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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 (A_0, control), 25 (A_1), 50 (A_2), 75 (A_3), and 100 mM of mixed alkali (Na_2CO_3:$NaHCO_3$, 1:2). A_2, A_3, and A_4 reduced net photosynthetic rate (P_N), stomatal conductance, and transpiration rate, but rose intercellular CO_2 concentration. Reduction in P_N occurred probably due to nonstomatal limitation. The decrease in efficiency of photosynthetic electron transport might be the main reason for the decrease of P_N. The concentrations of photosynthetic pigments were significantly reduced by high (A_3 and A_4) 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 alkalization is also a considerable phenomenon in Northeast China (Wang *et al*. 2009). In extensive alkaline soils over much of Northeast China, the neutral salts, NaCl and Na_2SO_4, and the alkaline salts, $NaHCO_3$ and Na_2CO_3, 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 CO_2 concentration in chloroplasts relative to that in substomatal cavities, can limit photosynthesis (Warren 2008). Wu *et al.* (2014) found that alkaline stress reduced F_v/F_m, 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 (F_v/F_m), PSII efficiency (Φ_{PSII}), photochemical quenching (q_P), nonphotochemical quenching (q_N), 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 P_N, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), maximal efficiency of PSII photochemistry, photochemical quenching coefficient (q_P), 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,

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Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DAT – days after treatment; E – transpiration rate; ETR – apparent rate of electron transport at the PSII level; F_v/F_m – maximum quantum yield of PSII; GR – glutathione reductase; g_s – stomatal conductance; P_N – net photosynthetic rate; POD – peroxidase; q_P – photochemical quenching; q_N – nonphotochemical quenching; ROS – reactive oxygen species; SOD – superoxide dismutase; Φ_{PSII} – PSII efficiency.
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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 synthetize 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 (A_0, control), 25 (A_1), 50 (A_2), 75 (A_3), and 100 (A_4) mM mixed alkaline (Na_2CO_3:$NaHCO_3$, 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 A_3 treatment was observed at 7 DAT. A randomized complete block design with three replications per treatment was used.

Gas exchange: Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), 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(CO_2) mol^{-1}, 25°C, rela-tive humidity of 65%, and light intensity of 800 μmol m^{-2} s^{-1}.

Treatment	A_0 (control)	A_1	A_2	A_3	A_4
pH	6.85 ± 0.02	9.16 ± 0.05	9.55 ± 0.01	9.67 ± 0.03	9.75 ± 0.05

Chl fluorescence parameters were determined using a portable modulated fluorometer *PAM-2500* (*WALZ*, Germany). Minimum fluorescence (F_0), maximum fluorescence (F_m), and variable fluorescence (F_v) after dark adaptation, and minimum fluorescence (F_0'), maximum fluorescence (F_m'), steady-state fluorescence (F'), and variable fluorescence (F_v') after light adaption were measured. Maximum quantum yield of PSII (F_v/F_m), PSII efficiency (Φ_{PSII}), photochemical quenching (q_P), nonphotochemical quenching (q_N), and apparent rate of electron transport at the PSII level (ETR) were automatically given by the instrument. The experiment was performed in the morning (8:00–11:00 h) with flag leaves dark-adapted for at least 30 min before measurements and then exposed to continuous red light. The measuring light was 0.1 μmol(photon) m^{-2} s^{-1} and the saturation pulse light used was 10,000 μmol(photon) m^{-2} s^{-1}. The experiment 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 acid for 2 h and rinsed three times with the same buffer solution. The samples were dehydrated in a graded acetone series, infiltrated, and embedded in freshly prepared epoxy resin, and polymerized at 60°C for 48 h. Ultrathin sections (50–60 nm) were cut with ultramicrotome (*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 aspired 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}(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 $KHCO_3$:100 mM KH_2PO_4: 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 Na_2CO_3, 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 H_2O_2, 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 A_0 treatment, Be464 showed higher P_N than that of KWS0143; however, KWS0143 had a higher P_N than Beta464 under A_1, A_2,

A_3, and A_4 treatments (Table 1). P_N of Beta464 gradually decreased with an increase in alkaline concentration, whereas P_N of KWS0143 increased to a maximum at A_1 and declined thereafter in response to alkaline stress. The decrease of P_N was sharper in Beta464 compared to KWS0143 under A_3 and A_4 treatments. Compared to the plants subjected to A_0 treatment, the P_N of KWS0143 decreased by 25.7 and 34.8% under A_3 and A_4 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 A_4 treatment compared to the plants subjected to A_0 treatment. Under A_3 and A_4 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 A_4 treatment. Under each treatment, Beta464 showed a higher C_i than that of KWS0143. A_2, A_3, and A_4 treatments significantly increased the C_i of both cultivars. Compared to the plants subjected to the A_0 treatment, the C_i of KWS0143 and Beta464 increased by 37.0 and 41.1% under the A_4 treatment, respectively.

Chl fluorescence: F_v/F_m of both cultivars gradually de-creased under different treatments from A_0 to A_4 (Table 2). The A_3 and A_4 treatments led to a significant reduction in F_v/F_m of both cultivars. The Φ_{PSII} and q_P of KWS0143 increased to a maximum at A_1 and declined thereafter, whereas those of Beta464 gradually decreased along with alkaline concentration. Under the A_0 treatment, Beta464 showed higher Φ_{PSII} and q_P compared to KWS0143, but Φ_{PSII} and q_P of KWS0143 were higher than that of Beta464 under A_1, A_2, A_3, and A_4 treatments. A sharper decrease in Φ_{PSII} (by 16.2%) and q_P (by 30.6%) was observed in Beta464 compared to the decrease in Φ_{PSII} (by 10.9%) and q_P (by 21.8%) of KWS0143 under the A_4 treatment.

The q_N of KWS0143 decreased to a minimum at A_2 and then increased in response to alkaline conditions, whereas that of Beta464 gradually increased (Table 2). The q_N of Beta464 was higher than that of KWS0143 under each treatment. The increase of q_N was sharper in Beta464 (by 17.2%) compared to KWS0143 (by 7.3%) under the A_4 treatment.

ETR of KWS0143 gradually decreased in response to alkaline conditions, whereas ETR of Beta464 showed the greatest value at A_1 and declined subsequently (Table 2). Under A_0, A_1, and A_2 treatments, Be464 exhibited a higher ETR than that of KWS0143; however, KWS0143 showed higher ETR than that of Beta464 under A_3 and A_4 treatments. The decrease of ETR was sharper in Beta464 compared to KWS0143 under A_3 and A_4 treatments. Compared to the plants subjected to A_0 treatment, the ETR of KWS0143 decreased by 5.4 and 5.6% under A_3 and A_4 treatments, respectively, whereas that of Be464 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 A_1 and declined thereafter in response to alkaline stress (Table 3). Under A_3 and A_4 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 A_4 treatment. Compared to the plants subjected to A_0 treatment, contents of Chl *a*, Chl *b*, and Car in KWS0143 decreased by 24.7, 16.3, and 29.3% under the A_4 treatment, respectively, whereas those of Beta464

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, * – significant at P<0.05; ** – significant at P<0.01; *** – significant at P<0.001.

Cultivar	Treatment	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [μmol(H_2O) m^{-2} s^{-1}]
KWS0143	A_0	7.42 ± 0.17^{Bb}	0.254 ± 0.065^{Aa}	212.7 ± 6.5^{Bd}	1.50 ± 0.01^{Aa}
	A_1	8.12 ± 0.32^{Aa}	0.217 ± 0.027^{Aab}	174.1 ± 2.2^{Be}	1.57 ± 0.08^{Aa}
	A_2	7.38 ± 0.45^{Ab}	0.174 ± 0.045^{Ab}	240.8 ± 9.6^{Bc}	1.39 ± 0.07^{Ab}
	A_3	5.51 ± 0.30^{Ac}	0.055 ± 0.002^{Ac}	260.5 ± 7.9^{Bb}	1.34 ± 0.05^{Ab}
	A_4	4.84 ± 0.35^{Ac}	0.040 ± 0.003^{Ac}	291.3 ± 5.6^{Ba}	1.15 ± 0.02^{Ac}
Beta464	A_0	8.78 ± 0.24^{Aa}	0.162 ± 0.026^{Ba}	264.7 ± 1.9^{Ad}	1.43 ± 0.04^{Aa}
	A_1	7.13 ± 0.33^{Bb}	0.144 ± 0.029^{Ba}	256.4 ± 7.5^{Ad}	1.33 ± 0.03^{Bb}
	A_2	5.87 ± 0.51^{Bc}	0.063 ± 0.005^{Bb}	275.7 ± 0.7^{Ac}	1.32 ± 0.08^{Ab}
	A_3	2.54 ± 0.23^{Bd}	0.055 ± 0.002^{Ab}	295.7 ± 4.0^{Ab}	0.90 ± 0.03^{Bc}
	A_4	2.51 ± 0.46^{Bd}	0.036 ± 0.003^{Ab}	373.6 ± 4.0^{Aa}	0.45 ± 0.04^{Bd}
Two – way *ANOVA*					
Treatment		189.16^{***}	58.67^{***}	377.25^{***}	204.90^{***}
Cultivar		93.33^{***}	40.89^{***}	705.21^{***}	306.03^{***}
Treatment × cultivar		31.23^{***}	6.85^{**}	22.12^{***}	48.00^{***}

Table 2. Chlorophyll fluorescence parameters of two sugar beet cultivars under different treatments. F_v/F_m – maximum quantum yield of PSII; Φ_{PSII} – PSII efficiency; q_P – photochemical quenching; q_N – 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.

Cultivar	Treatment	F_v/F_m	Φ_{PSII}	q_P	q_N	ETR
KWS0143	A_0	0.806 ± 0.017^{Aa}	0.604 ± 0.008^{Bc}	0.733 ± 0.010^{Aa}	0.246 ± 0.001^{Bb}	53.3 ± 0.6^{Ba}
	A_1	0.797 ± 0.009^{Aa}	0.679 ± 0.005^{Aa}	0.761 ± 0.007^{Ab}	0.191 ± 0.002^{Bc}	53.0 ± 0.4^{Ba}
	A_2	0.795 ± 0.009^{Aa}	0.635 ± 0.008^{Ab}	0.665 ± 0.012^{Ac}	0.158 ± 0.004^{Bd}	51.1 ± 0.7^{Bb}
	A_3	0.771 ± 0.010^{Ab}	0.566 ± 0.006^{Ad}	0.653 ± 0.007^{Ad}	0.231 ± 0.009^{Bb}	50.4 ± 0.5^{Ac}
	A_4	0.734 ± 0.019^{Ac}	0.538 ± 0.007^{Ae}	0.573 ± 0.008^{Ae}	0.264 ± 0.009^{Ba}	50.3 ± 0.6^{Ac}
Beta464	A_0	0.787 ± 0.009^{Aa}	0.628 ± 0.006^{Aa}	0.743 ± 0.009^{Aa}	0.274 ± 0.008^{Ab}	54.6 ± 0.5^{Aa}
	A_1	0.785 ± 0.009^{Aa}	0.560 ± 0.006^{Bb}	0.692 ± 0.010^{Bb}	0306 ± 0.008^{Aa}	55.1 ± 0.5^{Aa}
	A_2	0.776 ± 0.009^{Aa}	0.549 ± 0.006^{Bc}	0.622 ± 0.013^{Bc}	0.306 ± 0.034^{Aa}	54.5 ± 0.5^{Aa}
	A_3	0.760 ± 0.009^{Ab}	0.552 ± 0.013^{Bc}	0.547 ± 0.020^{Bd}	0.319 ± 0.010^{Aa}	45.8 ± 1.1^{Bb}
	A_4	0.732 ± 0.009^{Ac}	0.526 ± 0.001^{Bd}	0.516 ± 0.003^{Be}	0.321 ± 0.018^{Aa}	45.6 ± 0.1^{Bb}
Two – way *ANOVA*						
Treatment		36.40^{***}	$1{,}115.03^{***}$	$2{,}794.33^{***}$	33.36^{***}	1552.62^{***}
Cultivar		1.14^{ns}	1685.64^{***}	1613.33^{***}	580.65^{***}	138.34^{***}
Treatment × cultivar		21.07^{***}	679.66^{***}	156.14^{***}	32.95^{***}	$1{,}018.38^{***}$

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.

Cultivar	Treatment	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Chl (*a*+*b*) [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]
KWS0143	A_0	2.538 ± 0.088^{Ab}	0.867 ± 0.051^{Ab}	3.404 ± 0.080^{Ab}	0.368 ± 0.084^{Aab}
	A_1	2.758 ± 0.032^{Aa}	1.029 ± 0.039^{Aa}	3.787 ± 0.063^{Aa}	0.400 ± 0.081^{Aa}
	A_2	2.664 ± 0.028^{Aa}	0.925 ± 0.066^{Ab}	3.590 ± 0.080^{Ac}	0.379 ± 0.065^{Aa}
	A_3	2.242 ± 0.028^{Ac}	0.784 ± 0.074^{Ac}	3.026 ± 0.100^{Ad}	0.320 ± 0.058^{Aab}
	A_4	1.910 ± 0.030^{Ad}	0.726 ± 0.019^{Ac}	2.636 ± 0.024^{Ae}	0.260 ± 0.026^{Ab}
Beta464	A_0	2.629 ± 0.082^{Aa}	0.938 ± 0.023^{Ac}	3.568 ± 0.093^{Ab}	0.360 ± 0.049^{Aa}
	A_1	2.675 ± 0.043^{Aa}	1.041 ± 0.050^{Aa}	3.717 ± 0.088^{Aa}	0.401 ± 0.097^{Aa}
	A_2	2.672 ± 0.050^{Aa}	0.674 ± 0.042^{Bb}	3.347 ± 0.084^{Bc}	0.369 ± 0.077^{Aa}
	A_3	1.751 ± 0.037^{Bb}	0.527 ± 0.049^{Bd}	2.278 ± 0.046^{Bd}	0.248 ± 0.041^{Ab}
	A_4	1.467 ± 0.100^{Bc}	0.510 ± 0.052^{Bd}	1.977 ± 0.064^{Be}	0.228 ± 0.034^{Ab}
Two – way *ANOVA*					
Treatment		376.83^{***}	86.43^{***}	445.52^{***}	6.34^{**}
Cultivar		74.71^{***}	59.24^{***}	141.23^{***}	1.09^{ns}
Treatment × cultivar		31.58^{***}	17.84^{***}	43.57^{***}	0.31^{ns}

decreased by 44.2, 45.6, and 36.7%, respectively.

Chloroplast ultrastructure: Chloroplasts of both cultivars exhibited an orderly arrangement of granal and stromal thylakoids under the A_0 treatment (Fig. 1*A*,*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 A_3 treatment (Fig. 1*B*,*D*). The stacks of grana of both cultivars appeared loose and numerous osmiophilic drops were visible (Fig. 1*B*,*D*). Moreover, the inner structure of the chloroplast of Beta464 was disorganized and the outer membrane of some chloroplasts was broken (Fig. 1*D*).

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 A_1 significantly increased compared with that of the control at each DAT. In addition, the leaf proline content of KWS0143 at A_2 and A_3 significantly increased

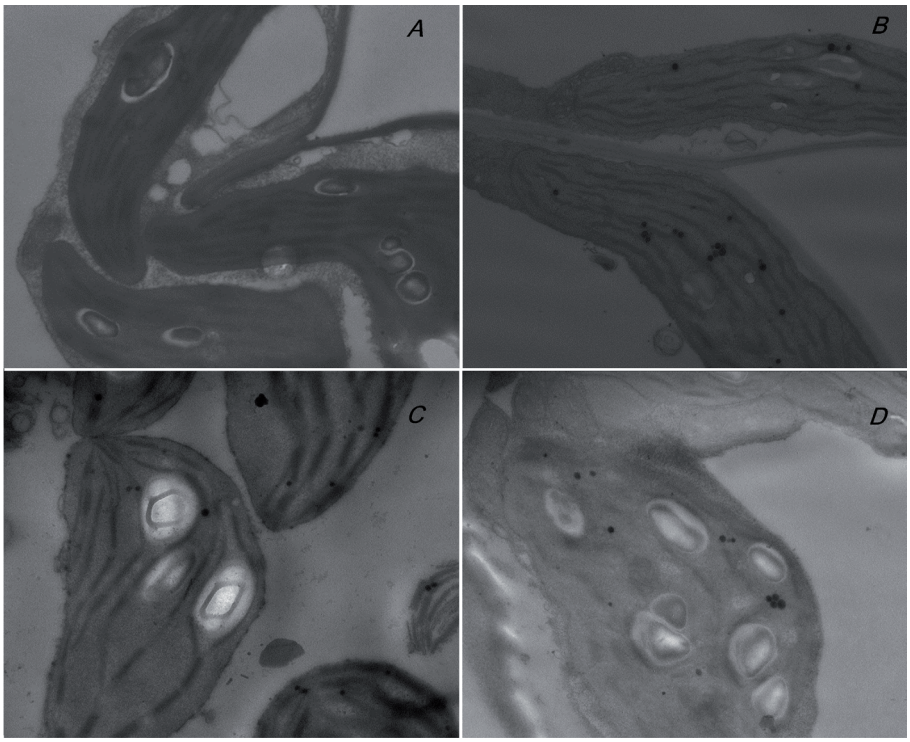

Fig. 1. Effects of alkaline stress on chloroplasts ultrastructure (×20,000) of two sugar beet cultivars. *A* and *B* present chloroplasts ultrastructure of KWS0143 under A0 and A3 treatment, respectively; *C* and *D* present chloroplasts ultrastructure of Beta464 under A0 and A2 treatment, respectively.

at each DAT. The leaf proline contents of KWS0143 under the A_2 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 A_1 treatment were 90.7, 24.3, 89.7, and 6.7% higher than those under the A_0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf proline content of KWS0143 were higher under A_2, A_3, and A_4 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 A_2 significantly increased compared with that of the control at each DAT. Moreover, the leaf betaine contents of KWS0143 at A_1, A_3, and A_4 significantly increased at each DAT. The leaf betaine contents of KWS0143 under the A_2 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 A_2 treatment were 161.1, 24.2, 36.6, and 45.7% higher than those under the A_0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf betaine content of KWS0143 were higher under A_3 and A_4 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 A_1 and A_2 significantly increased compared with that of the control at each DAT, whereas the leaf SOD activity of Beta464 at A_1 significantly increased at 1, 3, and 7 DAT. However, the leaf SOD activity of two cultivars at A_4 significantly decreased compared with that of the control. The leaf SOD activities of KWS0143 under the A_4 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 A_4 treatment were 39.3, 53.4, 27.0, and 28.5% lower than those under the A_0 treatment at 1, 3, 5, and 7 DAT, respectively.

Leaf POD activity first increased but then decreased with an increase in alkaline concetration (Table 7). The leaf POD activity of both cultivars at A_2 was significantly enhanced at each DAT. The leaf POD activities of KWS0143 under the A_2 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 A_2 treatment were 73.0, 82.1, 8.8, and 48.8% higher than those under A_0 treatment at 1, 3, 5, and 7 DAT, respectively. Moreover, the leaf POD activity of KWS0143 under the A_4 treatment was higher than that of Beta464 at each DAT.

Discussion

Energy imbalance and photosynthesis: High levels of alkaline stress (A_3 and A_4) reduced substantially P_N, g_s, and E, but significantly increased C_i 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 P_N in sugar beet by alkaline stress was not the

Table 4. Proline content [μg g^{-1}(FM)] in leaves of two sugar beet cultivars under different treatments. 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.

Cultivar	Treatment	Time after treatment [d] 1	3	5	7
KWS0143	A_0	26.8 ± 1.5^{Ac}	19.5 ± 2.6^{Ac}	22.7 ± 1.5^{Ac}	22.7 ± 2.6^{Ac}
	A_1	50.3 ± 4.0^{Aa}	25.3 ± 2.8^{Bb}	40.0 ± 2.6^{Aab}	31.2 ± 2.0^{Aa}
	A_2	55.7 ± 4.0^{Aa}	39.3 ± 2.5^{Aa}	47.0 ± 6.0^{Aa}	31.8 ± 1.2^{Aa}
	A_3	43.7 ± 4.9^{Ab}	27.5 ± 2.3^{Ab}	39.3 ± 2.5^{Ab}	27.7 ± 2.0^{Ab}
	A_4	22.7 ± 1.2^{Ac}	18.3 ± 0.3^{Ac}	14.3 ± 3.8^{Ad}	20.3 ± 1.0^{Ac}
Beta464	A_0	27.0 ± 1.7^{Ab}	27.2 ± 0.3^{Ab}	25.3 ± 1.2^{Ac}	27.0 ± 1.3^{Ab}
	A_1	51.5 ± 3.1^{Aa}	33.8 ± 5.1^{Aa}	48.0 ± 5.6^{Aa}	28.8 ± 2.9^{Aa}
	A_2	28.7 ± 2.6^{Bb}	28.2 ± 1.0^{Bb}	40.3 ± 6.7^{Ab}	29.5 ± 1.8^{Aa}
	A_3	26.0 ± 2.2^{Bb}	23.3 ± 1.5^{Ab}	17.2 ± 2.9^{Bd}	23.5 ± 1.3^{Ab}
	A_4	23.8 ± 3.3^{Ab}	16.2 ± 4.2^{Ac}	10.7 ± 1.8^{Ae}	18.0 ± 2.2^{Ac}
Two – way *ANOVA*					
Treatment		73.72***	31.81***	64.00***	32.41***
Cultivar		51.72***	0.07^{ns}	8.39**	3.52^{ns}
Treatment × cultivar		25.17***	9.98***	11.56***	4.06*

Table 5. Betaine content [μg g^{-1}(DM)] in leaves of two sugar beet cultivars under different treatments. 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.

Cultivar	Treatment	Time after treatment [d] 1	3	5	7
KWS0143	A_0	268.3 ± 23.9^{Bd}	284.0 ± 39.2^{Ac}	264.0 ± 25.2^{Ac}	268.0 ± 43.3^{Ac}
	A_1	376.0 ± 12.0^{Abc}	391.7 ± 19.9^{Ab}	368.7 ± 48.2^{Aab}	360.0 ± 27.0^{Ab}
	A_2	437.7 ± 7.1^{Aa}	399.0 ± 40.8^{Ab}	407.0 ± 32.2^{Aa}	407.7 ± 36.7^{Aab}
	A_3	414.7 ± 42.8^{Aab}	460.7 ± 14.4^{Aa}	376.0 ± 12.5^{Aab}	437.0 ± 35.5^{Aa}
	A_4	360.0 ± 19.1^{Ac}	391.0 ± 20.3^{Ab}	337.3 ± 34.1^{Ab}	430.7 ± 3.1^{Aa}
Beta464	A_0	337.0 ± 16.6^{Abc}	314.7 ± 20.0^{Ab}	303.0 ± 47.8^{Ab}	284.7 ± 40.8^{Ac}
	A_1	376.0 ± 31.7^{Aab}	361.0 ± 45.7^{Aab}	337.0 ± 8.9^{Ab}	345.7 ± 20.0^{Ab}
	A_2	391.3 ± 14.6^{Aa}	391.0 ± 25.2^{Aa}	414.0 ± 22.0^{Aa}	414.7 ± 37.4^{Aa}
	A_3	353.3 ± 36.1^{Aab}	315.7 ± 12.1^{Bb}	307.0 ± 23.3^{Bb}	391.3 ± 41.8^{Aab}
	A_4	299.3 ± 43.2^{Ac}	261.7 ± 24.9^{Bc}	291.3 ± 34.4^{Bb}	230.7 ± 15.5^{Bc}
Two – way *ANOVA*					
Treatment		17.15***	26.58***	12.40***	17.40***
Cultivar		4.26^{ns}	11.76***	2.80^{ns}	14.48**
Treatment × cultivar		6.60**	9.90***	2.56^{ns}	10.19***

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 (A_3 and A_4) lowered F_v/F_m, Φ_{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, Φ_{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.

Table 6. Leaf SOD activity [U g^{-1}(FM)] of two sugar beet cultivars under different treatments. SOD – superoxide dismutase. 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.

Cultivar	Treatment	Time after treatment [d]			
		1	3	5	7
KWS0143	A_0	190.5 ± 3.9^{Ad}	155.2 ± 5.9^{Bd}	237.4 ± 6.3^{Ac}	273.5 ± 4.0^{Ac}
	A_1	248.8 ± 5.7^{Aa}	229.7 ± 1.0^{Aa}	250.9 ± 6.7^{Aab}	289.7 ± 2.3^{Ab}
	A_2	203.2 ± 2.5^{Ac}	180.6 ± 5.8^{Bb}	258.8 ± 5.7^{Aa}	314.2 ± 2.6^{Aa}
	A_3	221.5 ± 1.3^{Ab}	168.1 ± 7.4^{Ac}	242.9 ± 2.3^{Abc}	294.7 ± 3.3^{Ab}
	A_4	112.2 ± 1.3^{Ae}	108.6 ± 8.5^{Ae}	163.5 ± 3.6^{Bd}	216.2 ± 4.3^{Ad}
Beta464	A_0	179.0 ± 7.5^{Bc}	181.0 ± 4.3^{Ac}	239.5 ± 7.2^{Aa}	268.3 ± 5.2^{Ac}
	A_1	211.3 ± 5.2^{Ba}	219.4 ± 3.1^{Ba}	243.2 ± 2.1^{Aa}	296.2 ± 2.9^{Aa}
	A_2	189.2 ± 3.1^{Bb}	197.2 ± 2.3^{Ab}	242.4 ± 7.4^{Ba}	278.4 ± 7.5^{Bb}
	A_3	165.3 ± 4.6^{Bd}	103.0 ± 4.3^{Bd}	234.1 ± 5.3^{Aa}	232.9 ± 3.4^{Bd}
	A_4	108.7 ± 8.1^{Ae}	84.4 ± 3.0^{Be}	174.9 ± 4.2^{Ab}	191.8 ± 2.2^{Be}
Two – way *ANOVA*					
Treatment		554.9^{***}	493.42^{***}	218.04^{***}	542.60^{***}
Cultivar		215.0^{***}	33.28^{***}	3.55^{ns}	287.44^{***}
Treatment × Cultivar		33.8^{***}	66.58^{***}	5.57^{**}	70.09^{***}

Table 7. Leaf POD activity [U g^{-1}(FM)] of two sugar beet cultivars under different treatments. POD – peroxidase. 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 are not significantly different *via* LSD test, * – significant at P<0.05; ** – significant at P<0.01; *** – significant at P<0.001.

Cultivar	Treatment	Time after treatment [d]			
		1	3	5	7
KWS0143	A_0	95.0 ± 5.0^{Ac}	118.3 ± 5.8^{Bb}	166.7 ± 7.6^{Bd}	158.3 ± 7.6^{Ad}
	A_1	108.3 ± 7.6^{Ab}	120.0 ± 3.6^{Bb}	213.3 ± 1.5^{Ab}	170.0 ± 8.7^{Bc}
	A_2	151.7 ± 2.9^{Aa}	153.3 ± 2.9^{Ba}	250.0 ± 5.0^{Aa}	245.0 ± 5.0^{Ab}
	A_3	95.0 ± 1.0^{Ac}	158.3 ± 5.8^{Ba}	183.3 ± 2.9^{Ac}	266.7 ± 2.9^{Aa}
	A_4	46.7 ± 2.9^{Ad}	113.3 ± 2.9^{Ab}	113.3 ± 5.8^{Ae}	145.0 ± 8.7^{Ae}
Beta464	A_0	86.7 ± 5.8^{Ab}	130.0 ± 7.6^{Ad}	190.0 ± 5.0^{Ab}	143.3 ± 2.9^{Bc}
	A_1	95.0 ± 8.7^{Bb}	171.7 ± 2.9^{Ac}	201.7 ± 1.5^{Ba}	181.7 ± 2.1^{Ab}
	A_2	150.0 ± 5.0^{Aa}	236.7 ± 2.9^{Aa}	206.7 ± 1.2^{Ba}	213.3 ± 2.9^{Ba}
	A_3	86.7 ± 5.8^{Ab}	196.7 ± 4.9^{Ab}	143.3 ± 1.2^{Bc}	141.7 ± 2.9^{Bc}
	A_4	40.0 ± 5.0^{Ac}	98.3 ± 4.7^{Be}	108.3 ± 2.9^{Ad}	128.3 ± 7.6^{Bd}
Two – way *ANOVA*					
Treatment		252.01^{***}	468.68^{***}	683.24^{***}	270.33^{***}
Cultivar		12.63^{**}	502.69^{***}	98.80^{***}	293.73^{***}
Treatment × cultivar		0.75^{ns}	122.44^{***}	63.20^{***}	129.69^{***}

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 (A_3 and A_4) alkalinity, while enhanced at low levels (A_1 and A_2) of alkalinty (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 Mg^{2+} 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

decrease in contents of photosynthetic pigments than that of Beta464. This indicated that photosynthetic pigment synthesis of Beta464 was hindered more heavily and pigment degrading was accelerated in response to alkaline stress compared with KWS0143.

Ultrastructure of chloroplasts: Alkaline stress apparently damaged the chloroplast ultrastructure in this study (Fig. 1). Observations under the control treatment revealed normal chloroplast ultrastructure of sugar beet (Fig. 1*A*,*C*). Due to alkaline stress, significant alterations in chloroplast ultrastructure of both cultivars occurred, which was accompanied by the increasing number of starch granules and osmiophilic droplets (Fig.1*B*,*D*). Previous studies demonstrated that increasing starch granules and osmiophilic droplets could maintain cytoplasmic concentration, relieve water loss of cells, and thus enhance the fitness of plants to stress conditions (Brown *et al*. 1983, Zheng *et al*. 1999). Altered thylakoid membrane structure may directly affect membrane functionality and inhibit photosynthesis when the integrity of the chloroplast ultrastructure in cells is destroyed under environmental stress (Chen *et al*. 2004). These changes were similar to the disorganization of thylakoid membranes observed in *Ocimum basilicum* (Bishehkolaei *et al*. 2011). 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 (A_1 and A_2) 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 (A_4), 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 (A_3 and A_4) 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 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 (A_1 and A_2) 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 (A_4), 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 (A_4) alkaline conditions. This suggested that KWS0143 posseses stronger antioxidation ability to eliminate ROS and reduce damage to photosystems caused by Na^+ and pH.

Conclusion: High levels (A_3 and A_4) 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 chlorophyll content. Thus, the loss of photosynthetic pigments and damage to the chloroplasts ultimately disturbed photosynthetic capacity. Low level (A_2) of alkaline stress significantly promoted osmolyte contents and antioxidant enzyme activity, whereas high level (A_4) of alkaline stress significantly decreased these parameters. Under high alkaline concentrations (A_3 and A_4), KWS0143 exhibited a better photosynthetic performance and showed stronger ability for osmotic adjustment and antioxidation than that of Beta464. Therefore, the improved performance of KWS0143 under alkaline stress might be associated with its more efficient osmotic and antioxidant systems to resist injury to photosynthetic apparatus from osmotic stress and ROS damage caused by alkaline stress. Our results may help provide a reference for the improvement of sugar beet production in alkaline soils.

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