

Doctoral Thesis

**Physiological studies on saline and alkaline toxicities and
tolerances in Swiss chard (*Beta vulgaris* L. subsp. *cicla*)**

(Summary)

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論文題目: Physiological studies on saline and alkaline toxicities and tolerances in Swiss chard (*Beta vulgaris* L. subsp. *cicla*)
(フダンソウ(*Beta vulgaris* L. subsp. *cicla*)の塩・アルカリ障害及び耐性に関する生理学的研究)

Chapter 1. General introduction

Saline soils (pH < 8.5) and alkaline soils (8.5 < pH < 10.5) are widespread adverse environmental problems globally, which significantly limit crop production. Saline soils affect plant growth in two phases: osmotic phase that inhibits the growth of young leaves, and ionic phase that accelerates the senescence of mature leaves. Alkaline soils exert similar reactions but with the added influence of stress due to high pH. Swiss chard (*Beta vulgaris* L. subsp. *cicla*) is a foliage vegetable closely related to beets with a large leaf blade, and thicker petiole. This plant is more tolerant to salinity than other leafy vegetables. However, there are few reports on Swiss chard resistance to salinity, and there is no information about its alkaline toxicity or alkaline tolerance in this plant. This study was conducted to examine toxic responses of Swiss chard to alkalinity via comparing the saline and alkaline toxicities in Swiss chard by determining its physiological characteristics, and identifying parameters that are more suppressed under alkaline conditions than under saline conditions.

Chapter 2. Physiological responses of Swiss chard to saline and alkaline stresses

To understand the difference between physiological responses of Swiss chard to salinity and alkalinity, Swiss chard plants were subjected to 50 mM and 100 mM of salinity (pH 6.5) and alkalinity (pH 9.0), respectively. The data revealed that although the relative water content (RWC) was higher under alkaline conditions, the plant growth under alkaline condition was worse than under saline conditions, indicating that the destructive effects caused by high pH, CO_3^{2-} and HCO_3^- toxicity, rather than water stress in this plants under alkaline conditions. Results of other physiological analysis demonstrated that chlorophyll (Chl) *a*, Chl *b*, photosynthetic rate (P_n), water use efficiency (WUE), K^+ content, and K^+/Na^+ ratio were more decreased, and thus resulted in the more serious inhibition of growth under alkaline conditions than under saline conditions. Among these parameters, decreases of P_n and WUE were in close connection with decreases of Chl and K^+ contents under alkaline conditions. Therefore, it was concluded that Chl and K^+ are limiting factors for the plant growth under alkaline conditions.

Chapter 3. Saline and alkaline tolerances of Swiss chard due to 5-aminolevulinic acid foliar applications

Following the serious reduction of Chl content under alkaline conditions, the objective of chapter 3 was to compare the possibility of saline and alkaline tolerance inductions in Swiss chard due to ALA foliar application (ALA is an essential precursor for biosynthesis of Chl). Twelve-week-old uniform seedlings were treated with ALA under saline and alkaline conditions. The observed results revealed that Chl content significantly increased due to ALA foliar application under alkaline conditions. Other physiological analyses revealed that shoot and root dry weights, RWC, osmotic potential (OP), K^+/Na^+ ratio, and total N content increased due to ALA foliar application under alkaline conditions, while these parameters more increased under saline conditions. On the other hand, alkalinity significantly increased malondialdehyde (MDA) content, but ALA foliar application effectively depressed the increase of MDA. This result clearly demonstrated that foliar-applied ALA had the potential to alleviate oxidative damage in alkalinity-stressed plants.

These results suggest that, although ALA foliar application effectively increased Chl content, it could not completely alleviate oxidative damage caused by osmotic stress and ionic stress, and thus less increase in alkaline tolerance.

Chapter 4. The alleviative function of potassium on Swiss chard under alkaline conditions

K is a major inorganic constituent for osmotic potential, ion homeostasis, and enzyme activation in plant cells. In chapter 2 and chapter 3, it was found that serious K^+ deficiency in Swiss chard leads to more destructive effects under alkaline conditions than under saline conditions. In light of this, Chapter 4 was conducted to investigate the impairments of water uptake, mineral elements, and antioxidant enzyme activities in the plant caused by alkalinity with or without K^+ application. The observed results revealed that the absence of K^+ somewhat intensified the effect of alkalinity on reducing plant growth, because RWC, K^+ content, micronutrient (Cl^- , BO_3^{3-} , Cu^{2+} , Fe^{3+} , Mn^{2+} , and Zn^{2+}) contents, and the activities of catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX), were more negatively affected under Alkaline-K treatment than under Alkaline+K treatment. Under Alkaline+K treatment, the GPX activity maintained the same values with control plants, and plant shoot DW was unaltered upon K^+ application. This finding indicates that GPX activity protected the plant against the oxidative damage caused by H_2O_2 , and K^+ application might help the plant by maintaining GPX activity under Alkaline+K treatment. Under Alkaline-K treatment, potassium use efficiency (KUE) increased 18 times compared with that of control, suggesting that the plant can survive under Alkaline-K treatment depending on its efficient use of K^+ through saving it for biomass production. After analysis for MDA, and percentage contributions of Na^+ and K^+ to osmotic potential at full turgor, results revealed that oxidative damage was slightly induced, while Na^+ replaced K^+ as the main ion contributing to osmotic potential at full turgor under

Alkaline-K treatment. This finding indicates that cytoplasmic K^+ content was maintained at a higher level, due to a high proportional substitution of vacuolar K^+ by Na^+ , which then markedly increased the KUE, somewhat reduced the oxidative damage caused by toxic Na^+ .

Chapter 5. General discussion

The results of this study show that alkalinity is more toxic than salinity, which is attributed mainly to the serious reductions in Chl and K^+ contents. ALA foliar application significantly increased Chl content more under alkaline conditions than under saline conditions. However, plant stress tolerance increased less, and K^+ content remained unaltered under alkaline conditions. In light of these observations, it was suggested that enhancement of plant alkalinity tolerance was consistent with the enhancement of K^+ content in the plant, but not enhancement of Chl content. The application of K under alkaline conditions showed that K supplementation enhanced the GPX activity and micronutrient contents, and the plant's survival under Alkaline-K treatment owes to high KUE, which is linked to a high proportional substitution of K^+ by Na^+ .