

## Effect of various salt–alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors

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### Abstract

Sunflower seedlings were treated under 30 different conditions of alkalinity and salinity, which were established by mixing NaCl, NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, and Na<sub>2</sub>CO<sub>3</sub>, at various proportions. The treatments included a salt concentration range of 50–250 mmol and pH values from 7.12 to 10.72. Several physiological indices of seedlings stressed—including relative growth rate (RGR), leaf area, electrolyte leakage rate, proline content, citric acid content, and contents of Na<sup>+</sup> and K<sup>+</sup>—were determined to analyze the characteristics of the stresses due to the salt–alkali mixes and their main stress factors.

The results showed that the physiological responses of sunflower closely correlated not only with salinity (the total concentration of stress salt) but also with the pH (or alkalinity) of the treatment solution. RGR, leaf area, and of K<sup>+</sup> content decreased with increasing salinity and pH. Electrolyte leakage rate, proline content, citric acid content, and Na<sup>+</sup> content increased with increasing salinity and pH. The deleterious effects of a high pH value or salinity alone were significantly less than those of high pH in combination with salinity. This result suggested that for a salt–alkali mix stress, a reciprocal enhancement between salt stress and alkali stress was a characteristic feature.

The buffer capacity of the treatment solution was taken as a stress factor in order to simplify the stress factor analysis. The results of the statistical analysis showed that for the stress factors of the salt–alkali mix stress, [CO<sub>3</sub><sup>2−</sup>] and [HCO<sub>3</sub><sup>−</sup>] could be fully represented by the buffer capacity; [Na<sup>+</sup>] could be fully represented by salinity; whereas [SO<sub>4</sub><sup>2−</sup>] was negligible. Therefore, four factors, salinity, buffer capacity, pH and [Cl<sup>−</sup>], could reflect all of stress factors. Perfect linear correlations were observed between all strain indices and the four stress factors. However, the effects of the four stress factors on the strain indices were significantly different in magnitude. Buffer capacity and salinity were dominant factors for all strain indices. Thus, it is reasonable to consider the sum of salinity plus buffer capacity as the strength value of salt–alkali mix stress. Furthermore, the relationships between different strain indices and various stress factors were shown to be different.

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## 1. Introduction

Salinity stress is a widespread environmental problem. Although considerable effort has been devoted to solve this problem, two very important aspects have been neglected, i.e. salt–alkali stress and complex salt stress. Even though the world's land surface occupies about  $13.2 \times 10^9$  ha, no more than  $7 \times 10^9$  ha are potentially arable, and only  $1.5 \times 10^9$  ha are currently cultivated. Of the cultivated area, about  $0.34 \times 10^9$  ha (23%) are saline and another  $0.56 \times 10^9$  ha (37%) are sodic (Tanji, 1990). Actually, the problem of soil alkalization due to  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$ , may be more severe than the problem of soil salinization caused by the neutral salts, such as  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$ . For example, in the northeast of China, alkalized grassland has reached more than 70% (Kawanabe and Zhu, 1991). Because soil salinization and alkalization frequently co-occur, the conditions in the naturally salinized and alkalized soil are very complex, the total salt contents and composition of salts and the proportion of neutral salts to alkaline salts may vary in different soils. Thus, the stresses imposed by these soil media on plants could be very complex and difficult to approach experimentally. Natural salt stresses are mostly mixed salts stresses, and most of them contain both neutral and alkaline salts. Therefore, the problems of alkaline stress and salt–alkali mixed stress ought to be recognized and investigated as thoroughly as salt stress.

To date, the research of salt stress still emphasizes  $\text{NaCl}$  as the main subject, but it is deeply developing towards various aspects such as  $\text{Na}^+$  metabolism (Serrano et al., 1999), molecular biology of salt-resistance genes (Holmström et al., 2000; Huang et al., 2000; Quesada et al., 2002), and salt stress signal transduction (DeWald et al., 2001), and so on. However, there are only a few reports about stress by alkali. However, there have been some studies about calcareous soils (Brand et al., 2002; Nuttall et al., 2003), alkaline soil (Hartung et al., 2002; Yin and Shi, 1993), alkaline salt stress (Campbell and Nishio, 2000; El and Shaddad, 1996; Shi and Yin, 1992, 1993), and mixed salt stress (Shi et al., 1998). Furthermore, some reports clearly demonstrated the existence of alkali stress and showed that it is more severe than salt stress (Shi and Yin, 1993; Tang and Turner, 1999). In previous studies, it was found that alkali salt stress and neutral salt stress

are actually two distinct kinds of stresses (Shi and Yin, 1993). Based on our results, alkaline salt stress is best called “alkali stress,” while “salt stress” only includes the neutral salt stress.

The resistance to alkali stress of sunflower (*Helianthus annuus* L.) is stronger than that of other crops. Some sunflower breeds are able to grow on alkalized soil. However, there are very few reports about sunflower resistance to salt stress or alkali stress (Liu and Baird, 2003). A cultivar of sunflower was selected as the material to investigate the features and acting factors of salt–alkali mixed stress.

The neutral salts  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  and the alkaline salts  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  are the main salt components in the extensive alkaline soil over much of northeast China (Ge and Li, 1990). Therefore, mixtures of the aforementioned salts, in various proportions, were used to simulate a range of mixed salt and alkaline conditions. Thirty kinds of the mixed salt and alkaline conditions with different salinities and pH values were obtained to investigate the effect of mixed salt and alkaline stresses on sunflower seedlings and to analyze the corresponding stress factors.

## 2. Materials and methods

### 2.1. Plant materials

*H. annuus* L. cv. Baikuiza 4 was provided by the Baicheng Academy of Agriculture Sciences, Jilin Province, China and was selected because of its tolerance to salt–alkaline conditions. Baikuiza 4 seeds were sown in 24 cm diameter plastic pots containing washed sand. Each pot contained six plants. All pots were placed outdoors avoiding rainfall. Seedlings were sufficiently watered with Hoagland nutrient solution every 2 days. Evaporated water was replenished with distilled water at other times.

### 2.2. Design of simulated salt and alkaline conditions

Two neutral salts ( $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$ ) and two alkaline salts ( $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$ ) were selected based on the salt components in the extent of salt–alkaline soil over northeast China (Ge and Li, 1990). The four selected salts were mixed in various proportions ac-

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