Growth characteristics of *Suaeda salsa* under different soil salinity gradients in controlled experiments

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Abstract: Xinjiang is the province with the largest saline-alkali land in China. The growth of halophytes will be stressed when their salt content reaches the threshold. In this study, the pot experiments were conducted and *Suaeda salsa*, a typical halophyte, was selected as the object to explore the relationship between growth characteristics and salt content under different soil salinity gradients. Salinity gradients were set according to the maximum soil salinity in the current years of Ebinur Lake Wetland National Nature Reserve (ELWNNR). They were classified using the following threshold values: ≤2 g/kg (Control Group), 10 g/kg, 20 g/kg, 30 g/kg, and 40 g/kg. The original spectrum, trilateral position and growth characteristics were used as indexes for analysis. With the increase of soil salt content, the red edge shift occurs first, followed by the blue edge shift. The position of yellow edge, green peak and blue edge were not sensitive to the change of soil salinity. This indicates that the red edge is the best indicator for evaluating plant growth. Compared with the Control Group, when the salt content is less than 10 g/kg, the growth status of plant seems not significantly affected. However, plant growth begins to be stressed when the salt content increases to 20 g/kg, which is a turning point for plant health. The increase of soil salt content can inhibit chlorophyll synthesis and plant growth. Plants begin to die when the salt content reaches 40 g/kg. Therefore, 40 g/kg could be regarded as the critical point of salt content which inhibits plant growth. The study also shows that the relationship between plant-height change rate and soil salt content is the most significant, indicating that the plant-height change rate is significantly impacted by soil salt content.

Keywords: *Suaeda salsa*, soil salt content, gradient, spectral, growth characteristics
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1 Introduction

Salinity is one of the major factors that affect plant growth and metabolism, leading to severe damage and a loss of productivity, approximately 20% (45 million hm^2) of irrigated land is salt-affected[1]. Salt stress, caused by either saline or sodic soils, represents a major threat to global food production, with estimates of up to $30 billion in agricultural losses annually. The problem is particularly acute in arid and semiarid environment[2-6]. The mechanism of salt accumulation in soil occurs when the reduction of water content leaves salt behind and the salt is stored[7].

According to the distribution of irrigation salinity risk in the world and Xinjiang Uygur Autonomous Region (XUAR)[8], by 2050, 50% of the world's arable land will be affected by salinity[9]. Xinjiang is the largest arid region in China, with a total area of approximately 1.66×10^6 km^2. It belongs to a typical continental arid climate. The annual precipitation and potential evaporation are 100-200 mm and 1500-2300 mm in the north, 16-85 mm and 2100-3400 mm in the south, respectively. The saline and alkaline soil areas reach 8.48×10^4 km^2, and 31.1% of the arable farmland is salt-affected[10,11]. *Suaeda salsa* is an annual mesophyll herb of Chenopodiaceae, the main halophytes in saline-alkali soil of China[12].

The response mechanism of plants to salt can reduce their growth[13]. However, hyperspectral remote sensing technology is an important technical method for early monitoring of crop stress[14]. Stress conditions include drought, nutrition deficiency, fungi and senescence. If the plant is inhibited in some way, its normal growth and development will be affected, which will cause chlorophyll content to decrease. The plant's light wave absorptivity in blue and red bands (i.e., about 650 nm and 425 nm) will also decrease and its reflection ability will enhance[15]. Xu et al.[16] found that under high temperature stress, the differential spectra of visible blue and red light are sensitive bands for the pigment content of *Phyllostachys edulis*. Yang et al.[17] studied the response of vegetation leaves to Sooty mold stress. He found that with the increase of the stress, the reflection peak disappeared at around 560 nm, and the inversion effect of the quadratic curve model was better used at 785 nm wave range. Corti et al.[18] found that red edge and red edge spectral regions, play the most important role in estimating biomass, moisture and nitrogen content

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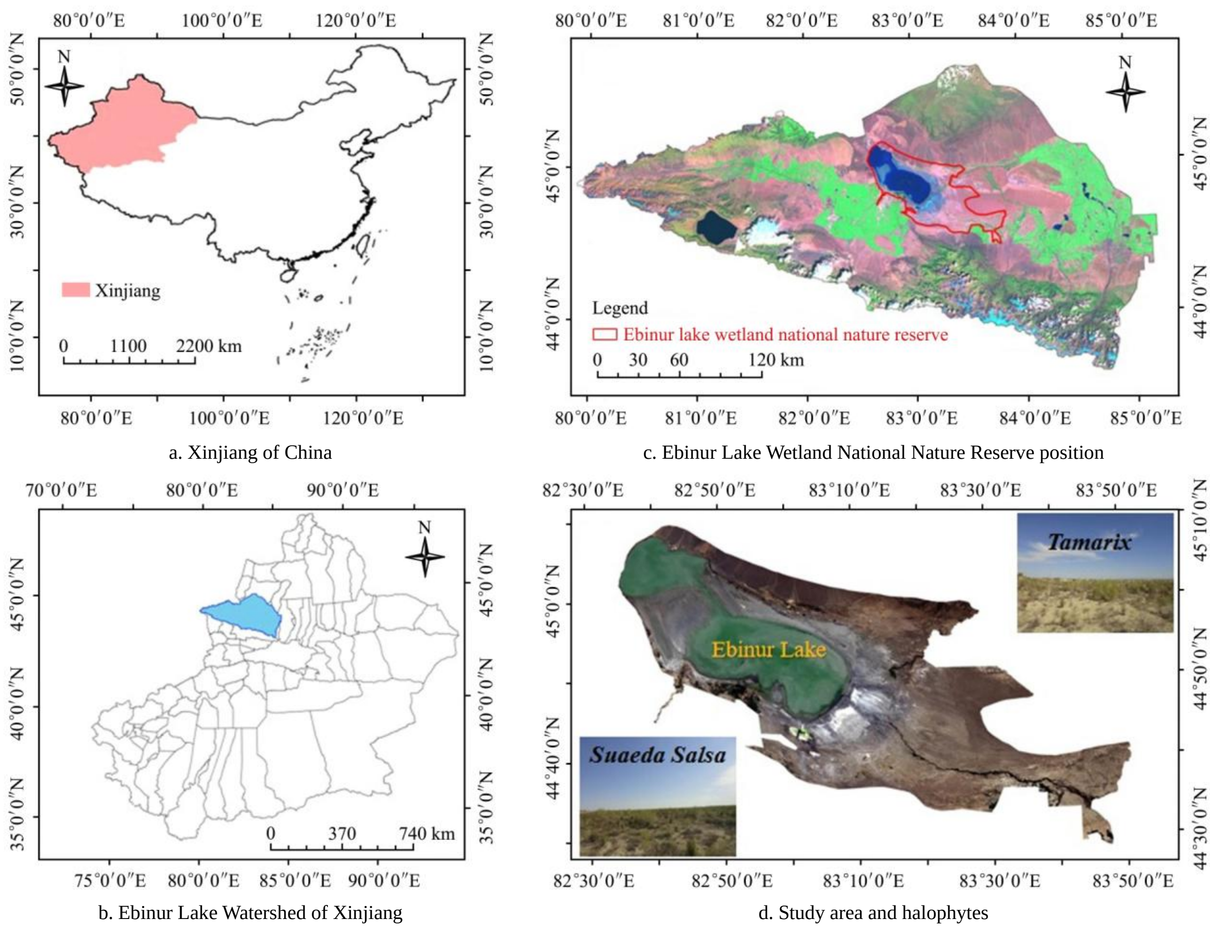
in canopy reflectance-based and HSG-based models. Oksana et al.[19] used HSI techniques to assess the changes in the early stages of salinity stress, select tolerant plant species or cultivars for the breeding process. Radanielson et al.[20] presented a method for quantitative analysis of genotype variations of plant growth biomass, leaf transpiration rate, and net photosynthetic rate in saline soil environment, and revealed the effects of salinity on plant growth. The benefit of derivative analysis is its ability to provide rapid determination of inflections points as well as reflectance maxima and minima. Derivative analysis has been applied to the analysis of vegetation stress monitoring[21,22]. Derivative spectra remove the effects of linear or near-linear regions of background and noise spectrum.

Through field investigation, it has been found that when soil salinity is high (Between moderate-salinization soil and saline soil) and the soil desertification is serious in the Ebinur Lake Watershed[23,24], there are short *Suaeda salsa* sporadic growth around other small shrubs. *Suaeda salsa*, as an annual plant with strong salt-alkali resistance and suitable for indoor cultivation, is also known as the "vanguard of saline-alkali land"[25]. *Suaeda salsa* can not only absorb a large amount of soil salt and store this in its thick leaves, but it can also accumulate and absorb heavy metals from the soil, which has effect of improving and restoring damaged wetland ecosystem[26,27]. Therefore, the *Suaeda salsa* can be used for implementing a salt control experiment. This study attempts to: (1) analyze the relationship between *Suaeda salsa* growth characteristics and salt content under different soil salinity gradients and (2) confirm salt content threshold during the *Suaeda salsa* growth and dying phases.

2 Materials and methods

2.1 Study site

Ebinur Lake Wetland National Nature Reserve (ELWNNR) is located in the northwest Xinjiang Uygur Autonomous Region at 44°54'-45°08'N and 82°35'-83°10'E. It includes the Ebinur Lake, which is also known as the largest saltwater lake in Xinjiang (Figure 1). The total area of the ELWNNR is 2670.8 km^2[28,29]. The climate of the ELWNNR is typically a continental arid climate, featuring hot summers and chilly winters, rare precipitation, and strong evaporation. The mean annual temperature varies from 4.0℃ to 8.1℃. The mean annual precipitation from the plains to the mountains varies from 102.6 to 229.4 mm. Therefore, soil salinization is aggravated by local climate factors. Vegetation types mainly include *Populus euphratic*a, *Tamarix ramosissima*, *Haloxylon ammodendron*, *Phragmites australis*, and *Suaeda salsa*[30-32]. As one of the pioneer plant in salt soil, *Suaeda salsa* can survive in soil with 3% salt content, compared with other halophytes, *Suaeda salsa* has a certain tolerance, which is an important germplasm resource, and has a crucial application potential in the research of plant resistance mechanism, development and utilization of salt-tolerant genes and soil ecological remediation[33].



a. Xinjiang of China

c. Ebinur Lake Wetland National Nature Reserve position

b. Ebinur Lake Watershed of Xinjiang

d. Study area and halophytes

Figure 1 Location map of the study area

2.2 Experimental design

The plump seeds of *Suaeda salsa* were collected from Ebinur Lake area. They were seeded in a greenhouse at the beginning of May, 2018. One hundred seedlings with the highest emergence rate and consistent growth rate were chosen for cultivation. Deionized water was used to wash the sandy soil until soil salinity was less than 2 g/kg. Each pot (15 cm×15 cm) was filled with 2 kg salt washed soil base material of *Suaeda salsa*. The seedlings were cultivated in pots in June, 2018. The plants were divided into 20 pots with five seedlings per pot (Figure 2).

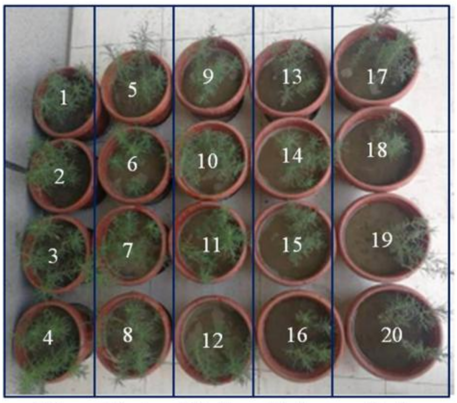


Figure 2 Gradients setting schematic diagram

Salinity gradients were classified using control group (≤2 g/kg), 10 g/kg, 20 g/kg, 30 g/kg and 40 g/kg (see Table 1). Four parallel samples under the same salt conditions were grouped together. After two weeks of slow seedling growth, the seedlings began to adapt to the new growth environment. In order to avoid salt shock, a control experiment of slow salt addition was implemented. During the control period, water, temperature and light for all samples were kept identical, and deionized water were mixed together as irrigation solutions with various concentrations representing different salt gradients. Use a watering can to irrigate near the root of *Suaeda salsa* three a week with 120 mL of irrigation solution each time. Sodium chloride (NaCl) after five weeks of cultivation, soil salinity in each pot reached the predetermined concentration. In mid-July, the indexes of spectral curve, plant height and chlorophyll were measured.

Table 1 Salinity gradient setting and number of plants

Soil salinity/g kg^{-1}	Number of pots	Plant per pot
≤2 (Control group)	4	5
10	4	5
20	4	5
30	4	5
40	4	5

2.3 Data collection

2.3.1 Spectral acquisition

The canopy spectral reflectance of *Suaeda salsa* was measured by portable ASD spectrometer (Analytical Spectral Devices Inc., Boulder, CO, USA). The spectral reflectance was originally measured at wavelengths of 1.4 nm and 2.2 nm sampling intervals from 350 to 1000 nm and 1000 to 2500 nm, respectively. However, the entire spectral range (350-2500 nm) was calculated automatically to resample to 1.0 nm continuous bands. To avoid noise interference and reduce errors, the collection of spectral reflectance began around 12:00 local time under sunny, cloudless and windless conditions. A standardized white panel, covered with a mixture of barium sulfate ($BaSO_4$), was employed to calibrate reflectance spectra before sampling. A probe was then placed 5 cm directly above the canopy of *Suaeda salsa*, making the canopy blades fill the whole field of view to reduce background soil noise.

2.3.2 Data acquisition of plant-height and chlorophyll

Plant height was measured after spectral curve acquisition. The longest distance from the root of the soil to the top of the leaf was measured with a ruler. Because the plant shape is slightly bent, there was a 2-4 mm error by manual measurement. The chlorophyll content of *Suaeda salsa* was measured by a hand-held SPAD-502Plus meter[34]. The leaves of five plants in each pot were tested. Each plant was measured three times and the average value was used as the chlorophyll value for the pot plant.

2.4 Data processing

2.4.1 Hyperspectral data processing

During the spectral measurement, the shape and trend of the spectral curve will inevitably be affected by the influence of illumination and soil background, although the error is minimized. It is necessary to minimize noise interference in spectral pretreatment[35]. The hyperspectral data were processed by ViewSpecPro software, and the spectral curve per pot was smoothed by Origin software. The average value of four pots of *Suaeda salsa* under the same gradient was calculated and a discontinuous smooth spectral curve was obtained for each gradient.

2.4.2 Calculation of the First Order Derivative

In order to extract spectrum to a greater extent, the first order derivative transformation was applied and the formulas are as follows[36]:

$$R'(\lambda_i)=[R(\lambda_{i+1})-R(\lambda_{i-1})]/(2\Delta\lambda) \quad (1)$$

where, λ_i is the wavelength of each waveband; $R'(\lambda_i)$ is the first order derivative for λ_i waveband; $\Delta\lambda$ is the interval from λ_{i-1} to λ_i, which is determined according to the wave bands.

2.4.3 "Trilateral" position determination

According to previous studies[37-40], green peak is the maximum reflectance within the range of 510-560 nm. The areas of red edge, yellow edge and blue edge are within the range of 670-737 nm, 550-582 nm and 490-530 nm, respectively. Their positions are defined by the maximum values of the first order derivatives in the three corresponding ranges as shown in Table 2.

Table 2 "Trilateral" parameters

Trilateral parameters	Definition	Description
D_r	Maximum value of 1st derivative within red edge	Red edge range covers 670-737 nm. D_r is maximum value of 1st order derivatives within the red edge of 61 bands
λ_r	Wavelength at D_r	λ_r is wavelength position at D_r
D_y	Maximum value of 1st derivative within yellow edge	Yellow edge range covers 550-582 nm. D_y is maximum value of 1st order derivatives within the yellow edge of 28 bands
λ_y	Wavelength at D_y	λ_y is wavelength position at D_y
R_g	Reflectance at green peak	R_g is maximum reflectance within the range of 510-560 nm
λ_g	Wavelength at R_g	λ_g is wavelength position at R_g
D_b	Maximum value of 1st derivative within blue edge	Blue edge range covers 490-530 nm. D_b is a maximum value of 1st order derivatives within the blue edge of 35 bands
λ_b	Wavelength at λ_b	λ_b is wavelength position at D_b

2.4.4 Calculation of plant height growth rate

At the beginning of the control experiment, the plants were measured in each pot, and the average value was taken as the height before the control experiment. After the control experiment, the plant height in each pot was measured as the plant height after the control. According to Shi et al.[41], the formula used was as follow:

$$CR=\frac{AH-BH}{BH}\times 100\% \quad (2)$$

where, AH (after height) and BH (before height) represent plant height after control and before control, respectively; CR represents change rate.

3 Results and discussion

3.1 Original spectral curves under different salinity gradients

There was much noise in the water absorption bands for the spectral curves of wild plants, such as 1353-1409 nm, 1801-1940 nm, and 2401-2500 nm, therefore the spectral noise was deleted in this paper. The original spectral curves were obtained under five salinity gradients, as shown in Figure 3. The red edge is a fairly wide feature. Hence it is often characterized by its maximum slope called the red edge position, so that it may be easily compared with measurements under different conditions and for comparison among different species[42].

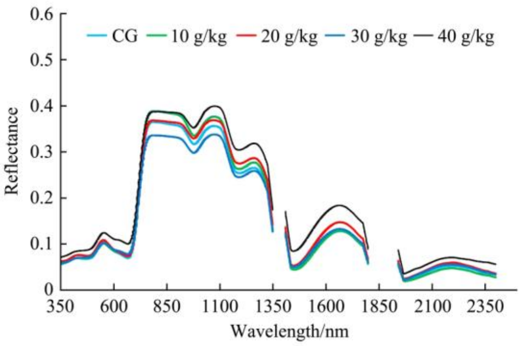


Figure 3 Spectral reflectance curves under five gradients

It is shown that the reflectance trends of *Suaeda salsa* are similar under different salinity gradients, the maximum of reflectance being in the range of 30%-40%. There are obvious absorption valleys in the wavelength area of 650-750 nm and reflection peaks in the wavelength area of 550-600 nm. However, it is very difficult to distinguish among them in detail.

3.2 Change of red edge position under different salinity gradient

Selection of the band related to visible spectrum absorption in the first derivative of spectrum. As shown in Figure 4, the first order derivative values are in the range of –0.001 to 0.008. The first order derivative map in the range of 350-760 nm under five gradients showed that first peak appeared at 700-750 nm and the second peak appeared at 500-550 nm.

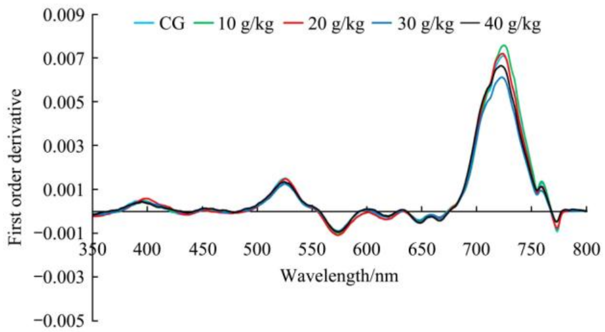


Figure 4 The first derivative of spectral reflectance

The trilateral position after calculating the first order derivative of the reflection of *Suaeda salsa* under different salinity gradients is listed in Table 3. When the salt content is less than 2 g/kg in the Control Group, the red edge position appears at 724 nm. When the salt content is up to 10 g/kg, the red shift phenomenon occurs, followed by the blue shift with the salt content greater than 20 g/kg. The yellow edge has the same value of 550 nm across the five gradients. Therefore, the yellow edge has no indication for the growth of plants under different salinity gradients.

In addition, in the range of 510-560 nm and 490-530 nm, the corresponding bands of the maximum of value are searched to determine the green peak and blue edge positions. When the salt content is less than 40 g/kg, the green peak and blue edge position both are 525 nm. Only when the salt content reaches 40 g/kg, the green peak and blue edge move to 524 nm. The positions of yellow edge, green peak and blue edge are not sensitive with the change of soil salt content, so, in subsequent analysis, only the red edge position is taken as the response index to study.

Table 3 Trilateral characteristics under different salt gradient (nm)

Salt content/g kg^{-1}	Red edge position	Yellow edge position	Green edge position	Blue edge position
≤2 (Control group)	724	550	525	525
10	725	550	525	525
20	723	550	525	525
30	722	550	525	525
40	722	550	524	524

The relationship between salt content and red edge is shown in Figure 5. It shows a negative correlation trend, with an R^2 value of 0.7206. With the increase of soil salinity, the wavelength of red edge position first increases and then decreases, from the control group (≤2 g/kg) to the second gradient (10 g/kg), the wavelength of red edge position increase and show the red shift. After that, the wavelength of red edge position decreases and reveals the blue shift. Due to the position of red edge can indicate the growth status of plants. Therefore, compared with the control group, when the salt content is up to 10 g/kg, the growth status of plant seems not significantly affected. However, plant growth begins to be stressed when the salt content reaches 20 g/kg, which is a turning point for plant health. Plants begin to die when the salt content reaches 40 g/kg. Therefore, 40 g/kg could be regarded as a critical salt content which stops plant growth.

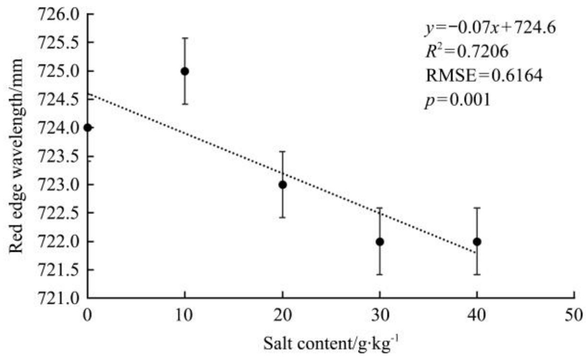


Figure 5 Relationship between red edge position and soil salt content

3.3 Change rate of plant height under different salinity gradients

Two groups of plant height data were obtained by measuring the plant height of *Suaeda salsa* before and after the control experiment. The plant height change rate under different salinity gradients can be calculated. Because the seedlings with the same growth status were selected at the beginning of the experiment, the plant height change rate could reflect the change degree of the height of *Suaeda salsa* in the control experiment. As shown in Figure 6, the change rate of plant height showed a negative correlation with salt content, the R^2 even equals to 0.9993.

During the control experiment, the seedlings of *Suaeda salsa* had the highest plant height and the highest change rate in the control group, and its growth status was different from that of the other four gradients. At the end of the cultivation, only the plants of control group showed flowering phenomenon. With the increase of soil salinity, the change rate of plant height decreased, which indicated that the growth of *Suaeda salsa* was inhibited by salinity.

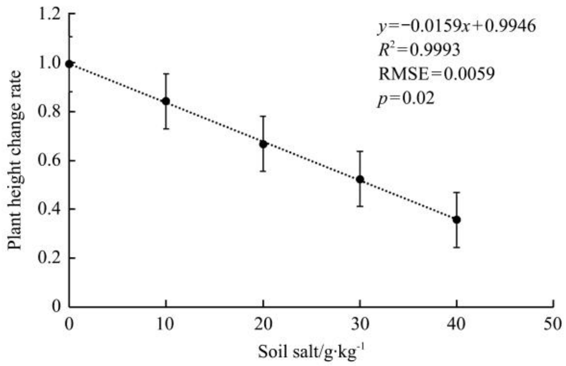


Figure 6 Relationship between plant height change rate and soil salt content

The research finding indicated that the change rate of plant height increased first, then inhibited plant growth during the process of stress, consistent with the conclusion of Li et al.[43] Reduced soil stress does not necessarily inhibit plant growth.

3.4 Change of chlorophyll content under different salinity gradients

The salinity stress not only has a negative impact on photosynthetic pigments and internal leaf structure, but it also leads to significant changes in plant water status due to the osmotic stress of salinity[44]. With the increase of soil salinity, the content of chlorophyll decreased. The increase of soil salinity inhibited the synthesis of chlorophyll, as excessive salt content can inhibit the root system and affect the photosynthesis as well as chlorophyll synthesis of plants. So, the chlorophyll content could affect the spectral reflectance characteristics. When salt content is up to 40 g/kg, a small part of the leaf surface appears white, called "crystal". The leaf's color becomes darker than that grown in a low soil salt content environment. The salting out phenomenon occurs at the roots above the surface of the soil, which later began to wither gradually after reaching the concentration. As shown in Figure 7, there is a negative correlation between soil salt content and chlorophyll content. With the increase of soil salt content, chlorophyll content shows a decrease. The R^2 equals to 0.9072.

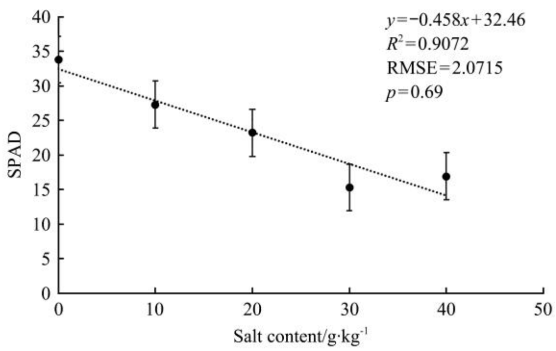


Figure 7 Relationship between chlorophyll content (SPAD) and soil salt content

From Figures 5-7, it is clearly illustrated that relationship between plant height change rate and salt content is much better than the red edge position and chlorophyll content.

Soil water retention capacity improved with the increase of salinity content. Under the natural environment in arid areas, evaporation is large and water shortage is common[45]. The growth of halophytes also needs salt. Soil containing a certain amount of salt can not only provide nutrition for the growth of halophytes, but also significantly improve the growth ability of *Suaeda salsa*, this is consistent with Guo et al.[46] The appropriate amount of soil salt can also improve soil water retention[47]. However, if excessive salinity exceeds the adjustable growth threshold of plants, plant death will also occur. The growth of *Suaeda salsa* will be inhibited under high salt environment, the same result as Zhou et al.[48] *Suaeda salsa* began to die gradually when the soil salt content reached 40 g/kg.

4 Conclusions

The author explores the *Suaeda Salsa* growth characteristics and salt content relationship under different soil salinity gradients based on the control experiment. The main results are as follows:

(1) In this experiment, with the increase of soil salinity content, the red edge position of *Suaeda salsa* first shows a trend of red shift, followed by blue shift. The red edge position shows a linear relationship with soil salinity content.

(2) The position of the yellow edge, green peak and blue edge are not sensitive to the plant growth with the change of soil salinity. Taking the red edge as an indicator to evaluate plant growth is sufficient.

(3) Compared with the Control Group, when the salt content is under 10 g/kg, the growth status of plant seems to not be significantly affected. However, plant growth begins to be stressed, when the salt content is up to 20 g/kg, which is a turning point for plant health. Plants begin to die when the salt content reaches 40 g/kg. Therefore, 40 g/kg could be regarded as a critical point of salt content which stops plant growth.

(4) The plant height change rate and chlorophyll content of *Suaeda salsa* were significantly negatively correlated with soil salinity content. The R^2 equals to 0.9993, and 0.9072, respectively. The change rate of plant height decreased with the increase of soil salinity, which indicates the growth of *Suaeda salsa* was inhibited by salinity. The increase of soil salinity inhibits the synthesis of chlorophyll. Therefore, excessive salt content can inhibit the *Suaeda salsa* growth and influence the photosynthesis and chlorophyll synthesis of plants.

The major drawback of this study was the lack of control undertaking an experiment of water and salt interaction and the limited number of pots and plants per pot. Future work will mainly include building a universal hyperspectral diagnosis and recognition model for the halophytes community under water and salt stress, and deciding the best combination of water and salt content suitable for plant growth and water retention effect.

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