



Full Length Article

Beneficial Effects of Potassium on Growth, Water Relations, Mineral Accumulation and Oxidative Damage of *Beta vulgaris* L. in Sodic-alkaline Condition

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Abstract

Potassium (K^+) plays a vital role for plant growth and therefore the amount of K^+ seems to be a key of plant development. Therefore, this study investigated whether the sodic-alkalinity tolerance of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) could be increased, via greater water uptake, higher concentrations of mineral elements and higher antioxidant enzyme activities in sodic-alkaline stress conditions with K^+ supplementation. Forty-seven-day-old uniform seedlings were pre-treated in hydroponic medium with or without K^+ for 7 days, and then treated in non-alkaline or alkaline conditions for 9 days. The absence of K^+ somewhat intensified the effect of alkalinity on reducing the growth of Swiss chard, the total dry weight (DW) was reduced by 34% in plants treated in sodic-alkaline conditions without K^+ when compared with plants with K^+ . Relative water content (RWC) of leaves was increased by 10% by K^+ supplementation in sodic-alkaline conditions. Additionally, the osmotic adjustment was retorted; the contents of K^+ , Cl^- , BO_3^- , Fe^{3+} and Mn^{2+} , and the activities of catalase, guaiacol peroxidase, and glutathione reductase were increased. On other hand, K^+ supplementation in sodic-alkaline conditions helped to maintain guaiacol peroxidase activity similar to that of control plants, and reduced malondialdehyde content when compared with plants treated in sodic-alkaline conditions without K^+ . In conclusion, the K^+ supplementation resulted in favorable changes in plants with higher micronutrient contents and a reduction of oxidative damage induced by sodic-alkalinity in Swiss chard. © 2016 Friends Science Publishers

Keywords: Micronutrients; Osmotic adjustment; Potassium; Sodic-alkalinity; Swiss chard; Reactive oxygen species

Introduction

Potassium (K^+) is major inorganic constituent of living cells and required in large amounts for plant growth and development, being taken from soil then translocated to various organs for many processes in plant cells (Kanai *et al.*, 2011; Chérel *et al.*, 2014). Plants need a high content of K^+ for specific functions in the cytoplasm and 90% is localized in vacuoles (Wakeel *et al.*, 2011). However, in conditions with high contents of Na^+ , Na^+ reduce the activity of K^+ , and also compete with K^+ for uptake sites at the plasma membrane (Shabala and Cuin, 2007). When positively charged Na^+ crosses the plasma membrane, significant membrane depolarization makes passive K^+ uptake through inward-rectifying K^+ channels impossible, and K^+ leakage increases through depolarization-activated outward-rectifying K^+ channels (Shabala and Cuin, 2007). For particular plant species of the Chenopodiaceae, K^+ localized in vacuoles was substituted by Na^+ to a high degree, and plants grow on soils with high K-fixing capacity have more potential for this substitution (Subbarao *et al.*, 2000; Wakeel *et al.*, 2010), and supplementation of Na^+ in

reduced amounts eliminated K^+ -deficient symptoms in conditions of limited K^+ supply (Wakeel *et al.*, 2011). Therefore, some species of Chenopodiaceae showed particularly strong stimulation by low Na^+ application in either low- or high-K conditions (El-Sheikh *et al.*, 1967; Subbarao *et al.*, 2003).

Many saline soils are also sodic-alkaline due to the presence of $NaHCO_3$ and Na_2CO_3 , which resulted in high soil pH, usually greater than 8.5 (Lyubimova *et al.*, 2009; Bui, 2013). Sodic-alkalinity is more deleterious to plant growth than salinity alone, and results in laboratory-bred $NaCl$ -tolerant cultivars failing to achieve good yield in field conditions (Nishiuchi *et al.*, 2010; Bui, 2013). Overproduced reactive oxygen species (ROS) can activate plasma membrane K^+ and Ca^{2+} -permeable conductance, which mediate K^+ efflux and Ca^{2+} influx, respectively, resulted in a concomitant elevation of cytosolic Ca^{2+} activity and K^+ loss (Demidchik *et al.*, 2003; Demidchik *et al.*, 2007). Alongside toxic Na^+ influx, cytosolic ionic homeostasis is seriously impaired, and cellular tissue damage occurs via a number of destructive processes following a rise in ROS levels (Ashraf, 2009;

Velarde-Buendía *et al.*, 2012). The most abundant types of ROS in plants are hydrogen peroxide (H_2O_2), superoxide radical, and hydroxyl radical. To detoxify ROS, plant cells and their organelles employ both enzymatic and non-enzymatic mechanisms (Zepeda-Jazo *et al.*, 2011). Enzymatic antioxidants include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (GPX; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.1), and glutathione reductase (GR; EC 1.6.4.2) (Gill and Tuteja, 2010). APX, followed by CAT, is the key enzyme in detoxifying H_2O_2 (Ashraf, 2009). It plays a vital role in plant defense against oxidative stress (Ashraf, 2009; Hernandez *et al.*, 2012). GPX not only scavenges H_2O_2 , but also involves in the biosynthesis of cell-wall components and tissue lignification (Cavalcanti *et al.*, 2004). The common non-enzymatic antioxidants are glutathione, ascorbate, carotenoids, phenolic compounds and non-protein amino acids (Ashraf, 2009; Gill and Tuteja, 2010).

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) is a foliage vegetable closely related to beets with a large leaf blade, thicker petiole, and no root enlargement (Welbaum, 2015). The leaf of Swiss chard contains nutritionally significant contents of magnesium, calcium and phosphorus, and has potential to protect humans against several chronic diseases such as diabetes (Pyo *et al.*, 2004; Maynard and Hochmuth, 2007; Sacan and Yanardag, 2010). However, the growth of Swiss chard is severely suppressed by alkaline conditions owing to serious impairment of intracellular Na^+ and K^+ homeostasis, and serious K^+ starvation in tissues (Liu *et al.*, 2013).

As mentioned above, although sodic-alkaline conditions and K^+ starvation have been studied extensively, impact of the combination of these conditions on plants was not examined. The main objective of this study is to examine the influence of K^+ on the responses of Swiss chard to sodic-alkaline constraints, and to explore differences in physiological parameters in Swiss chard seedlings subjected to sodic-alkaline conditions with or without K^+ supplementation.

Materials and Methods

Plant Material and Treatment Conditions

The description of Swiss chard (*Beta vulgaris* L. subsp. *cicla*) seeds used in this study was the same as described by Liu *et al.* (2013). The seeds were sown in a plastic seed bed (96 wells per bed) and irrigated with tap water in a greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University, Japan. Forty-five days after sowing, uniform seedlings that have shed their cotyledons from the mother plants were transferred to deionized water for discarding nutrients on the root surface. After two days, seedlings were transplanted to water culture solution with or without K^+ for 1 week (K^+ pre-treatment). The nutrient medium with K^+ contained the following

macronutrients: 0.6 mM NH_4NO_3 , 0.2 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.34 mM K_2SO_4 , 0.34 mM KCl, 0.38 mM $\text{Ca}(\text{NO}_3)_2$, and 0.2 mM MgSO_4 , and the following micronutrients: 4 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 8 μM H_3BO_3 , 0.12 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.3 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 μM MoO_3 , and 20 μM Fe-EDTA. For no K^+ treatment, K_2SO_4 and KCl were omitted from the nutrient medium. 70 L plastic containers (12 plants for each container) were maintained under natural sunlight conditions of the greenhouse.

After K^+ pre-treatment, the seedlings were transplanted to 20 L plastic containers (six plants for each container) filled with nutrient mediums as mentioned above, and treated by both sodic-alkalinity and non-sodic control, respectively (for 9 days). All 4 treatments were as follow: 0 mM K, 2 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K. The nutrient solutions of 2 mM K and Alkalinity+2 mM K treatments contained the same micronutrients and macronutrients with K^+ -pretreatment except for K^+ , which was simulated by adding 0.68 mM K_2SO_4 and 0.68 mM KCl. For 0 mM K and Alkalinity+0 mM K treatments, the omission of K_2SO_4 and KCl from the nutrient medium was continued. The sodic-alkalinity was simulated by a mixture of NaHCO_3 and Na_2CO_3 ($\text{NaHCO}_3:\text{Na}_2\text{CO}_3 = 9:1$ molar ratio) as described by our previous study (Liu *et al.*, 2013). The electrical conductivity (EC) of the nutrient medium was gradually increased from 186.8 mS m^{-1} to 340 mS m^{-1} to 493 mS m^{-1} for Alkalinity+2 mM K treatment, while from 208 mS m^{-1} to 354 mS m^{-1} to 506 mS m^{-1} for Alkalinity+0 mM K treatment, through the gradual increase of Na^+ content from 20 mM to 40 mM to 60 mM every 3 days. 1 M NaOH was used to adjust the pH (5.5-6.0) of the basal nutrient solution, which was used for 0 mM K and 2 mM K treatments. For the Alkalinity+0 mM and Alkalinity+2 mM treatments, the pH ranges of the nutrient medium were increased to 8.8-9.1 after adding NaHCO_3 and Na_2CO_3 to the basal nutrient solution. The nutrient medium was renewed every 3 days. The pots were still maintained under natural sunlight condition in the greenhouse. The average temperature was 23°C, and the average of relative humidity was 58% during treatment period.

Physiological Parameters

At the end of the treatment period, plants were carefully separated into leaf blade, petiole and root, and each plant part was weighted and freeze-dried. The freeze-dried samples were used for the estimation of dry weight (DW), and young leaf blades were sampled for analyses. The relative water content (RWC) was measured as described by Turner (1981). The saturated osmotic potential (Ψ_π^{100}) was calculated using the following equation (Saneoka *et al.*, 1995): $\Psi_\pi^{100} = (\text{osmotic potential} [\text{RWC} - \text{apoplastic water content}] / [1.0 - \text{apoplastic water content}])$. The apoplastic water content is assumed as 0.15, and osmotic potential of the cell sap was measured by Wescor 5500 vapor pressure osmometer (Wescor Inc., Logan, UT, USA).

For mineral analysis, young leaf blades were freeze-dried and then ground into fine powder. Sample digestion for cations, and measurements of the Na^+ and K^+ contents were conducted as described by Liu *et al.* (2013), Mg^{2+} and Ca^{2+} contents were determined as described by Saneoka *et al.* (1999), and BO_3^{3-} , Cu^{2+} , Fe^{3+} , Mn^{2+} , and Zn^{2+} were determined using Inductively Coupled Plasma (iCAP 6000, Thermo Fisher Scientific Inc., UK). Anions were extracted in tightly closed micro tube with distilled water and finely ground powder at 100°C for 1 h and the supernatant obtained after centrifugation was used to determine anion concentration by ion chromatography (Dionex ICS-900, Nippon Dionex K.K., Osaka, Japan). The contribution of each ion to total osmotic potential at full turgor (Ψ_π^{100}) was calculated as described by Ming *et al.* (2012).

The malondialdehyde (MDA) content was measured using a modified version of the thiobarbituric acid (TBA) procedure (Draper and Hadley, 1990). The fine powder (ground with liquid nitrogen) of young leaf blade was homogenized by an extraction buffer containing 3.3 mM HEPES (pH 7), 0.25% TBA, 10% trichloroacetic acid (TCA), 0.2 M HCl, 0.01% butylated hydroxytoluene, and 1.3% ethanol. The absorbance of the supernatant acquired after centrifugation was measured at 535 nm and 600 nm after incubating at 95°C , and the extinction coefficient ($1.56 \times 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$) was used to calculate the MDA content.

The crude extract for measuring enzyme activity and protein concentration was prepared as described by Koca *et al.* (2007), with modifications. A frozen sample (0.5 g) was extracted with ice-cold 25 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM ethylenediaminetetraacetic acid (EDTA), 2% polyvinylpyrrolidone, and 1 mM ascorbic acid. For CAT activity, it was assayed by measuring the initial rate of H_2O_2 disappearance at 240 nm (Aebi, 1984). For APX activity, the absorbance of ascorbic acid was determined at 290 nm (Nakano and Asada, 1981). For GPX activity, the absorbance of tetraguaiacol was measured at 470 nm (Velikova *et al.*, 2000). For GR activity, the absorption of oxidized NADPH was monitored at 340 nm as described by Foyer and Halliwell (1976). The soluble protein content in the enzyme extract was determined as described by Assaha *et al.* (2015).

Statistical Analysis

All data were examined by one-way ANOVA using the IBM SPSS statistical package version 21. Test of significance was performed using Duncan's test at the 5% significance level. The values are means (\pm SE) of six replicates.

Results

Plant Growth

The root DW of Swiss chard was 23% less, and shoot DW

was not changed with Alkalinity+2 mM K treatment compared to those of 2 mM K treatment (Fig. 1). Additionally, the root DW was 43% less, shoot DW was 30% less with 0 mM K treatment, and root DW was 45% less, shoot DW was 33% less with Alkalinity+0 mM K treatments when compared with the root and shoot DW with 2 mM K treatment. Therefore, the reductions in plant growth caused by 0 mM K and Alkalinity+0 mM K treatments were greater than of the plants subjected to Alkalinity+2 mM K treatment. Also, leaf chlorosis phenomenon was observed in plants after treatment of Alkalinity+0 mM K.

Water Relations

The results indicated that the RWC was 6%, 8%, and 16% less, respectively, with 0 mM K, Alkalinity+2 mM K, and Alkalinity+0 mM K treatments when compared to 2 mM K treatment (Fig. 2). In addition, the Ψ_π^{100} was 15% less with Alkalinity+2 mM K treatment, 18% more with 0 mM K treatment, and 13% more with Alkalinity+0 mM K treatments compared to 2 mM K treatment.

Cation and Anion Contents

Plants subjected to 0 mM K and Alkalinity+0 mM K treatments, Ca^{2+} content in young leaves were higher than in plants subjected to 2 mM K and Alkalinity+2 mM K treatments (Table 1). Ca^{2+} content was 75% more with 0 mM K, 48% more with Alkalinity+0 mM K; while 19% less with Alkalinity+2 mM K treatments when compared to 2 mM K treatment. Mg^{2+} contents in leaf tissues of Swiss chard was 90%, 107%, and 52% more with 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively compared to 2 mM K treatment. The presence of a mixture of NaHCO_3 and Na_2CO_3 in the culture medium resulted with 32- and 34-fold greater leaf Na^+ content in Alkalinity+0 mM K and Alkalinity+2 mM K treatments, respectively. It is 4-fold greater in 0 mM K treatment compared to 2 mM K treatment. Potassium content was 82%, 57%, and 55% lower in young leaves of Swiss chard with Alkalinity+0 mM K, Alkalinity+2 mM K, and 0 mM K treatments, respectively compared to 2 mM K treatment. Consequently, the lowest Na^+/K^+ ratio was observed with 2 mM K treatment. For example, the Na^+/K^+ ratio was 7-fold, 182-fold, and 79-fold higher in young leaves of Swiss chard with 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively compared to 2 mM K treatment.

On the other hand, it was observed that Cl^- content was significant lower with all treatments compared to 2 mM K treatments. It was 92%, 96% and 84% lower after treatments with Alkalinity+0 mM K, Alkalinity+2 mM K, and 0 mM K, respectively, compared to 2 mM K treatment. The NO_3^- content was 7-fold, 4-fold and 1.6-fold greater in Alkalinity+0 mM K, Alkalinity+2 mM K, and 0 mM K treatments, respectively compared to 2 mM K treatment.

Table 1: Changes in the contents of cations and anions (mg g⁻¹ DW), and Na⁺/K⁺ ratio in young leaves in response to alkaline and K⁺ treatments

Measurements	Treatments			
	0 mM K	2 mM K	Alkalinity+0 mM K	Alkalinity+2 mM K
Cation				
Ca ²⁺	4.03±0.22 ^a	2.30±0.28 ^{bc}	3.4±0.52 ^{ab}	1.86±0.21 ^c
Mg ²⁺	8.80±0.66 ^a	4.62±0.46 ^b	9.59±1.31 ^a	7.00±0.74 ^{ab}
Na ⁺	8.35±0.84 ^b	2.25±0.22 ^c	73.58±3.30 ^a	79.18±5.21 ^a
K ⁺	34.78±0.93 ^b	78.06±4.43 ^a	14.15±1.36 ^c	33.45±1.79 ^b
Na ⁺ /K ⁺	0.24±0.03 ^c	0.03±0.00 ^d	5.50±0.66 ^a	2.39±0.19 ^b
Anion				
Cl ⁻	1.80±0.15 ^c	23.57±2.09 ^a	0.84±0.09 ^d	3.74±0.41 ^b
NO ₃ ⁻	3.37±0.90 ^b	2.09±0.86 ^{bc}	15.32±4.49 ^a	8.29±3.62 ^{ab}
PO ₄ ³⁻	23.68±1.73 ^a	27.19±1.57 ^a	5.79±0.59 ^b	3.46±0.34 ^b

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) grown either in a nutrient solution without (0 mM K) or with (2 mM K) K⁺, or in a sodic-alkaline nutrient solution without (alkalinity+0 mM K) or with (alkalinity+2 mM K) K⁺. The values are means (±SE) of six replicates. Followed by the same letters are not significantly different at P ≤ 0.05

Table 2: Changes in boron (BO₃³⁻), copper (Cu²⁺), iron (Fe³⁺), manganese (Mn²⁺), and zinc (Zn²⁺) contents (μg g⁻¹ DW) in young leaves in response to alkaline and K⁺ treatments

Measurements	Treatments			
	0 mM K	2 mM K	Alkalinity+0 mM K	Alkalinity+2 mM K
BO ₃ ³⁻	27.53±1.27 ^b	41.97±1.87 ^a	17.40±2.03 ^c	27.75±1.82 ^b
Cu ²⁺	2.91±0.53 ^{ab}	3.31±0.39 ^a	2.68±0.32 ^{ab}	1.59±0.19 ^b
Fe ³⁺	169.63±20.83 ^a	79.56±4.57 ^b	43.24±2.35 ^c	52.89±2.48 ^{bc}
Mn ²⁺	139.06±12.22 ^b	234.09±29.11 ^a	157.79±14.28 ^b	181.40±16.67 ^{ab}
Zn ²⁺	13.41±0.95 ^b	21.93±1.34 ^a	9.23±0.77 ^c	10.19±0.92 ^c

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) grown either in a nutrient solution without (0 mM K) or with (2 mM K) K⁺, or in a sodic-alkaline nutrient solution without (alkalinity+0 mM K) or with (alkalinity+2 mM K) K⁺. The values are means (±SE) of six replicates. Followed by the same letters are not significantly different at P ≤ 0.05

PO₄³⁻ content was unchanged by 0 mM K treatment, and significant lower with Alkalinity+2 mM K (79%) and Alkalinity+0 mM K (87%) treatments compared to 2 mM K treatment.

Micronutrient Contents

Borate (BO₃³⁻) contents was 34%, 59% and 34% less in treatments of 0 mM K, Alkalinity+0 mM K and Alkalinity+2 mM K, compared to 2 mM K treatment (Table 3). Copper (Cu²⁺) content was 12%, 19%, and 52% less in 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively, compared to 2 mM K treatment. Iron (Fe³⁺) content was 2-fold greater with 0 mM K treatment, while 0.46- and 0.34-fold lower with Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively, compared to 2 mM K treatment. Manganese (Mn²⁺) content was 0.41-, 0.33-, and 0.23-folds lower with 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively compared to 2 mM K treatment. Zinc (Zn²⁺) content was 0.39-, 0.58-, and 0.54-fold lower with 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments, respectively compared to 2 mM K treatment.

Contribution of Each Ion to Osmotic Potentials of Ions at Full Turgor

The percentage contribution of each ion at Ψ_{π}^{100} from

highest to lowest was as follows: K⁺, Na⁺, Mg²⁺, PO₄³⁻, Ca²⁺, NO₃⁻, Cl⁻ and micronutrients for 0 mM K plants (Table 3). The order for 2 mM plants was K⁺, Cl⁻, PO₄³⁻, Mg²⁺, Na⁺, Ca²⁺, NO₃⁻, and micronutrients. However, the percentage contribution of each ion at Ψ_{π}^{100} from highest to lowest changed as follows: Na⁺, Mg²⁺, K⁺, NO₃⁻, Ca²⁺, PO₄³⁻, Cl⁻, and micronutrients for Alkalinity+0 mM K plants, and Na⁺, K⁺, Mg²⁺, NO₃⁻, Cl⁻, Ca²⁺, PO₄³⁻, and micronutrients for Alkalinity+2 mM K plants.

Malondialdehyde Content and Antioxidant Enzyme Activities

The lowest value of MDA content was observed in leaf tissues of 2 mM K treatment and it was 8% more in Alkalinity+2 mM K treatment, 26% more in Alkalinity+0 mM K treatment, and 88% more in 0 mM K treatment compared to 2 mM K treatment (Fig. 3).

For the antioxidants enzymes, the CAT, APX, GPX, and GR activities decreased in treatments without K (Table 4). For example, CAT, APX, GPX and GR activities were 77%, 51%, 45% and 41% lower, respectively in 0 mM K treatment compared to 2 mM K treatment and were also 82%, 58%, 57%, and 87% less, respectively in Alkalinity+0 mM K treatment compared to 2 mM K treatment. With Alkalinity+2 mM K treatment, CAT, APX, and GR activities were 69%, 33%, and 75% lower, respectively, whereas GPX activity was 7% higher compared to the 2 mM K treatment.

Table 3: Percentage contribution of each ion to total osmotic potential at full turgor in young leaves in response to alkaline and K⁺ treatments

Measurements	Treatments			
	0 mM K	2 mM K	Alkalinity+0 mM K	Alkalinity+2 mM K
Ca ²⁺ (%)	4.84±0.22 ^a	1.72±0.20 ^{bc}	1.94±0.39 ^b	0.94±0.05 ^c
Mg ²⁺ (%)	17.42±1.49 ^a	5.70±0.52 ^c	9.00±1.37 ^b	5.86±0.67 ^c
Na ⁺ (%)	17.48±1.78 ^b	2.93±0.08 ^c	73.09±1.45 ^a	70.05±1.66 ^a
K ⁺ (%)	42.81±2.54 ^b	59.85±2.09 ^a	8.27±1.13 ^d	17.40±0.89 ^c
Cl ⁻ (%)	2.45±0.31 ^b	19.92±1.59 ^a	0.54±0.08 ^c	2.15±0.37 ^b
NO ₃ ⁻ (%)	2.61±0.50 ^b	1.01±0.63 ^{bc}	5.64±2.02 ^a	2.72±1.52 ^{ab}
PO ₄ ³⁻ (%)	12.00±0.84 ^a	8.58±1.26 ^b	1.39±0.20 ^c	0.74±0.12 ^c
Micronutrients (%)	0.40±0.03 ^a	0.30±0.01 ^b	0.12±0.00 ^c	0.14±0.01 ^c

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) grown either in a nutrient solution without (0 mM K) or with (2 mM K) K⁺, or in a sodic-alkaline nutrient solution without (alkalinity+0 mM K) or with (alkalinity+2 mM K) K⁺. The values are means (±SE) of six replicates. Followed by the same letters are not significantly different at P ≤ 0.05

Table 4: Changes in activity of catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) in young leaves in response to alkaline and K⁺ treatments

Measurements	Treatments			
	0 mM K	2 mM K	Alkalinity+0 mM K	Alkalinity+2 mM K
CAT (mM min ⁻¹ mg ⁻¹ protein)	54.86±8.09 ^b	130.62±14.56 ^a	23.30±4.49 ^c	39.86±6.95 ^{bc}
APX (μmol min ⁻¹ mg ⁻¹ protein)	1096.10±181.63 ^b	2246.28±152.61 ^a	948.37±127.47 ^b	1505.08±278.96 ^b
GPX (μmol min ⁻¹ mg ⁻¹ protein)	190.34±40.03 ^{bc}	346.49±32.52 ^a	148.95±16.32 ^c	371.31±82.39 ^a
GR (μmol min ⁻¹ mg ⁻¹ protein)	494.55±122.57 ^b	843.21±148.09 ^a	113.25±6.92 ^c	214.07±35.90 ^{bc}

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) grown either in a nutrient solution without (0 mM K) or with (2 mM K) K⁺, or in a sodic-alkaline nutrient solution without (alkalinity+0 mM K) or with (alkalinity+2 mM K) K⁺. The values are means (±SE) of six replicates. Followed by the same letters are not significantly different at P ≤ 0.05

These results suggested that, reduction in enzymatic antioxidant capacity in Swiss chard seedlings caused by Alkalinity+0 mM treatment was higher than by 0 mM K and Alkalinity+2 mM K treatments.

Discussion

The effect of potassium on growth and antioxidant enzymes of *Beta vulgaris* L. in sodic-alkaline conditions seems to be an important study to investigate the role of K⁺ as a physiological parameter in plant cell. High pH and excessive Na⁺ in sodic-alkaline soil medium result in serious K⁺ deprivation in living plant cells (Liu *et al.*, 2013). Several studies have reported that a combination of salinity and K⁺ starvation had a greater effect on the growth and productivity of barley and maize (Degl'Innocenti *et al.*, 2009; Hafsi *et al.*, 2010; Gong *et al.*, 2011; Qu *et al.*, 2012). This study showed that the lowest biomass production and chlorosis with Alkalinity+0 mM K treatment, which indicates that the absence of K⁺ partially intensified the effect of alkalinity on reducing the growth of Swiss chard. As well, the higher biomass production with Alkalinity+2 mM K treatment than with Alkalinity+0 mM K suggest that pre-treated-plants with K⁺ could resist the detrimental effects of sodic-alkaline conditions. Therefore, K⁺ plays a crucial role in NaHCO₃- and Na₂CO₃-treated Swiss chard. At the same time, excessive CO₃²⁻ has its own toxic effect on plant growth because of adverse effects on protein synthesis, respiration, and the absorption of nutrients (Wu and Xing, 2012). High contents of HCO₃⁻ strongly affect the availability of several micronutrients,

especially Fe, and it is often considered to be the primary factor inducing Fe chlorosis in leaves (Colla *et al.*, 2010). Also, this study, with Alkalinity+0 mM K treatment, the combined stress caused by Na⁺ toxicity, CO₃²⁻ and HCO₃⁻ toxicities, high pH, and absence of K⁺, induced the greatest adverse effects on plant growth when compared with other treatments.

Water deficit is the rapid and main consequence that plants experience when exposed to external excessive Na⁺, and plants can adapt to water deficit by accumulating osmolytes to maintain leaf turgor and physiological activities at relatively low leaf water potential (Ming *et al.*, 2012). From the results here, young leaves of Swiss chard were exposed to water stress in all treatments when compared with 2 mM K treatment and with Alkalinity+0 mM K treatment water stress conditions were greater. Furthermore, osmotic adjustment was not achieved in young leaves of Swiss chard with Alkalinity+0 mM K treatment, indicating that the harmful effects caused by Alkalinity+0 mM K treatment were more serious than by Alkalinity+2 mM K treatment. Hence, the greater reduction in RWC, increase in Na⁺/K⁺ ratio, and lack of osmotic adjustment with Alkalinity+0 mM K treatment provides strong evidence that absence of K⁺ somewhat intensified the effect of sodic-alkalinity on reducing growth of Swiss chard.

The depletion of K⁺ in the cytoplasm was crucial for triggering programmed cell death (Shabala, 2009). Following K⁺ deprivation together with sodic-alkaline treatment, K⁺ deficiency in living cells of Swiss chard was significantly expedited owing to excessive Na⁺ accumulation, high pH, and reduction in cytoplasmic K⁺.

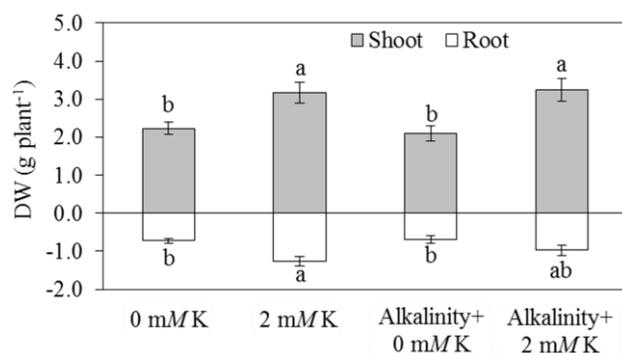


Fig. 1: Changes in shoot dry weight (DW) and root DW in Swiss chard (*Beta vulgaris* L. subsp. *cicla*) in response to alkaline and K⁺ treatments. Seedlings were grown either in a nutrient solution or in a sodic-alkaline nutrient solution. The values are means (\pm SE) of six replicates. Followed by the same letters are not significantly different at $P \leq 0.05$

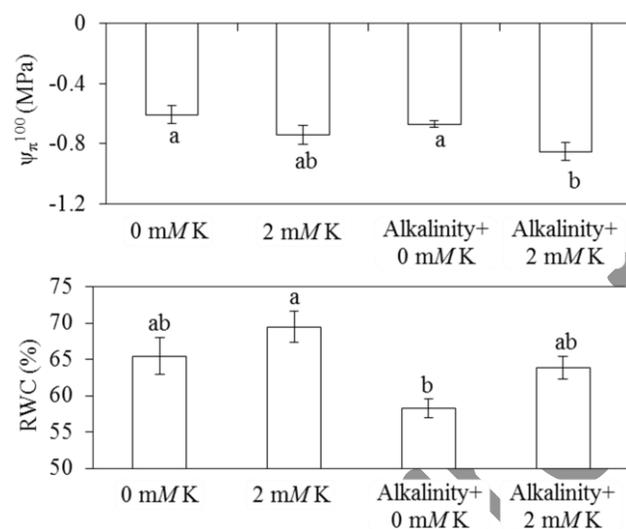


Fig. 2: Changes in relative water content (RWC) and osmotic potential at full turgor (Ψ_{π}^{100}) in young leaves in response to alkaline and K⁺ treatments. Swiss chard (*Beta vulgaris* L. subsp. *cicla*) seedlings were grown either in a nutrient solution or in a sodic-alkaline nutrient solution. The values are means (\pm SE) of six replicates. Followed by the same letters are not significantly different at $P \leq 0.05$

Plant cells of Swiss chard may have sensed the low content of K⁺ and initiated a series of severe physiological reactions, such as water deficit, ionic imbalance, and oxidative damage, and then seriously limited plant growth in Alkalinity+0 mM K treatment. In the other hand, with Alkalinity+2 mM K treatment, K⁺ content in living cells of Swiss chard just decreased in the sodic-alkaline treatment period, and maintained at a higher level than that of Alkalinity+0 mM K treatment. Additionally, in some species of Chenopodiaceae, such as sugar beet and red beet, Na⁺ can substitute for K⁺ localized in vacuoles for osmotic function

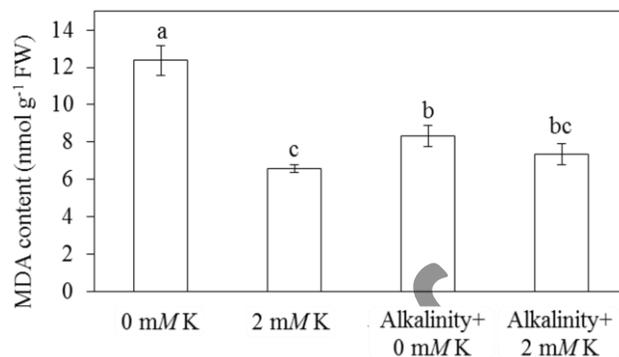


Fig. 3: Changes in malondialdehyde (MDA) content in young leaves in response to alkaline and K⁺ treatments. Swiss chard (*Beta vulgaris* L. subsp. *cicla*) seedlings were grown either in a nutrient solution or in a sodic-alkaline nutrient solution. The values are means (\pm SE) of six replicates. Followed by the same letters are not significantly different at $P \leq 0.05$

to a high degree (Subbarao *et al.*, 2000; Wakeel *et al.*, 2010). For further clarification in this study, in the Alkalinity+2 mM K treatment, Na⁺/K⁺ ratio in young leaves of Swiss chard was increased significantly, however, shoot dry weight was not significantly affected, indicating that K⁺ ions in vacuoles might be substituted by Na⁺ ions for maintenance of osmotic adjustment. Furthermore, K⁺ content in living cells were maintained at a higher level, and Na⁺ toxic effect in the cytoplasm was somewhat avoided. In terms of Na⁺ influx and K⁺ efflux from vacuoles to the cytoplasm (Wang and Wu, 2010; Chérel *et al.*, 2014). The ability of Swiss chard to control the cytoplasmic K⁺ concentration in Alkalinity+2 mM K and Alkalinity+0 mM K conditions could possibly have been regulated by the over-expression of NHXs or tonoplast-located K⁺ channels and transporters, however, these phenomena need to be further investigated. Furthermore, Na⁺ was taken up by high-affinity K⁺ transporters (HKTs) from the soil solution to reduce K⁺ requirements (Rodríguez-Navarro and Rubio, 2006). Therefore, the higher value of Na⁺ content in 0 mM K treatment than in 2 mM K treatment indicates that Na⁺ influx component mediated by the HKT transporter into roots to maintain growth in Swiss chard in 0 mM K treatment.

Elevated cytoplasmic Ca²⁺ can stimulate NADPH oxidase-mediated production of ROS, and increased ROS levels in turn activate Ca²⁺-permeable ion channels leading to further Ca²⁺ influx under K⁺ deprivation condition (Wang and Wu, 2010). Therefore, the higher Ca²⁺ content in Swiss chard in 0 mM K and Alkalinity+0 mM K treatments may

have been caused by the hyperpolarization of cell membranes. This phenomenon was consistent with the significant increase in MDA content in 0 mM K and Alkalinity+0 mM K treatments in Swiss chard. Also, Mg^{2+} and Ca^{2+} in most tissues tends to be sequestered in vacuoles, where they contribute to turgor generation (Maathuis, 2009). The increased Ca^{2+} content together with Mg^{2+} was observed in 0 mM K and Alkalinity+0 mM K treatments to compensate the reduction in osmolarity induced by K^+ deficiency in young leaves of Swiss chard. Wakeel *et al.* (2009) reported that Ca^{2+} uptake and translocation through xylem sap was inhibited in Na^+ -treated sugar beet (*Beta vulgaris* L.). Hence, value of Ca^{2+} content in Alkalinity+0 mM K treatment was lower than in 0 mM K treatment, and value of Ca^{2+} content in Alkalinity+2 mM K treatment was lower than in 2 mM K treatment.

Although higher concentrations of Cl^- is toxic to plants, it is considered as an essential nutrient that regulates enzyme activities in the cytoplasm, acts as a counter anion to stabilize membrane potential, and is involved in turgor and pH regulation (Teakle and Tyerman, 2010). Therefore, the reduction in Cl^- in 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments clearly contributed an adverse effect to the natural physiological metabolism processes of Swiss chard plants in these treatments. From a Cl^- perspective, the efflux of Cl^- could be balanced by the uptake of another anion. NO_3^-/Cl^- interactions are analogous to K^+/Na^+ interactions and many anion channels are more selective for NO_3^- than Cl^- (Teakle and Tyerman, 2010). In Alkalinity+2 mM K and Alkalinity+0 mM K treatments, the obvious inductions of NO_3^- would likely offset the scarcity of anions caused by sharp reductions in Cl^- . Whereas, in the 0 mM K treatment, the scarcity of anions caused by sharp reductions in Cl^- was offset by PO_4^{3-} . In addition, NO_3^- is the predominant form of inorganic nitrogen in high-pH soils, and sodium enhances root-to-leaf nitrate translocation in Swiss chard (Maathuis, 2009; Emi *et al.*, 2015). The obvious inductions of NO_3^- in Alkalinity+2 mM K and Alkalinity+0 mM K treatments may also have affected due to high pH and excessive Na^+ , caused by $NaHCO_3$ and Na_2CO_3 . The PO_4^{3-} contents decreased sharply, because of the precipitation of PO_4^{3-} at higher pH in the environment surrounding the roots under Alkalinity+2 mM K and Alkalinity+0 mM K treatments.

Micronutrients are essential for plant growth but are required in much smaller amounts than macronutrients (Hänsch and Mendel, 2009). They involve in all metabolic and cellular functions, and deficiency in any one of these trace elements reduces plant growth and crop yield (Hänsch and Mendel, 2009; White and Brown, 2010). However, Cu^{2+} , Fe^{3+} , Mn^{2+} , and Zn^{2+} contents in young leaves of Swiss chard decreased, because of the limited phytoavailability of these elements at high pH in Alkalinity+2 mM K and Alkalinity+0 mM treatments. Furthermore, BO_3^{3-} can promote the structural integrity of bio-membranes (Brown *et al.*, 2002;

Hänsch and Mendel, 2009). The significant decrease of BO_3^{3-} content in Alkalinity+2 mM K and Alkalinity+0 mM K treatments might contribute to damage to the cell membrane in young leaves of Swiss chard in these treatments.

Osmotic adjustment helps plants to maintain turgor under water stress. Although organic components were the major components that contribute to osmotic adjustment in plants, inorganic components also involved (Kusaka *et al.*, 2005). In this study, K^+ was the major inorganic component contributing to osmotic adjustment in living cells of Swiss chard in 0 mM K and 2 mM K treatments. In contrast, in Alkalinity+0 mM K and Alkalinity+2 mM treatments, influx of excess Na^+ causes ionic imbalance in the living cells, and Na^+ was used to substitute for K^+ for osmotic adjustment in young leaves of Swiss chard.

Oxidative stress occurs owing to the rapid accumulation of ROS in response to nutritional starvation and salinity (Zhu, 2001; Shin *et al.*, 2005), and results in the damage of cell membrane. From the results of this study, the greater increase in MDA content by 0 mM K treatment than that by Alkalinity+0 mM K and Alkalinity+2 mM K treatments, suggests that cell membrane damage was severe in 0 mM K treatment than in Alkalinity+0 mM K and Alkalinity+2 mM K treatments. This phenomenon is consistent with the results of Pi *et al.* (2014), who reported that MDA content was significantly lower in the replacement of K^+ by Na^+ groups than in K^+ -deficient groups in sugar beet with either a salt-sensitive genotype or a salt-tolerant one. They suggested that the substitution of K^+ by Na^+ in the living cells alleviates damage to the cytomembrane. This phenomenon may prove the hypothesis that K^+ ions in vacuoles are replaced by excessive cytoplasmic Na^+ ions for the maintenance of cytoplasmic K^+ , and then the protection of cytomembrane in Swiss chard. Among all the treatments, the MDA content was the highest in 0 mM K treatment. Previous reports already suggested that the iron-catalyzed Haber-Weiss reaction is considered as the major mechanism for generating OH^- in biological systems, which together with H_2O_2 induces K^+ efflux and Ca^{2+} influx from the plasma membrane (Kehrer, 2000; Demidchik *et al.*, 2003; Demidchik *et al.*, 2007; Zepeda-Jazo *et al.*, 2011). Therefore, higher Fe^{3+} and Ca^{2+} contents, less K^+ content, and lower enzyme activities (CAT, APX, GPX and GR) in 0 mM K treatment compared to 2 mM K treatment, provided a strong evidence for the overproduction of OH^- and H_2O_2 in Swiss chard in 0 mM K treatment.

APX, followed by CAT, are the key enzymes in detoxifying H_2O_2 . However, the results here indicated the activities of APX, CAT and GR were decreased in 0 mM K, Alkalinity+0 mM K, and Alkalinity+2 mM K treatments compared to those of 2 mM K treatment. The reductions in their activities may be caused by the clear reductions in K^+ , Cl^- , Fe^{3+} , and Mn^{2+} contents in living cells. This is because K^+ is involved in the activation of enzymes as the most

abundant cation in the cytoplasm, and other elements are also involved with the oxidative enzymes. Among all the treatments, the integrality of cell membrane was most damaged in leaf tissues in 0 mM K treatment, while the lowest activities of CAT, APX, GPX and GR were observed in leaf tissues in Alkalinity+0 mM K treatment. It suggested that the significantly increased Fe³⁺ content may supply sufficient electrons for OH[·] formation, which may cause major ROS, inducing cell membrane damage in Swiss chard in 0 mM K treatment. In sugar beet and wild beet, increased GPX was found to protect the plants against oxidative stress during NaCl stress (Bor *et al.*, 2003). In our study, the reduction in GPX activity coincided with the decrease in shoot DW in 0 mM K and Alkalinity+0 mM K treatments. Unaltered GPX activity in Alkalinity+2 mM K treatment coincided with unaltered shoot DW of Swiss chard. Also, GPX protected the plant against oxidative stress in Alkalinity+2 mM K treatment, while this protective function was lacking in 0 mM K and Alkalinity+0 mM K treatments. Therefore, GPX in Swiss chard may be involved in scavenging of H₂O₂.

In conclusion, K⁺ supplementation effectively alleviated tolerance to sodic-alkaline stress in Swiss chard seedlings, with its ability to increase cell extension in young leaves. Increased concentrations of K⁺, Cl⁻, BO₃⁻, Fe³⁺ and Mn²⁺, and maintenance of GPX activity also contributed positively. In addition, the favorable changes of Ca²⁺ influx, K⁺ efflux, tissue water status, and greater enzyme activities (CAT, APX, GPX and GR) also contributed to the increased tolerance to sodic-alkaline stress conditions exhibited in plants with K⁺ supplementation.

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