Photosynthetic capacity, osmotic adjustment and antioxidant system in sugar beet (*Beta vulgaris* L.) in response to alkaline stress

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Abstract

Photosynthetic characteristics of sugar beet cultivars KWS0143 and Beta464 were studied under alkaline conditions, including 0 (A0, control), 25 (A1), 50 (A2), 75 (A3), and 100 mM of mixed alkali (Na2CO3:NaHCO3, 1:2). A1, A3, and A4 reduced net photosynthetic rate (PN), stomatal conductance, and transpiration rate, but rose intercellular CO2 concentration. Reduction in PN occurred probably due to nonstomatal limitation. The decrease in efficiency of photosynthetic electron transport might be the main reason for the decrease of PN. The concentrations of photosynthetic pigments were significantly reduced by high (A1 and A3) alkalinity. Changes in chloroplast ultrastructure are the reason for the decrease in chlorophyll content. Sugar beet could resist injury from alkali owing to osmotic substances and antioxidant enzymes if alkaline stress was at a lower level. The better performance of KWS0143 under alkalinity might be associated with its more efficient osmotic and antioxidant systems to resist injury of photosynthetic apparatus caused by alkalinity.

Additional key words: absolute electron transfer rate; betaine; carotenoids; proline; starch; superoxide dismutase.

Introduction

Soil salinization is a major and increasingly severe environmental problem throughout the world (Jesus et al. 2015). In general, high salt concentrations can compromise plant growth and development by causing ion imbalance, osmotic stress, and oxidative damage (Parida et al. 2004, Wakeel et al. 2011). Nasr et al. (2013) and Nedjimi et al. (2014) reported that salinity significantly reduced the rate and final percentage of germination, which, in turn, can lead to uneven plant establishment. With the exception of salinization, soil alkalinization is an also considerable phenomenon in Northeast China (Wang et al. 2009). In extensive alkaline soils over much of Northeast China, the neutral salts, NaCl and Na2SO4, and the alkaline salts, NaHCO3 and Na2CO3, are the main salt components (Bai et al. 2016). Alkaline salt stress is referred to as “alkaline stress”, whereas “salt stress” refers to neutral salt stress (Shi and Sheng 2005). Alkaline stress shares many factors with the salt stress, such as excessive Na+ and water deficit, and both stress types trigger similar responses in plants (Gong et al. 2014). Alkaline stress may be more severe than salt stress because of its high pH (Liu and Shi 2010, Yang et al. 2011). 

Photosynthesis plays a pivotal role in plants with leaves being key organs for photosynthesis. Photosynthesis is influenced by numerous environmental and physiological regulators. Changes in mesophyll conductance, which reduces the CO2 concentration in chloroplasts relative to that in substomatal cavities, can limit photosynthesis (Warren 2008). Wu et al. (2014) found that alkaline stress reduced Fv/Fl, PSII efficiency, electron transport rates, and net photosynthetic rate. Photosynthetic pigments, such as chlorophyll (Chl) and carotenoids (Car), and Chl fluorescence parameters, such as maximum quantum yield of PSII (Fv/Fl), PSII efficiency (ΦPSII), photochemical quenching (qP), nonphotochemical quenching (qN), and apparent rate of electron transport at the PSII level (ETR), serve as indicators of plant stress (Netto et al. 2005). Studies on sunflower (Liu and Shi 2010) and poplars (Wang et al. 2013) suggested that salt or alkaline stress affected Chl and Car contents and decreased PN, stomatal conductance (gs), intercellular CO2 concentration (Ci), maximal efficiency of PSII photochemistry, photochemical quenching coefficient (qP), electron transport rate, and actual PSII efficiency significantly, while increased nonphotochemical quenching. The morphology and internal structure of mesophyll cells, a fundamental component of photosynthesis, play an important role in photosynthetic capacity. Under adverse circumstances, the Chl content,
and photosynthetic capacity are significantly reduced, mainly due to damaged chloroplast morphology and damaged ultrastructure of functional leaves (Giles et al. 1976, Niki et al. 1978). Chloroplasts (Weston et al. 2000) and mitochondria (Xu et al. 2008) in mesophyll cells are the most sensitive to light quantity, and their morphology and internal structure change in response to environmental variations (Pessarakli 2005).

Osmotic adjustment plays an important role in tolerance of plants to salt and alkaline stress (Gorham et al. 1982, Ludlow and Muchow 1990). Substances involved in osmotic regulation are inorganic ions and small organic molecules, which can decrease cell water potential, maintain the stability of membrane and ultrastructure, protect biological macromolecules including protein, and eliminate reactive oxygen species (ROS). Higher plants can synthesize various osmotic substances; studies regarding osmotic substances have been mainly focused on proline, betaine, soluble sugar, etc. Proline and hydroxyproline synthetase activity of leaf mustard increased, but proline oxidase activity decreased, and thus the proline content significantly increased under salt stress (Iqbal et al. 2014). With increasing salinity, the betaine concentration in shoots of Kochia sieversiana increased rapidly and to similar extents under salt and alkali stress (Yang et al. 2007). It is reported that a total soluble sugar content in the flag leaf of Pokkali rice was enhanced if exposed to EC = 13.25 dS m⁻¹ (salt stress) for 3 d (Boriboonkaset et al. 2013).

One of the biochemical changes in plants under salt or alkaline stress is the excessive generation of ROS (An et al. 2016, Quan et al. 2016). ROS can have detrimental effects on metabolism through oxidative damage to lipids, proteins, and nucleic acids (Szabó et al. 2005, Sharma and Dubey 2007). In order to quench ROS and overcome oxidative stress, plants are equipped with antioxidative defense systems comprising superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (POD, EC 1.11.1.7), and enzymes of ascorbate glutathione cycle, such as ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) (Prochazkova et al. 2001). Yan et al. (2011) reported that the activities of SOD, POD, and catalase (CAT, EC 1.11.1.6) in roots of Spiraea × bumalda all increased first and then decreased with the increases of the salinity and pH value.

Sugar beet (Beta vulgaris L.) is one of the most important commercial crops. Sugar beet is used not only in the food industry but also for the production of bioethanol as a source of renewable energy (Magaña et al. 2011). Sugar beet is considered a cash crop and requires careful agronomic practices and breeding techniques for adaptation to biotic and abiotic stresses. It is important to understand the mechanisms through which plants adapt to alkaline conditions and select sugar beet genotypes better suited to alkaline stress. Previous studies have investigated the alkaline tolerance of different sugar beet cultivars (Chen et al. 2010, Zou et al. 2018). However, few studies have examined the photosynthetic characteristics of sugar beet species with different resistance to alkaline conditions. A better understanding of the factors involved in alkaline tolerance of sugar beet, particularly in the maintenance of high photochemical efficiency, may help in designing strategies for improvement of sugar beet yield under alkaline conditions.

The goal of this research was to examine the photosynthetic performance and physiological responses of alkaline-tolerant and sensitive sugar beet species and to provide a theoretical reference for the improvement of photosynthetic capacity of sugar beet under alkaline conditions.

**Materials and methods**

**Plant material and treatment:** Two sugar beet cultivars (Beta vulgaris L.) with contrasting alkaline tolerance were selected as tolerant – KWS0143 and as sensitive – Beta464. Pelleted seeds of the cultivar KWS0143 (KWS, Germany) and Beta464 (BETASEED, USA), were germinated in vermiculite with distilled water for a week, then irrigated with Hoagland solution for three weeks, and finally transplanted to hydroponic device filled with Hoagland solution at relative humidity of 65%, temperature of 25/20°C (day/night), light intensity of 450 μmol(photon) m⁻² s⁻¹, and a photoperiod of 16-h light/8-h dark. After one more week, seedlings were treated with Hoagland solution, containing 0 (A₀, control), 25 (A₁), 50 (A₂), 75 (A₃), and 100 (A₄) mM mixed alkaline (Na₂CO₃:NaHCO₃, 1:2). The pH values of each treatment is shown in the table below. Hoagland solution containing different concentrations of mixed alkaline was replaced every day to stabilize the pH values of each treatment. Osmotic substances contents and antioxidant enzyme activities in leaves of sugar beet were determined at 1, 3, 5, and 7 d after treatments (DAT). Leaf photosynthetic characteristics, contents of photosynthetic pigments, and Chl fluorescence were measured, and chloroplast ultrastructure of two cultivars under the control and A₄ treatment was observed at 7 DAT. A randomized complete block design with three replications per treatment was used.

**Gas exchange:** Net photosynthetic rate (Pₖ), stomatal conductance (gₛ), intercellular CO₂ concentration (Cᵢ), and transpiration rate (E) were measured simultaneously by CI-340 portable photosynthesis system (CID Bio-Science, Inc., USA) at 8:00–11:00 h. Experiments were carried out in triplicates, where each replicate included 15 leaves of different plants. The leaves were the first two fully developed ones from the top. All photosynthetic measure-ments were measured at 400 μmol(CO₂) mol⁻¹, 25°C, rela-tive humidity of 65%, and light intensity of 800 μmol m⁻² s⁻¹.
Chl fluorescence parameters were determined using a portable modulated fluorometer PAM-2500 (WALZ, Germany). Minimum fluorescence (F₀), maximum fluorescence (Fₘ), and variable fluorescence (Fᵥ) after dark adaptation, and minimum fluorescence (F₀'), maximum fluorescence (Fₘ'), steady-state fluorescence (Fₛ'), and variable fluorescence (Fᵥ') after light adaption were measured. Maximum quantum yield of PSII (ΦPSII) according to the ethanol/acetone method (Zhang 2009).

15 leaves of different plants, was carried out in triplicates and each replicate included 15 leaves of different plants.

Contents of photosynthetic pigments were measured in accordance with the ethanol/acetone method (Zhang 2009). Photosynthetic pigments were extracted from fresh samples by 80% acetone. The extract was centrifuged at 3,000 × g for 5 min. The absorbance of the supernatant was measured at 470, 645, and 663 nm with a UV-754 spectrophotometer (Zealquest Scientific, Shanghai, China). Contents of Chl a, Chl b, Chl (a+b), and Car were calculated using adjusted extinction coefficients. Pigment contents were expressed as mg g⁻¹ (fresh mass, FM).

Chloroplast ultrastructure: Samples were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 6.8) at 4°C overnight. After 15-min rinses with buffer solution three times, samples were postfixed in 1% osmium tetroxide (LKB, Sweden), stained with uranyl acetate and citric acid, and finally, examined using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan).

Osmotic substances: Determination of proline content was done according to Bates et al. (1973). Leaf samples (0.5 g) from each group were homogenized in 3% (w/v) sulfosalicylic acid and homogenate filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100°C for 1 h in water bath. Reaction was then stopped by using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspirated from liquid phase was read at 520 nm using a UV-754 spectrophotometer (Zealquest Scientific, Shanghai, China). Proline concentration was determined using calibration curve and expressed as μg g⁻¹ (fresh mass, FM).

Glycine betaine (betaine) content was measured using the method of Nishimura et al. (2001). Five milliliters of distilled water were added to 0.1 g of pulverized dried leaf samples and mixed well. The supernatant was separated and filtered with a filter membrane (pore size 0.2 μm). An esterification method by Gorham (1984) was modified in the following esterification reaction. In a microtube, 100 μL of plant extract or standard betaine solution was placed and mixed with 50 μL of a buffer solution (100 mM KHCO₃:100 mM KH₂PO₄: acetonitrile = 1:1:4, v/v). To the solution, 300 μL of a p-bromophenacyl bromide solution (20 mg mL⁻¹ in acetonitrile) was added. The tube was capped and heated at 80°C for 90 min. The reaction mixture was evaporated to dryness with a centrifugal evaporator at 80°C. After running electrolyte solution, samples were injected in the hydrostatic mode (10 cm, 10 s). The applied potential was 15 kV. The peak was monitored at 254 nm. The betaine content concentration of the plant extract was obtained by a calibration curve of standard betaine solutions. Leaf betaine content was expressed as μg g⁻¹ (DM).

Antioxidant enzymes: After homogenizing 0.5 g of plant material in 0.05 M PBS, and centrifugation at 10,000 × g for 20 min at 4°C, the supernatant was used to determine activities of SOD and POD (Fu et al. 2014). Enzyme activity assays were performed in 3-ml reaction volumes at 25°C and determined spectrophotometrically (Zealquest Scientific, Shanghai, China).

SOD activity was measured according to Stewart and Bewley (1980). The reaction mixture included 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM Na₂CO₃, and 100 μL of the enzyme extract. The reaction was allowed to proceed for 15 min under illumination of two 20-W fluorescent tubes. Absorbance of the reaction mixture was read at 560 nm. One unit of activity was defined as the amount of enzyme required to inhibit 50% of the initial reduction of NBT under light. Enzyme activity was expressed as unit g⁻¹ (FM). One unit of activity was defined as the amount of enzyme required to inhibit 50% of the initial reduction of NBT under light.

POD activity was assayed by monitoring the formation of guaiacol at 470 nm according to the method of Fu et al. (2014). A reaction mixture consisted of 16 mM guaiacol, 0.15 M phosphate buffer (pH = 6.1), 2 mM H₂O₂, and 100 μL of enzyme extract. Enzyme activity was expressed in mmol(guaiacol) min⁻¹ g⁻¹ (FM).

Statistical analyses were performed with software statistical package SPSS 20.0 (IBM, New York, USA). Two-way analyses of variance (ANOVA) were used to test the effects of alkaline stress, cultivar, and their interaction on physiological and biochemical variables. Differences were considered significant at P<0.05. Data were expressed as mean ± standard deviation (SD) from three individual experiments.

Results

Photosynthetic characteristics: Under the A₀ treatment, Be464 showed higher Pₛ than that of KWS0143; however, KWS0143 had a higher Pₛ than Beta464 under A₁, A₂,
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A1, and A4 treatments (Table 1). \( P_s \) of Beta464 gradually decreased with an increase in alkaline concentration, whereas \( P_n \) of KWS0143 increased to a maximum at A1 and declined thereafter in response to alkaline stress. The decrease of \( P_n \) was sharper in Beta464 compared to KWS0143 under A1 and A4 treatments. Compared to the plants subjected to A0 treatment, the \( P_n \) of KWS0143 decreased by 25.7 and 34.8% under A1 and A4 treatments, respectively, whereas that of Beta464 decreased by 71.1 and 71.4%, respectively.

For both cultivars, \( g_s \) and \( E \) gradually decreased with increasing alkaline concentration, whereas \( C_i \) increased with alkaline concentration (Table 1). The \( g_s \) of KWS0143 and Beta464 decreased by 84.3 and 77.8%, respectively, under the A4 treatment compared to the plants subjected to A0 treatment. Under A1 and A4 treatments, KWS0143 exhibited higher \( E \) than that of Beta464. A sharper decrease in \( E \) (by 68.7%) was observed in Beta464 compared to the 23.3% decrease in KWS0143 under the A4 treatment. Under each treatment, Beta464 showed a higher \( C_i \) than that of KWS0143. A2, A3, and A4 treatments significantly increased the \( C_i \) of both cultivars. Compared to the plants subjected to the A0 treatment, the \( C_i \) of KWS0143 and Beta464 increased by 37.0 and 41.1% under the A4 treatment, respectively.

**Chl fluorescence**: \( F_v/F_m \) of both cultivars gradually decreased under different treatments from A0 to A4 (Table 2). The A3 and A4 treatments led to a significant reduction in \( F_v/F_m \) of both cultivars. The \( \Phi_{PSII} \) and \( q_P \) of KWS0143 increased to a maximum at A1 and declined thereafter, whereas those of Beta464 gradually decreased along with alkaline concentration. Under the A0 treatment, Beta464 showed higher \( \Phi_{PSII} \) and \( q_P \) compared to KWS0143, but \( \Phi_{PSII} \) and \( q_P \) of KWS0143 were higher than that of Beta464 under A1, A2, A3, and A4 treatments. A sharper decrease in \( \Phi_{PSII} \) (by 16.2%) and \( q_P \) (by 30.6%) was observed in Beta464 compared to the decrease in \( \Phi_{PSII} \) (by 10.9%) and \( q_P \) (by 21.8%) of KWS0143 under the A1 treatment.

The \( q_P \) of KWS0143 decreased to a minimum at A2 and then increased in response to alkaline conditions, whereas that of Beta464 gradually increased (Table 2). The \( q_P \) of Beta464 was higher than that of KWS0143 under each treatment. The increase of \( q_P \) was sharper in Beta464 (by 17.2%) compared to KWS0143 (by 7.3%) under the A0 treatment.

ETR of KWS0143 gradually decreased in response to alkaline conditions, whereas ETR of Beta464 showed the greatest value at A1 and declined subsequently (Table 2). Under A0, A1, and A2 treatments, Be464 exhibited a higher ETR than that of KWS0143; however, KWS0143 showed higher ETR than that of Beta464 under A3 and A4 treatments. The decrease of ETR was sharper in Beta464 compared to KWS0143 under A1 and A4 treatments. Compared to the plants subjected to the A0 treatment, the ETR of KWS0143 decreased by 5.4 and 5.6% under A1 and A4 treatments, respectively, whereas that of Beta464 decreased by 16.1 and 16.5%, respectively.

**Photosynthetic pigments**: Chl \( a \), Chl \( b \), Chl \( (a+b) \), and Car contents of both cultivars rose to a maximum at A1 and declined thereafter in response to alkaline stress (Table 3). Under A1 and A4 treatments, KWS0143 exhibited higher contents of Chl \( a \), Chl \( b \), Chl \( (a+b) \), and Car than that of Beta464. The decrease of contents of Chl \( a \), Chl \( b \), Chl \( (a+b) \), and Car was sharper in Beta464 compared to KWS0143 under the A4 treatment. Compared to the plants subjected to A0 treatment, the ETR of Beta464 showed higher \( \Phi_{PSII} \) and \( q_P \) compared to KWS0143, but \( \Phi_{PSII} \) and \( q_P \) of KWS0143 were higher than that of Beta464 under A1, A2, A3, and A4 treatments. A sharper decrease in \( \Phi_{PSII} \) (by 16.2%) and \( q_P \) (by 30.6%) was observed in Beta464 compared to the decrease in \( \Phi_{PSII} \) (by 10.9%) and \( q_P \) (by 21.8%) of KWS0143 under the A1 treatment.

**ANOVA**

The values followed by the letters not present in the table are not significantly different via LSD test. * – significant at \( P<0.05 \); ** – significant at \( P<0.01 \); *** – significant at \( P<0.001 \).

Table 1. Leaf characteristics of two sugar beet cultivars under different treatments. \( P_n \) – net photosynthetic rate; \( g_s \) – stomatal conductance; \( C_i \) – intercellular \( CO_2 \) concentrations; \( E \) – transpiration rate. Data are presented as means ± SD (\( n = 3 \)). The different capital letters followed the mean values denote significant differences between different cultivars under the same treatments (\( P<0.05 \)). The different lowercase letters denote significant differences (\( P<0.05 \)) between different alkaline treatments under the same cultivars (\( P<0.05 \)). The \( F \) values followed by the letters not present in the table are not significantly different via LSD test.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>( P_n ) [( \mu mol(CO_2) m^{-2} s^{-1} )]</th>
<th>( g_s ) [( \mu mol(H_2O) m^{-2} s^{-1} )]</th>
<th>( C_i ) [( \mu mol(CO_2) mol^{-1} )]</th>
<th>( E ) [( \mu mol(H_2O) m^{-2} s^{-1} )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KWS0143</td>
<td>A0</td>
<td>7.42 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.254 ± 0.06A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>212.7 ± 6.5B&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.50 ± 0.01A&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>A1</td>
<td>8.12 ± 0.32A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.217 ± 0.027A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>174.1 ± 2.2B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.57 ± 0.08A&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>A2</td>
<td>7.38 ± 0.45A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.174 ± 0.045A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>240.8 ± 9.6B&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.39 ± 0.07A&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>A3</td>
<td>5.51 ± 0.30A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.055 ± 0.002A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.5 ± 7.9B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.34 ± 0.05A&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>A4</td>
<td>4.84 ± 0.35A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.040 ± 0.003A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>291.3 ± 5.6B&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.15 ± 0.02A&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Beta464</td>
<td>A0</td>
<td>8.78 ± 0.24A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.162 ± 0.026A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264.7 ± 1.9A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.43 ± 0.04A&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>A1</td>
<td>7.13 ± 0.33A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.144 ± 0.029A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>256.4 ± 7.5A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.33 ± 0.03A&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>A2</td>
<td>5.87 ± 0.51A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.063 ± 0.005A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>275.7 ± 0.7A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.32 ± 0.08A&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>A3</td>
<td>2.54 ± 0.23A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.055 ± 0.002A&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>A4</td>
<td>2.51 ± 0.46A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.036 ± 0.003A&lt;sup&gt;ab&lt;/sup&gt;</td>
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Two – way ANOVA

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<th>( F ) value</th>
<th>( P ) value</th>
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<td>204.90***</td>
<td>Treatment × Cultivar</td>
<td>306.03***</td>
<td>306.03***</td>
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Table 2. Chlorophyll fluorescence parameters of two sugar beet cultivars under different treatments. Fv/Fm – maximum quantum yield of PSII; ΦPSII – PSII efficiency; qP – photochemical quenching; qN – nonphotochemical quenching; ETR – apparent rate of electron transport at the PSII level. Data are presented as means ± SD (n = 3). The different capital letters followed the mean values denote significant differences between different cultivars under the same treatments (P<0.05). The different lowercase letters denote significant differences (P<0.05) between different alkaline treatments under the same cultivars (P<0.05). The F value followed by the letters ns is not significantly different via LSD test, * – significant at P<0.05; ** – significant at P<0.01; *** – significant at P<0.001.

Table 3. Contents of photosynthetic pigments in leaves of two sugar beet cultivars under different treatments. Chl – chlorophyll; Car – carotenoids. Data are presented as means ± SD (n = 3). The different capital letters followed the mean values denote significant differences between different cultivars under the same treatments (P<0.05). The different lowercase letters denote significant differences (P<0.05) between different alkaline treatments under the same cultivars (P<0.05). The F values followed by the letters ns are not significantly different via LSD test, * – significant at P<0.05; ** – significant at P<0.01; *** – significant at P<0.001.

Table 4. Chloroplast ultrastructure of two sugar beet cultivars under different treatments. Chloroplasts of both cultivars exhibited an orderly arrangement of granal andstromal thylakoids under the A0 treatment (Fig. 1A,C). The starch granule volumes in leaves of KWS0143 decreased and the starch granules of Beta464 increased, while chloroplasts in leaves of both cultivars appeared to be longer under the A3 treatment (Fig. 1B,D). The stacks of grana of both cultivars appeared loose and numerous osmiophilic drops were visible (Fig. 1B,D). Moreover, the inner structure of the chloroplast of Beta464 was disorganized and the outer membrane of some chloroplasts was broken (Fig. 1D).

Chloroplast ultrastructure: Chloroplasts of both cultivars exhibited an orderly arrangement of granal and stromal thylakoids under the A0 treatment (Fig. 1A,C). The starch granule volumes in leaves of KWS0143 decreased and the starch granules of Beta464 increased, while chloroplasts in leaves of both cultivars appeared to be longer under the A3 treatment (Fig. 1B,D). The stacks of grana of both cultivars appeared loose and numerous osmiophilic drops were visible (Fig. 1B,D). Moreover, the inner structure of the chloroplast of Beta464 was disorganized and the outer membrane of some chloroplasts was broken (Fig. 1D).

Osmotic substances: The leaf proline content of both cultivars first increased but then decreased with increasing alkaline concentration (Table 4). The leaf proline content of both cultivars at A1 significantly increased compared with that of the control at each DAT. In addition, the leaf proline content of KWS0143 at A2 and A3 significantly increased...
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at each DAT. The leaf proline contents of KWS0143 under the A2 treatment were 107.8, 101.5, 107.0, and 40.1% higher than those of the control at 1, 3, 5, and 7 DAT, respectively, whereas the leaf proline contents of Beta464 under the A1 treatment were 90.7, 24.3, 89.7, and 6.7% higher than those under the A0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf proline content of KWS0143 were higher under A2, A3, and A4 treatments than those of Beta464 at each DAT.

The leaf betaine content of two cultivars first increased but then decreased with the increase in alkaline concentration (Table 5). The leaf betaine content of two cultivars at A2 significantly increased compared with that of the control at each DAT. Moreover, the leaf betaine contents of KWS0143 at A1, A3, and A4 significantly increased at each DAT. The leaf betaine contents of KWS0143 under the A2 treatment were 63.1, 40.5, 54.2, and 52.1% higher than those of the control at 1, 3, 5, and 7 DAT, respectively, whereas the leaf betaine contents of Beta464 under the A2 treatment were 161.1, 24.2, 36.6, and 45.7% higher than those under the A0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf betaine content of KWS0143 were higher under A3 and A4 treatments than those of Beta464 at each DAT.

Antioxidant enzymes: Leaf SOD activity first increased but then decreased with increasing alkaline concentration (Table 6). The leaf SOD activity of KWS0143 at A1 and A2 significantly increased compared with that of the control at each DAT, whereas the leaf SOD activity of Beta464 at A1 significantly increased at 1, 3, and 7 DAT. However, the leaf SOD activity of two cultivars at A4 significantly decreased compared with that of the control. The leaf SOD activities of KWS0143 under the A1 treatment were 41.1, 30.0, 31.1, and 21.0% lower than those of the control at 1, 3, 5, and 7 DAT, respectively, whereas the leaf SOD activities of Beta464 under the A4 treatment were 39.3, 53.4, 27.0, and 28.5% lower than those under the A0 treatment at 1, 3, 5, and 7 DAT, respectively.

Leaf POD activity first increased but then decreased with an increase in alkaline concentration (Table 7). The leaf POD activity of both cultivars at A2 was significantly enhanced at each DAT. The leaf POD activities of KWS0143 under the A2 treatment were 59.7, 29.6, 50.1, and 14.9% higher than those of the control at 1, 3, 5, and 7 DAT, respectively, whereas the leaf POD activities of Beta464 under the A2 treatment were 73.0, 82.1, 8.8, and 48.8% higher than those under the A0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf POD activity of KWS0143 under the A4 treatment was higher than that of Beta464 at each DAT.

Discussion

Energy imbalance and photosynthesis: High levels of alkaline stress (A3 and A4) reduced substantially PN, gs, and E, but significantly increased Ci of both sugar beet cultivars (Table 1), indicating that photosynthesis of sugar beet was inhibited under alkaline stress. From the analysis of gas-exchange parameters, we concluded that the inhibition of PN in sugar beet by alkaline stress was not the
result of stomatal closing, but occurred due to nonstomatal limitations. The greater reduction of $P_N$ could be due to high pH injury. We found that high concentrations of alkali (A3 and A4) lowered $F_v/F_m$, $\Phi_{PSII}$, $q_P$, and ETR in two sugar beet cultivars (Table 2). This indicated that the stress affected the efficiency of photosynthetic electron transport and the capture of light energy by PSII, which might be the main reason for the decrease of $P_N$ under conditions of alkaline stress. Our findings regarding photosynthetic characteristics and Chl fluorescence parameters were similar to previous studies (Liu and Shi 2010, Wu et al. 2014). Moreover, compared to Beta464, KWS0143 showed higher $P_N$, $F_v/F_m$, $\Phi_{PSII}$, $q_P$, and ETR under alkaline stress (Tables 1, 2). This suggested that KWS0143 could maintain relatively stable photosynthetic capacity and photosynthetic electron transport in response to alkaline stress. The sharper decrease in $P_N$ of Beta464 under more alkaline conditions revealed that photosynthetic apparatus of Beta464 might be damaged more severely by alkali.
Photosynthetic pigments are important indexes of the photosynthetic capacity of plants. Photosynthetic pigment concentrations were determined after 7 DAT, thus, the results could reflect the response of seedlings to alkaline stress. The concentrations of Chl and Car were significantly reduced by higher (A3 and A4) alkalinity, while enhanced at low levels (A1 and A2) of alkalinity (Table 3). Similar results were obtained for maize when grown under alkaline stress (Rui et al. 2017). The decreasing photosynthetic pigment concentrations with increasing stress indicated that alkali salt may enhance the activity of the Chl-degrading enzyme chlorophyllase (Yang et al. 2011). Another possible reason might be due to the precipitation of Mg²⁺ at high pH, hence inhibiting Chl synthesis (Shi and Zhao 1997). Elstner (1982) reported that the disturbance in the balance of certain ions (i.e., Na⁺) under saline and alkaline conditions could inhibit proteinase activity and alter the Chl concentration in leaves, leading to reduced photosynthesis in the plants. In present study, KWS0143 showed higher contents of Chl and Car and suffered a relatively slighter
A relatively high level of antioxidative activity under environmental stress to eliminate ROS and reduce damage (Parihar et al. 2015). In order to avoid oxidative damage by ROS, antioxidants, including key enzymes (i.e., SOD, POD, CAT, APX, and GR) catalyze ROS detoxification (Vuleta et al. 2016). Our results showed that leaf SOD and POD activities all increased under low levels (A1 and A2) of alkaline stress (Tables 6, 7). These results suggested that antioxidant enzymes, including SOD and POD in leaves of sugar beet, could participate in oxygen scavenging to resist injury from Na⁺ and pH when alkaline stress are at relatively low levels. However, when alkaline stress reached a higher level (A4), the activities of leaf SOD and POD decreased (Tables 6, 7). It indicated that the antioxidant enzyme system of sugar beet lost its intrinsic balance and was unable to resist effectively to the further generation of excessive ROS. Compared to Beta464, KWS0143 showed higher activities of SOD and POD at different stages under high (A4) alkaline conditions. This suggested that KWS0143 possesses stronger antioxidation ability to eliminate ROS and reduce damage to photosystems caused by Na⁺ and pH.

Conclusion: High levels (A1 and A2) of alkaline stress decreased the photosynthetic capacity, photosynthetic electron transport efficiency, and photosynthetic pigment contents of sugar beet. Alkaline stress caused significant alterations in chloroplast ultrastructure of sugar beet. Changes in chloroplast ultrastructure are another important reason for the decrease in the Chl content. Thus, the loss of photosynthetic pigments and damage to the chloroplasts ultimately disturbed photosynthetic capacity (Qiao et al. 2013).

Osmotic substances: Increasing osmolyte contents, such as proline and betaine, can reduce the osmotic potential of cell protoplasm. This reduction is beneficial for retaining water uptake to maintain cell turgor, improve the stability of the plasma colloid (Clifford et al. 1998), and ultimately relieve ion toxicity and physiological drought resulting from salt alkaline stress (Patade et al. 2011). Our results showed that leaf proline and betaine contents of sugar beet were substantially enhanced when exposed to low levels (A1 and A2) of alkaline stress (Tables 4, 5). These findings indicate that sugar beet can reduce cell osmotic potential by osmotic adjustment when alkaline stress was at lower level. However, when alkaline stress reached the higher level (A4), osmolyte contents began to decrease, especially, the leaf proline content in Beta464 (Tables 4, 5). These results suggested that excessively high alkaline stress is beyond the osmotic adjustment ability of sugar beet, resulting in membrane damage and Chl degradation, ultimately hindering photosynthesis. Moreover, KWS0143 exhibited higher betaine content than that in Beta464 at different stages under high levels (A1 and A2) of alkaline stress (Table 5). This indicated that KWS0143 has a stronger ability of osmotic adjustment to relieve injury to photosynthetic apparatus from osmotic stress caused by alkalinity.

Antioxidant enzymes: Similar to other abiotic stresses, alkaline stress triggers oxidative damage resulting from the production of ROS (An et al. 2016). Plants maintain...


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