP in acetone. After acetone evaporated, the spiked soils were mixed thoroughly with uncontaminated soils, sieved again to homogenize the soils, and aged for two weeks under dark condition. The soil without DBP spiked was used as a control. A total of 3.0 kg (DW) of the above soil was put into a ceramic pot (20 cm in diameter, and 14 cm in height) and mixed with 6.0 g of compound fertilizer (N:P:K = 4:3:4). Next, 20 seeds of each cultivar were sown into the soil of each pot. All treatments were in three replicates. Pots were randomly arranged in a glasshouse at natural temperatures (25–32 °C), and the soils were watered daily with deionized water to maintain moderate moisture. After germination, seedlings were gradually thinned with five seedlings remained (day 15) in each pot. At the end of experiment (day 45), six to eight leaves of each pot were used for measuring pigments, gas exchange, OJIP transients, antioxidant enzyme activities, and methane dicarboxylic aldehyde (MDA) content. Finally, plant samples were washed with deionized water and then divided into roots and shoots, and fresh weights were recorded.

2.3. Analysis of DBP

The procedures of ultrasonic-assisted sample extraction using silicagel column and DBP analysis using gas chromatography coupled with mass spectrometry (GC/MS, Shimadzu QP2010 Plus, Japan) were conducted following the method adopted in our previous study (Cai et al., 2007) with minor modifications (see Supplementary M1). The detection limit of DBP was 1.2 μg kg⁻¹. The average concentration of DBP was 3.2 (ranged from 1.27 to 7.80) μg kg⁻¹ in all procedural blanks (n = 12), which was subtracted from sample values. The recoveries of DBP in plant samples ranged from 87.4% to 107.2%.

2.4. Chlorophyll content

Leaf chlorophyll content was measured following the method described by Hu et al. (2014), with minor modifications (see Supplementary M1).

2.5. Transmission electron microscope (TEM)

Ultrastructural studies were performed on leaf tissues of the two cultivars by TEM following the protocol of Zhang et al. (2015a). Briefly, the samples with different DBP treatments were collected and cut to 1 mm² cubes, fixed in 4% (v/v) glutaraldehyde and then 1% (v/v) osmium tetroxide, and dehydrated successively in ethanol series (30%–100%), v/v and embedded in Epon-812 Resin. After making ultrathin sections (50–70 nm) with a Leica UCT ultramicrotome, the sections were mounted on copper grids and stained with 4% (w/v) uranyl acetate and lead citrate, and then examined at 100 kV using a FEI-Tecnai G2 12-type TEM (Eindhoven, Netherlands).
2.6. Measurement of gas exchange

The gas exchange system (LI-6400XT; Li-Cor Inc., USA) was used to determine net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs), and intercellular CO2 concentration (Ci) following the method described by Li et al. (2013). The third or fourth leaf of each treatment was measured for 11 times. LI-6400XT (6400-02B) was used to control chamber conditions. Light intensity, leaf temperature, and CO2 concentration inside the leaf chamber were 1000 μmol m-2 s-1, 30 °C, and 380 μmol mol-1, respectively.

2.7. Leaf chlorophyll a fluorescence transient

Chlorophyll a fluorescence parameters were estimated immediately after the gas exchange measurements using a Handy Plant Efficiency Analyser (Hansatech, U.K.) according to Tomar and Jajoo (2013). The leaves (also used for gas exchange measurements) were first dark-adapted for 20 min and then measured for 11 times. The fluorescence signal was received by the sensor head during recording and was digitized in the control unit using a fast digital converter. The control leaves exhibited a polyphasic rise known as OJIP Chl a fluorescence transient: O to J phase (ended at 2 ms), J to I phase (ended at 30 ms), and I to P phase (ended at 500 ms). The JIP test was named after the basic steps in the fluorescence transient when plotted on a logarithmic time scale (Tomar and Jajoo, 2013). The OJIP transient was induced by red light of about 3000 μmol m-2 s-1 provided by an array of three light-emitting diodes (peak 650 nm), which focused on the leaf surface to give homogenous illumination over the exposed area of the leaf. From the OJIP transient, the extracted parameters (F0, Fm, F0/Fl, F100, F500, F1000, Fl, Fm, F0m, etc.) led to the calculation and derivation of a range of new parameters, according to previous studies (Jiang et al., 2008; Tsimilli-Michael and Strasser, 2008) (Table 1).

2.8. Activities of antioxidant enzymes and MDA content

Plant samples were extracted according to the method of Ma et al. (2014) with minor modifications. The activities of antioxidant enzymes (including SOD, CAT, and GST) and MDA content were measured with a spectrophotometer (Shimadzu UV-2450, Japan) according to the manufacturer’s instructions of the corresponding kits (Nanjing Jiancheng Bioengineering Institute, China) (see Supplementary M1).

2.9. Statistical analysis

Results are expressed as means ± standard error (SE). One-way analysis of variance (ANOVA) followed by Duncan’s test was performed with Microsoft Excel 2003 and SPSS 17.0.

3. Results

3.1. Plant biomass and DBP concentration

DBP concentrations in shoots and roots of both cultivars increased with an increase in the concentration of DBP spiked in soil (Fig. S1A). Similar as our previous study (Zhao et al., 2015), Lvbao70 had lower DBP concentrations in shoots but higher in roots than those of Huaguan under the same DBP treatment (Fig. S1A). Thus, Lvbao70 should be a desirable choice in terms of food safety. Compared with the control, low-DBP exposure promoted the growth of Lvbao70 with a significant increase in shoot biomass (P < 0.05), but no significant effects were observed on shoot and root biomass of Huaguan (Fig. S1B). However, the shoot and root biomass of the two cultivars decreased significantly in high DBP treatment (P < 0.05). Especially, the shoot biomass of Lvbao70 was significantly lower (P < 0.05) than that of Huaguan. On the other hand, the opposite correlations were observed in low DBP
treatment (Fig. S1B). Thus, the shoot biomass cannot be used as an indicator to distinguish the low- and high-DBP accumulators.

3.2. Chlorophyll concentration and leaf ultrastructure

Total Chl concentrations in leaves of Huaguan presented no significant changes \((P > 0.05)\) with increasing DBP levels in soil (Table 2). However, compared with the control, total Chl concentration in leaves of Lvbao70 increased significantly \((P < 0.05)\) under low DBP treatment, but decreased significantly \((P < 0.05)\) under high DBP treatment. Furthermore, as shown in Table 2, the Chl a/Chl b ratio, which is a stress indicator, did not differ significantly \((P > 0.05)\) between Lvbao70 and Huaguan under both levels of DBP exposure.

TEM observation revealed that the leaves of both of the cultivars presented well-organised structures containing definite cell walls, chloroplasts, mitochondria, and clear nuclei in both CK (Fig. 1A and D) and low DBP treatments (Fig. 1B and E). The shuttle-like chloroplasts were of integrity, with tightly stacking lamellae of grana. There were few differences in chloroplast structures between the two cultivars, except that starch grains in the chloroplasts of Lvbao70 were smaller. However, obvious changes in cell ultrastructures were observed in both cultivars under high DBP treatment. Especially, the chloroplasts and mitochondria swelled, and the amount of integrated chloroplasts decreased (Fig. 1C and F). The results provided direct evidence that Lvbao70 performed a lower tolerance to DBP toxicity than Huaguan did.

3.3. Leaf gas exchange

The leaf gas exchange parameters (including Pn, Gs, Ci, and E) of

<table>
<thead>
<tr>
<th>DBP treatments (mg kg(^{-1}))</th>
<th>Cultivars</th>
<th>Pn ((\mu)mol m(^{-2}) s(^{-1}))</th>
<th>Ci ((\mu)mol mol(^{-1}))</th>
<th>Gs (mol m(^{-2}) s(^{-1}))</th>
<th>E (mmol m(^{-2}) s(^{-1}))</th>
<th>Total chl content (mg g(^{-1}))</th>
<th>Chl a/b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>Lvbao70</td>
<td>11.18 ± 0.72a</td>
<td>193 ± 11b</td>
<td>0.18 ± 0.02b</td>
<td>3.02 ± 0.16b</td>
<td>1.24 ± 0.16b</td>
<td>2.44 ± 0.16a</td>
</tr>
<tr>
<td></td>
<td>Huaguan</td>
<td>10.87 ± 0.65a</td>
<td>186 ± 17b</td>
<td>0.19 ± 0.01b</td>
<td>3.87 ± 0.23a</td>
<td>1.18 ± 0.13b</td>
<td>2.58 ± 0.13a</td>
</tr>
<tr>
<td>10</td>
<td>Lvbao70</td>
<td>12.75 ± 1.12a</td>
<td>199 ± 14b</td>
<td>0.18 ± 0.01b</td>
<td>3.16 ± 0.22b</td>
<td>1.47 ± 0.14a</td>
<td>2.57 ± 0.20a</td>
</tr>
<tr>
<td></td>
<td>Huaguan</td>
<td>11.74 ± 0.59a</td>
<td>202 ± 12b</td>
<td>0.24 ± 0.02a</td>
<td>4.24 ± 0.22a</td>
<td>1.31 ± 0.16 ab</td>
<td>2.62 ± 0.22a</td>
</tr>
<tr>
<td>100</td>
<td>Lvbao70</td>
<td>5.62 ± 0.34c</td>
<td>248 ± 16a</td>
<td>0.10 ± 0.01d</td>
<td>2.54 ± 0.14c</td>
<td>0.89 ± 0.08c</td>
<td>2.71 ± 0.17a</td>
</tr>
<tr>
<td></td>
<td>Huaguan</td>
<td>8.79 ± 0.82b</td>
<td>196 ± 13b</td>
<td>0.13 ± 0.01c</td>
<td>3.19 ± 0.21b</td>
<td>1.14 ± 0.11b</td>
<td>2.74 ± 0.23a</td>
</tr>
</tbody>
</table>

\(^a\) Means (±SE, \(n = 6–10\)) followed by the same letters within a column are not significantly different \((P > 0.05)\).

Fig. 1. Transmission electron microscopy of mesophyll cell of two Chinese flowering cabbage cultivars in different DBP treatments. (A–C) Huaguan treated with 0, 10, and 100 mg kg\(^{-1}\) DBP, respectively; (D–F) Lvbao70 treated with 0, 10, and 100 mg kg\(^{-1}\) DBP, respectively. CW: cell wall; C: chloroplast; M: mitochondrion; N: nucleus; S: starch grain; T: thylakoid lamellae; V: vacuole; P: plastoglobuli.
both cultivars were comparable between low DBP treatment and the control, but the values of Pn and Gs of both cultivars decreased significantly under high DBP exposure. Moreover, Pn, Gs, and E values of Lvba70 decreased more sharply (49.73%, 44.44%, and 15.89%, respectively, compared with the control) than those of Huaguan (Table 2). These results indicated that CO2 assimilation in leaves of Lvba70 was inhibited more seriously under high DBP exposure. Additionally, it should be noted that the E and Gs values of Huaguan were significantly higher than those of Lvba70 under the same DBP exposure (26–34% for E, and 33–36% for Gs under two levels of DBP exposure) (Table 2). The differences in E and Gs might play a role in DBP accumulation variation in the two cultivars.

3.4. Chlorophyll a fluorescence transient and related parameters

The leaves in both the control and DBP treatments showed typical polyphasic increases in Chl a fluorescence (Fig. 2A). The OJIP transients of both cultivars under low DBP treatment were similar to those of the control (except a slight increase at the P-step of Lvba70) (Fig. 2B, presenting OJIP data as the kinetics of relative variable fluorescence at any time, \( V_t = (F_t - F_0) / (F_m - F_0) \)). However, under high DBP exposure, the OJIP transients of both cultivars showed an increase at the O-step and a decrease at the P-step (Fig. 2A), and an increase in the K-band (300 μs) was observed in the relative variable fluorescence (especially for Lvba70) (Table 3). The values of fluorescence parameters derived from transient changes were analyzed in detail and the results were presented in Table 3 and Fig. 3.

No significant influences of low DBP exposure were observed on \( F_0, F_m, F_{50\mu s}, F_{100\mu s}, F_{300\mu s}, F_{t_m}, t_f, t_m, \) and Area in leaves of both cultivars compared with the control (except some increases in \( F_m, F_f, \) and \( F_t \) for Lvba70) (Table 3). However, the values of \( F_0, F_{50\mu s}, F_{100\mu s}, F_{300\mu s}, F_{t_m}, \) and Area of both cultivars increased, while those of \( F_m, F_f, \) and Area decreased under high DBP exposure. Moreover, the values of \( F_0, F_{50\mu s}, F_{100\mu s}, F_{300\mu s}, F_{t_m}, \) and Area for Lvba70 were higher, but its \( F_m \) and \( F_t \) were lower than those of Huaguan. The fluorescence parameters can be derived in several ways, such as based on changes per \( C_{S_0} \) or changes per \( R_C \). Several parameters including ABS (absorbance), ET0 (electron transport), TR0 (trapping), and DI0 (dissipation) per \( C_{S_0} \) were calculated from the OJIP curves (Table 1 and Fig. 3). Fig. 3A showed the behavior of 17 biophysical parameters of PSII for each parameter, its value was normalized to the control value of Huaguan. Under two levels of DBP exposure, the data of Lvba70 and Huaguan could yield consistent estimates for most of the parameters. In general, slight
increases in TR₀/RC, M₀, DI₀/CS₀, DI₀/RC, ABS/CS₀, ABS/RC, TR₀/CS₀, ET₀/CS₀, RC/CS₀, ET₀/RC, PI₁₀, PI₁₀/CS₀, PI₁₀/RC, and Ψ₀ of both cultivars were observed under low DBP exposure, while significant increases in DI₀/RC, DI₀/CS₀, ABS/CS₀, ABS/RC, TR₀/CS₀, M₀, and TR₀/CS₀, and significant decreases in PI₁₀, PI₁₀/CS₀, PI₁₀/RC, and Ψ₀, were observed under high DBP exposure. Notably, there were significant differences \( (P < 0.05) \) in both DI₀/RC (Fig. 3B) and DI₀/CS₀ (Fig. 3C) between the two cultivars under high DBP exposure, which indicated that the two cultivars had different tolerance to photoinhibition.

### 3.5. Responses of antioxidases and MDA in plants

As shown in Fig. 4, the activities of SOD and GST in the shoots and roots of both cultivars increased significantly under DBP exposures. The activities of SOD and GST in the shoots and roots of Lvbao70 were higher than or similar to those of Huaguan under low DBP exposure, but significantly lower under high DBP exposure. However, no significant difference in CAT activities was recorded between the two cultivars.

The responses of MDA to DBP treatments were approximately

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**Table 3**

Effects of DBP (mg kg⁻¹) reflected in data extracted from the recorded chlorophyll a fluorescence transient of two cultivars of *B. parachinensis*.

<table>
<thead>
<tr>
<th>DBP Cultivars</th>
<th>F₀</th>
<th>Fₘ</th>
<th>F₅₀ₜₜₜ</th>
<th>F₁₀₀ₜₜₜ</th>
<th>F₃₀₀ₜₜₜ</th>
<th>F₅₀ₜₜₜ</th>
<th>F₇</th>
<th>F₉</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Lvbao70</td>
<td>546 ± 27a</td>
<td>3434 ± 59b</td>
<td>792 ± 45c</td>
<td>1160 ± 38c</td>
<td>1618 ± 45b</td>
<td>2747 ± 79b</td>
<td>289 ± 17c</td>
<td>63949 ± 2461a</td>
<td></td>
</tr>
<tr>
<td>Huaguan</td>
<td>569 ± 22c</td>
<td>3452 ± 66b</td>
<td>654 ± 24c</td>
<td>1173 ± 46c</td>
<td>1870 ± 54a</td>
<td>2799 ± 54b</td>
<td>302 ± 14c</td>
<td>62254 ± 2194a</td>
<td></td>
</tr>
<tr>
<td>10 Lvbao70</td>
<td>566 ± 29c</td>
<td>3699 ± 84a</td>
<td>672 ± 30c</td>
<td>1166 ± 34c</td>
<td>1824 ± 39a</td>
<td>2907 ± 82a</td>
<td>271 ± 23c</td>
<td>63017 ± 2156a</td>
<td></td>
</tr>
<tr>
<td>Huaguan</td>
<td>587 ± 35c</td>
<td>3374 ± 73b</td>
<td>689 ± 32c</td>
<td>1207 ± 47c</td>
<td>1826 ± 42a</td>
<td>2801 ± 91ab</td>
<td>290 ± 12c</td>
<td>63866 ± 1899a</td>
<td></td>
</tr>
<tr>
<td>100 Lvbao70</td>
<td>816 ± 57a</td>
<td>2957 ± 62d</td>
<td>1084 ± 36a</td>
<td>1342 ± 55a</td>
<td>1589 ± 57a</td>
<td>1611 ± 56b</td>
<td>2424 ± 63d</td>
<td>43482 ± 1563b</td>
<td></td>
</tr>
<tr>
<td>Huaguan</td>
<td>684 ± 36b</td>
<td>3211 ± 56c</td>
<td>939 ± 41b</td>
<td>1167 ± 34b</td>
<td>1396 ± 59b</td>
<td>1578 ± 25b</td>
<td>2617 ± 68c</td>
<td>52086 ± 1714c</td>
<td></td>
</tr>
</tbody>
</table>

* Means ± SE \((n = 6 – 10)\) followed by the same letters within a column are not significantly different \((P > 0.05)\).
dose-dependent, and the MDA content in both shoots and roots increased with increasing DBP concentration in soil, implying a certain lipid peroxidation damage of plant cells induced by DBP. The highest MDA content in the roots of Lvbao70 was nearly 3.5-fold compared with the control. The MDA content in both shoots and roots of Lvbao70 was lower than those in Huaguan under low DBP exposure, but significantly higher under high DBP exposure.

4. Discussion

Ultrastructural investigation showed that Lvbao70 suffered greater damage in the structures of mesophyll cells than Huaguan did (Fig. 1C and F), which provide a direct evidence that Lvbao70 is more sensitive to DBP toxicity. Especially, lots of starch grains disappeared and many chloroplasts exhibited remarkable disintegration, suggesting that DBP in Lvbao70 induced more disturbances in
metabolic functions and lipid composition of membranes (Zhang et al., 2015a). Meanwhile, more plastoglobules were observed in the chloroplasts of Lvbao70, which indicated that the function of the chloroplast in Lvbao70 decreased to a greater extent because the pathways to form protein-containing cell structures were suppressed (Hoziner et al., 2009). In comparison, the cell structure of Huaguan displayed a larger increase in cell wall thickness and cytoplasmic vacuolization, suggesting a better cellular defense or detoxification mechanism against DBP exposure. It is well documented that a high tolerance of plants to contaminants coupled with an increased capacity of contaminant accumulation may attribute to an efficient detoxification mechanism in plant cells (Rascio and Navari-Izzo, 2011; Nehnevajova et al., 2012). Therefore, it was likely to cause toxic damages to the organelles of the low-tolerance Lvbao70 when DBP levels in the mesophyll cells were high enough to result in phytotoxicity. However, the sensitive Lvbao70 minimized DBP accumulation in mesophyll cells to protect its organelles from toxicity. As showed in Fig. S1A, the DBP concentrations of the roots in Lvbao70 were significantly higher than those in Huaguan, which is consistent with our previous results that Lvbao70 possesses a greater ability to prevent DBP from translocating from the roots to the shoots (Zhao et al., 2015). Especially, thinned cell wall and the irregular cytoplasmic vacuolization in mesophyll cells of Lvbao70 (Fig. 1F) suggested a worse cellular defense or detoxification mechanism against DBP stress, which was not favorable for DBP accumulation in its cell walls or vacuoles (Zhang et al., 2015b). This inference was confirmed by the observation that Lvbao70 had a lower proportion of DBP in the cell walls of the shoot than Huaguan did (Zhao et al., 2015). Overall, Lvbao70 showed a lower tolerance to DBP toxicity than Huaguan did, which might account for its lower DBP accumulation to protect itself from toxicity.

As an important marker of internal plant metabolism, photosynthesis under any inhibition would adversely affect physiological activities during plant growth (Li et al., 2013). The gas exchange of many semivolatile organic compounds between plant leaves and air was considered to partly control the uptake and accumulation of these chemicals by plants (Tao and Hornbuckle, 2001). In this study, the high DBP exposure resulted in significant decreases in Pn, Gs, and E (Table 2), suggesting that a stomatal effect could be involved in the photosynthetic response of plants to DBP stress (Su et al., 2013). Higher Gs observed in Huaguan indicated less stomatal resistance, which was directly associated with the diffusion of organic pollutants through the stomata (Oguntimehin et al., 2008). It was such effect that might lead to enhanced uptake and accumulation of DBP by Huaguan through the stomata. In addition, the Ci of leaves increased significantly with the decrease in Gs and E, suggesting that the decrease of Pn in leaves of Lvbao70 might be mainly attributable to non-stomatal limitation (Ahammed et al., 2012). Compared with Lvbao70, the unaffected Ci and a lower decrease of Gs and E in the leaves of Huaguan might lead to its higher Pn. The higher photosynthetic efficiency of Huaguan might correlate with its elevated DBP-tolerant mechanisms, which allowed Huaguan to accumulate more DBP. In addition, plant transpiration might play an important role in the uptake and translocation of organic contaminants (Gao and Collins, 2009; Dodgen et al., 2015). Some studies reported that high transpiration induced greater uptake of contaminants by plants than low transpiration did (Liao et al., 2006; Dodgen et al., 2015). In this study, the difference of DBP accumulation in the two cultivars could partly attribute to the difference in transpiration (Table 2). However, the quantitative contribution of transpiration to the uptake and accumulation of DBP by plants merits further investigation.

Chlorophyll is an important component that plays a role in energy production through photosynthesis. Compared with Huaguan, Lvbao70 showed a significant decrease in the total chlorophyll content (Table 2), which might be due to its easier degradation or decreased synthesis when exposure to DBP (Vánová et al., 2009). Correspondingly, their ultrastructural observations also displayed obvious structural changes in chloroplasts, including modification in the quantity, size, and the organization of thylakoids (Fig. 1). Therefore, the reduction in Pn in Lvbao70 was likely to be attributable to the reduction of leaf pigment and the damage of chloroplast (Su et al., 2013). Furthermore, our results clearly showed that DBP inhibited the photoactivation of PSII. The decreases in fluorescence intensity in both cultivars under high DBP exposure were pronounced within the IJ and IP phases (Fig. 2A). The intensity during the OJ phase associated with accumulation of QA shifted to a lesser degree. The decrease in the IP phase detected in this study could be associated with blocking of the electron transfer from QA to Qb, likely due to the increase in the fraction of QA-nonreducing centers (Tomar and Jajoo, 2013). Meanwhile, the decreased extents of Fv, Fm, and Fm′ were higher in Lvbao70 than in Huaguan (Table 3), suggesting that electron transport at the donor side of PS II was inhibited more seriously in the former (Aksmann and Tukaj, 2008). Besides, the increased Fv′/Fm for Lvbao70 exposed to DBP (Fig. 2A and Table 3) might be due to the increased number of inactive RCs (where electrons cannot be transferred through the primary electron transport chain, especially in Lvbao70), while reduced the low energy transfer from the light-harvesting complex II to the PSII reaction center, and thus higher fluorescence from light-harvesting complex II (Tomar and Jajoo, 2013). From the kinetics of the relative variable fluorescence, a positive K-step appeared at 300 μs in the OJIP transients of Lvbao70 under high DBP exposure (Fig. 2B). This suggested that the oxygen-evolving complex in the leaves of Lvbao70 was more easily damaged, which inhibited the electron donation from water to the secondary electron donor of PSII (Hakala et al., 2005). Similar results were also observed in Desmodesmus subsppicatus exposed to atrazine (Baścik-Remiszewicz et al., 2011).

Furthermore, fluorescence parameters suggested that the inhibition of photosynthetic activity of both cultivars exposed to high levels of DBP resulted from the impairment of primary photochemical processes (Fig. 3A). Most of the parameters indicated that PSII was more photo-inhibited in Lvbao70 than in Huaguan. Specifically, DBP decreased the total electron acceptor capacity in the leaves of Lvbao70 more strongly than that in Huaguan, as indicated by the decreased Sm (Fig. 3A), which could measure the pool of electron transporters between PSII and the acceptor side of PSI (Pinior et al., 2005). The leaves exposed to DBP also presented a lower ESA/CS0 than those in the control, but an increased TR0/CS0 (Fig. 3A), indicating that DBP affected the activity of electron transport chain, especially in Lvbao70 (jiang et al., 2008). As demonstrated by Strasser et al. (2000), the decrease in the performance index (PI) might result from a disruption of energy absorption, trapping, or transfer beyond QA quinone. In this study, the decreases in both PABS and PEC in both cultivars exposed to DBP were due to low quantum efficiency of electron trapping and transport (V0, φPSII, φEF0) (Fig. 3A). The efficiency by which a trapped electron can move further ahead of QA is equal to 1 − Vj (i.e. φ0) (Tomar and Jajoo, 2013). Moreover, a significant decrease was observed in the value of φ0 in both cultivars under high DBP exposure, especially in Lvbao70 (Fig. 3A), suggesting that QA electron transport at the acceptor side of PSII was blocked. Besides, the reduction in Fv/Fm (i.e. φ0), which is a good indicator of photo-inhibitory effects on PSII, was caused by both a decrease in Fm′ and an increase in Fv′ (Fig. 2A) (Maxwell and Johnson, 2000). In addition, the decreased RC/CS0 (Fig. 3A), implied that elevated Fv′ might be induced by the inactivation of some of the PSII RCs (Yamane et al., 1997) or be related to the accumulation of reduced QA, caused by the fractional reduction of QA to Qb (Bukhov et al.,
the control and low-DBP treatment (Fig. 4), which con-
observed for MDA content in the shoots of
Lvbao70 activities of SOD and GST were recorded in both the shoots and
under high DBP exposure, the activities of SOD and GST in the
responses under certain conditions (Hashmi et al., 2014). However,
could trigger antioxidant defenses that induced hormetic re-
the antioxidant enzymes, the CAT activities of both cultivars
absorption of light energy in photosynthetic electron transport, as
in MDA content were observed in both the shoots and the roots of
Huaguan70 was significantly greater (P < 0.05) than that in Huaguan. Compared
with the Huaguan, Lvbao70 utilized only a smaller fraction of the
absorbed light energy in photosynthetic electron transport, as
indicated by more decreases in \( \text{T}_{\text{O}} / \text{RC} \), \( \varphi_{\text{O}} \), and \( \varphi_{\text{p}} \) (Fig. 3A), suggesting more excess excitation energy existed in Lvbao70, which
could further lead to the production of ROS, and caused damage to
photosynthetic apparatus (Song et al., 2006). These results revealed
that PSII of Lvbao70 was photodamage-inhibited more seriously than that in
Huaguan (Yang et al., 2012). Therefore, the lower DBP-tolerance of
Lvbao70 could be associated with its poor photosynthesis per-
formance, which may function as a mechanism for accumulation of a
lower amount of DBP.

To combat ROS induced by DBP, plants are endowed with an
efficient enzymatic antioxidant system including CAT, SOD, and
GST (Zhang et al., 2015a). Under low DBP exposure, the higher
activities of SOD and GST were recorded in both the shoots and
the roots of Lvbao70 compared to Huaguan (Fig. 4), suggesting that SOD and GST might play critical roles in the hormetic
response of Lvbao70 to DBP. Accordingly, a “stimulatory effect”
occurred on the shoot biomass of Lvbao70 (Fig. S1B). The result
suggested that the organic chemical-induced ROS production
could trigger antioxidant defenses that induced hormetic re-
sponses under certain conditions (Hashmi et al., 2014). However,
under high DBP exposure, the activities of SOD and GST in the
both shoots and the roots of Lvbao70 were significantly lower than those of Huaguan (Fig. 4), indicative of a loss of protective
capacity in Lvbao70 against cellular superoxide toxicity under increased DBP concentration. This may result from the break-
down of the antioxidant system when DBP level was high enough
to cause phototoxicity (He et al., 2013). These results are
consistent with our observation that Lvbao70 is more sensitive to
DBP toxicity than Huaguan due to lack of a more powerful anti-
oxidant capability. Additionally, it should be noted that among
the antioxidant enzymes, the CAT activities of both cultivars
decreased under high DBP exposure and were not significantly
different (Fig. 4). The decreases of CAT activities might be related to the fact that the CAT was more sensitive to DBP toxicity than other antioxidant enzymes, and it is likely that there is a
chloroplast-mediated CAT inactivation caused by oxidative
damage (Yang et al., 2012). Besides, no significant difference was
observed for MDA content in the shoots of Lvbao70 between in
the control and low-DBP treatment (Fig. 4), which confirmed the
beneficial effects on the photosynthetic system under low DBP
exposure, as mentioned above. However, the significant increases
in MDA content were observed in both the shoots and the roots of
the two cultivars under high DBP exposure, especially in Lvbao70
(Fig. 4). Suggestive of greater damage to Lvbao70 than Huaguan as a result of lipid peroxidation. Overall, these results indicated that
a weaker antioxidant capacity of Lvbao70 might be associated
with its lower DBP accumulation.

5. Conclusions

Our data support the hypothesis that the physiological re-
sponses of Chinese flowering cabbage to DBP stress differ signifi-
cantly between the low- and high-DBP cultivars. Compared with
Huaguan, Lvbao70 suffered more adverse effects under high DBP
exposure which resulted in greater damage to the photosynthetic
system. The result showed that Lvbao70 was more sensitive to DBP
toxicity than Huaguan was, which might account for its decreased
DBP accumulation to protect against toxicity. However, it requires further study on the functional mechanism that underlies physi-
ological differences in DBP-accumulation between low and high
accumulators.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://
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