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# The Classification Criteria for $NO_3^-$ Type Soil Secondary Salinization in Cucumber Cultivated Greenhouse

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**Abstract** The use of nitrogen fertilizers in intensive agricultural systems in China has dramatically increased for protected vegetale farming in recent years. The excessive use of nitrogen fertilizer has obviously resulted in salinizing soil, namely soil secondary salinization (short for SSS), which has become a significant environmental stress for crops, such as cucumber cultivated in greenhouse, in the protected farming in China. Therefore it is urgent and necessary to propose some guidelines for farmers to manage of the SSS problem in the areas of facility agriculture. In this study, the criteria of SSS classification based on the concentration of $NO_3$- was studied in cucumber cultivating greenhouses (*Cucumis sativus* L. *cv*. Dongfangmingzhu) in Taizhou, Jiangsu province, neighboring Shanghai of East China. After the soils in the tested greenhouses were treated with different concentrations of nitrate, the SSS levels expressed by electrical conductivity (EC) were detected at 0.89 ds/m for control, 2.78 ds/m for treatment 1 (T1), 3.65 ds/m for treatment 2 (T2), 4.66 ds/m for treatment 3 (T3) and 6.15 ds/m for treatment 4 (T4). The results showed that there were significant relationships between $NO_3^-$ contents and soil EC in the surface soil, which indicated that the changes of soil characters resulting from the changes of nitrate concentrations in soil in the greenhouse should be used as criteria for classifying the ranks of the $NO_3^-$ type SSS. The ranks of the $NO_3^-$ type SSS was significantly related with the plant height and yield of cucumber ($p < 0.05$). In this research, we proposed the criteria of classification for $NO_3^-$ type SSS in cucumber cultivated greenhouse in Delta of Yangtze River with five ranks based on the effects of SSS on the plant height and yield of cucumber, that is: level Ⅰ: 0.89 ds/m < EC < 2.03 ds/m, level Ⅱ: 2.03 ds/m < EC < 3.76 ds/m, level Ⅲ: 3.76 ds/m < EC < 5.32 ds/m, level Ⅳ: 5.32 ds/m < EC < 6.15 ds/m, and level Ⅴ: EC > 6.15 ds/m. This study might provide a practical guideline for evaluating $NO_3$- type SSS in facility agriculture, and also might be importance of the protected vegetable farming, particular in cucumber cultivated in greenhouse and plastic covering house, to monitor and adjust salinity in soil for .

**Keywords** Soil secondary salinization (SSS); $NO_3^-$ type SSS; Classification criteria; Protected farming; Facility agriculture; Cucumber (*Cucumis sativus* L.)

## Background

In recent years, The use of nitrogen fertilizer has been dramatically increased in intensive agricultural systems, particular in protected vegetable farming in China, which results in soil salinization due to large amount accumulation of nitrate in soil, that is so-called soil secondary salinization (SSS) in the protected farm (Kitamura et al., 2006). Nowadays, SSS becomes one of the major negative factors that hinder sustainable development of agricultural production in the area of protected farming in China (Yu et al., 2005). Cucumber (*Cucumis sativus* L.) is one of the major vegetable crops cultivated in greenhouse and protected farm, which is considered to be a moderately sensitive crop to salinity (Ayers and Westcot., 1985; Dorota, 1997). It was reported that overuse of nitrate will retard the growth and development of cucumber in the greenhouse (Yang et al., 2010; Kotsiras et al., 2002). There are some studies on the response of cucumber to salinity reported (Trajkova et al., 2006). However, little information involved in the classification of the $NO_3^-$

type SSS based on cucumber farming, that would be the reason why it is lack of some guidelines for the application of nitrogen fertilizers in long-term successive cropping areas in China.

So far, some guidelines of SSS management in agricultural ecosystem have been proposed, which can be summarized as follows. The SSS level can be decreased by (1) taking some agricultural measures such as irrigation and precise fertilization (Mustafa et al., 2006; Rouphael et al., 2006; Yuan et al., 2004); (2) soil replacement and amendments (Sui et al., 2009; Liang et al., 2005; Conde et al., 2005); (3) establishing flexible cropping system (Darwish et al., 2005; Graeme and Ellen, 2009). Although the above mentioned approached would be effective for alleviating SSS, it might be difficult to be employed in different greenhouses with different types of SSS. Therefore, it is necessary to build "standard or uniform" criteria to classify the types of SSS and to manage soil and fertilizers in the system of facility agriculture.

Jiangsu Province, neighboring Shanghai of East China, is a typical representative region of facility agriculture in China, where the nitrate ($NO_3^-$) is a common salt widely used in greenhouse soil (Ge et al., 2010). $NO_3^-$ based soil salinization become dominant limiting factor for the sustainable development in facility agriculture. In this study, the major objective is to develop a guideline for classification of SSS in greenhouse and to provide insights to improve the yield and quality of cucumber in greenhouse.

## 1 Results and Analysis

### 1.1 Soil $NO_3^-$ contents

Soil $NO_3^-$ contents of 0~8 cm soil layer at different times are shown in Table 1. The $NO_3^-$ concentrations in the control (ck) soil were lower than those in the treatments. The initial $NO_3^-$ contents in the treatment groups increased with the increase of salinity concentrations from T1 to T4. The trend of the soil $NO_3^-$ contents at the surface soils from 0 to 8 cm in the control and treatments were to lower values with experimental time.

Table 1 $NO_3^-$ contents of the surface soil layer (0~8cm) at different periods (Means, n=4)

| Treatments | $NO_3^-$ (mg/kg) | | | |
|---|---|---|---|---|
| | T7ds | P40ds | FBA | H7ds |
| ck | 92.45 | 85.43 | 40.15 | 23.22 |
| T1 | 325.43 | 264.24 | 159.85 | 111.40 |
| T2 | 680.15 | 519.40 | 294.60 | 258.26 |
| T3 | 998.15 | 848.30 | 568.50 | 445.60 |
| T4 | 1488.50 | 1406.80 | 932.60 | 843.50 |

Note: T7ds: Soil $NO_3^-$ contents after one week of salt treatment; P40ds: 40 days after planting; FBA: Full bearing age; H7ds: 7 days after full harvest

### 1.2 SSS expressed by electrical conductivity (EC)

Soil electrical conductivity (EC) was determined at four times, that is, 7 days after salt treatment, 40 days after planting, 70 days after planting, and 7 days after full harvest (Figure 1). The levels of SSS expressed by electric conductivity (EC) were 2.78 ds/m for T1, 3.65 ds/m for T2, 4.66 ds/m for T3 and 6.15 d ds/m for T4, but 0.91 ds/m for the ck. It is clear that the EC values of the ck were always lower than those in the treatments during the experimental period. The rates of decrease in soil salinization (EC/sampling time) for the different treatment groups (T1, T2, T3 and T4) were almost the same although the difference in initial levels of salinity existed. On the 7th day after full harvest (day 77), the soil EC had decreased to 0.31 ds/m for ck, 1.62 ds/m for T1, 2.45 ds/m for T2, 3.28 ds/m for T3, and 4.76 ds/m for T4. Compared with the initial value， EC values were dramatically reduced by 61.8% for ck, 41.7% for T1, 32.9% for T2, 29.6% for T3 and 22.6% for T4, respectively.

### 1.3 Plant heights and fruit yields of Cucumber

In the T4 (EC = 6.15 ds/m) the seeding was dead totally after 7 days of planting due to its high salinity. So the data of T4 were not shown. As Figure 2 showed, the cucumber plant heights

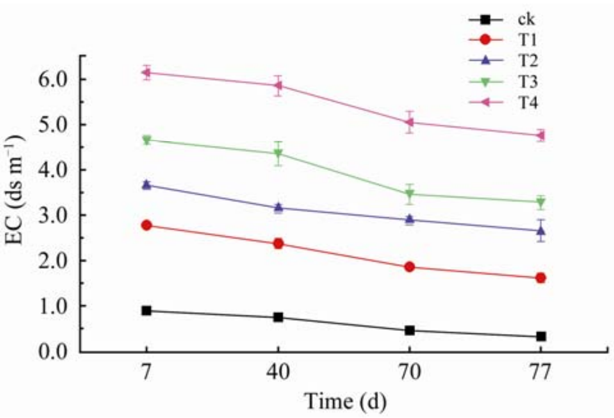


Figure 1 Soil EC (0~8 cm) dynamic variation of four treatments (T1, T2, T3 and T4) and ck at different periods
Note: Each datum point is the mean of six measurements with SD; The sample times are: 7 d: 7 days after salt treatment; 40 d: 40 days after planting; 70 d: 70 days after planting, which is also cucumber full bearing age; and 77 d: 7 days after full harvest

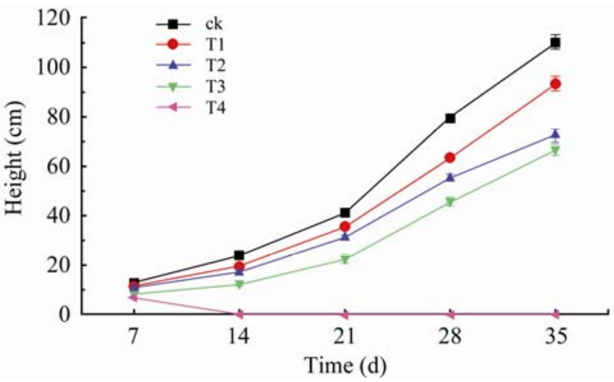


Figure 2 Height of cucumber plants for 5 treatments at different times
Note: p10, p20, p30, p40, p50, p60, p70: The day after planting 10 d, 20 d, 30 d, 40 d, 50 d, 60 d, 70 d; Each datum point is the mean of six measurements with standard deviation

in treatments were lower than those of ck at each sampling time. The heights of T1, T2, and T3 at the end of the experiment had decreased by 33.5%, 56.0%, 66.7% compared to ck, respectively (Table 2). The Y/P, WSF values for T1, T2, and T3 decreased significantly compared to those of ck ($p$ <0.05) (Table 2). The Y/P reduced by 12.3%, 31.7% and 61.9% for T1, T2, and T3 respectively. The same trend of weight per fruit (WSF) occurred with the increasing salinity. The WSF decreased 13.0% for T1, 26.7% for T2 and 47.1% for T3. The difference of the fruit number per plant between treatments was not significant.

### 1.4 Cucumber fruit quality

The parameters of cucumber quality including fruit VC, total soluble sugar content (TSC), total soluble protein (TSP) and crude fiber (CF) were studied (Table 3). In generally, the quality parameters except TSC, CF and TSP in treatments were higher than those of the ck, while VC decreased with the increase of salinity in soil.

## 2 Discussion

### 2.1 The relationship between Soil $NO_3^-$ contents and EC

With the growth of the cucumber, $NO_3^-$ contents of the ck and treatments decreased. The reduction might be due to the uptake by plants and the infiltration into deeper layers (Shi et al., 2009). Soil initial $NO_3^-$ increased with the enhancement of nitrate in the soil. It was found that there were significant relationships between $NO_3^-$ contents and top soil EC (Table 4). These results implied that the change in soil quality

Table 2 Cucumber height, fruit yield, number and weight in ck and treatments (n=6, means ± SD)

| Item | CK | Treatments | | |
|---|---|---|---|---|
| | | T1 | T2 | T3 |
| Height (cm) | 110.33±2.87a | 93.48±3.10b | 72.15±1.64c | 66.78±2.96d |
| Stem diameter | 1.64±0.04a | 1.57±0.04b | 1.50±0.01c | 1.40±0.04d |
| TY(kg) | 24.58±1.33a | 21.36±1.56b | 16.78±0.94c | 9.34±0.41d |
| Y/P (kg) | 1.76±0.09a | 1.53±0.11b | 1.29±0.08c5 | 0.93±0.15d |
| NF | 8 | 8 | 8 | 8 |
| WSF(g) | 220.00±11.50a | 191.3±13.5b | 161.3±10.3c | 116.3±18.6d |

Notes: TY: Total yield per treatment; Y/P: fruit yield per plant; NF: Number of fruits per plant; WSF: Weight of single fruit; Letters (a, b, c, d) represent the test results of different significance at the 0.05 level

Table 3 Fruit vitamin C (VC), soluble sugar content (TSC), total titratable acid (TTA) and sugar-acid ratio (TSC/TTA) in the ck and treatments (n=6, means ± SD)

| Property | Samples | | | |
|---|---|---|---|---|
| | ck | T1 | T2 | T3 |
| VC (mg/100g) | 3.33±0.09 | 3.11±0.07 | 2.98±0.22 | 2.83±0.08 |
| TSC (g/100g) | 1.86±0.11 | 1.99±0.02 | 2.15±0.0.7 | 2.36±0.13 |
| TSP (g/kg) | 1.26±0.08 | 1.29±0.04 | 1.31±0.08 | 1.35±0.10 |
| CF (g/100g) | 15.6±1.25 | 16.3±2.29 | 16.9±2.01 | 17.5±1.18 |

Table 4 The relationships between electrical conductivity (EC) and $NO_3^-$ contents in 0~8 cm horizon of the control (ck) and treatments (T)

| Item | Linear regression equation | r | *p* |
|---|---|---|---|
| ck | $NO_3^-$ = 0.008 39 EC + 0.132 31 | 0.986 | 0.010 |
| T1 | $NO_3^-$ = 0.004 65 EC + 1.232 49 | 0.981 | 0.020 |
| T2 | $NO_3^-$ = 0.002 71 EC + 1.983 89 | 0.997 | 0.004 |
| T3 | $NO_3^-$ = 0.002 53 EC + 2.070 91 | 0.996 | 0.005 |
| T4 | $NO_3^-$ = 0.002 70 EC + 2.059 06 | 0.984 | 0.020 |

should result from the difference in nitrate content and could be characterized as $NO_3^-$ type SSS. This finding is consistent with previous studies (Albiach et al., 2000; Shi et al., 2009).

The soil is considered to be saline if the electrical conductivity of a solution, extracted from a saturated soil paste, reaches to a value of 4 mmhos/cm (equal to 2.56 g/L dissolved salt). (Maas and Hoffman, 1977; Nele et al., 2011). According to this threshold, the soils in the T2 was very closer to the secondarily saline, T3 and T4 were definitely secondary saline, which then defined as $NO_3^-$ type SSS in this study.

## 2.2 Relationships among soil EC and plant height and yield of Cucumber

During the experimental period, from the whole trend line, the plant height of cucumber in the T1, T2and T3 decreased with the increase of EC in soils. There was a significant relationship between EC in soil and plant height of cucumber (Height = −12.23EC + 122.31, r = −0.975, $p$ = 0.025). When the plant height decreased by 15%, 30% and 60%, the corresponding soil EC were 2.33 ds/m, 3.69 ds/m, 6.39 ds/m, respectively.

The correlation between Y/P of cucumber and the soil EC (ds/m) was negative, following the linear equation: Y/P = −0.214 EC + 2.017 (R = −0.964, $p$ = 0.0036). When Y/P decreased by 10%, 30%, 50% and 70%, the corresponding soil EC were 2.03 ds/m, 3.67 ds/m, 5.32 ds/m and 6.97 ds/m, respectively. Number of fruits per plant (NF) was not obviously affected by the different concentrations of soil salinity (Table 3). There was a linear relationship between the weight of single fruit (WSF) and soil EC [WSF = −25.099EC + 250.02 (R = −0.954, $p$ = 0.046)]. When WSF decreased by 10%, 30%, 50% and 70%, the soil EC values were 2.09 ds/m, 3.83 ds/m, 5.59 ds/m and 7.34 ds/m, respectively.

## 2.3 Relationships between EC and cucumber quality

Salt treatment can slightly affect cucumber fruit quality. In our study total soluble sugar contents (TSC), total soluble protein (TSP) and crude fiber (CF) were linearly increased by varying degrees with the increasing soil EC (TSC = 0.130 EC + 1.702, r = 0.961, $p$ = 0.039; TSP = 0.023EC + 1.233, r = 0.972, $p$ = 0.028; CF = 0.503 EC + 15.07, r = 0.989, $p$ = 0.011). While fruit VC contents linearly decreased with the increasing soil EC. (VC =−0.132 EC + 3.458, r = −0.998, $p$ = 0.002).

## 2.4 The proposed classification of $NO_3^-$ type SSS

It is noticeable that the height of harvested fruits per plant decreased with salinity that was a contributing factor to loss fruit yield. This result was consistent with the previous studies (Adams and Ho, 1989; Kareem and Taiwo, 2007). So it might be potential possible that classification criteria for $NO_3^-$ type SSS can be performed based on the height and yield of harvested fruits per plant.

We can sum up that increasing salinity will significantly affect cucumber fruit quality including fruit total soluble sugar, total soluble protein and crude fiber contents, whereas the superfluous nitrate will decrease fruit VC contents. The conclusion in the present study has been consistent with that of other reported studies (Elates et al., 2002). The increasing of TSC and TSP could indicate the improvement in quality, whereas the increasing CF and the decreasing VC indicate the deterioration of fruit quality. However, the improvement in quality was unable to economically compensate for the reduction in fruit yield (Magán et al., 2008).

Plants exposing to saline environments are subjected to several adverse conditions, which can be categorized as shown in Table 5 (Abrol et al., 1988). Similarly, based on the results of the relationships and analyses mentioned above, the classification of $NO_3^-$ type SSS in greenhouse should be proposed according to the parameters of height and yield of cucumber. It is then suggested that the $NO_3^-$ type SSS in this study was classified into five catalogues from level Ⅰ (light salinity) to level Ⅴ (heavy salinity). When the height and yield are not affected by the salinity in soils, the $NO_3^-$ type SSS classification is defined as level Ⅰ. When the height and yield reduced by 0%~15% caused by salinity in soils, the $NO_3^-$ type SSS classification is defined as level Ⅱ. When the height and yield reduced by 15%~30% caused by salinity in soils, the $NO_3^-$ type SSS classification is defined as level Ⅲ. When the height and yield reduced by 30%~60% caused by salinity in soils, the $NO_3^-$ type SSS classification is defined as level Ⅳ. As long as the height and yield reduced more than 60% caused by salinity in soils, the $NO_3^-$ type SSS classification is defined as level Ⅴ. Correspondingly, level Ⅰ means no secondary salinization; level Ⅱ stands for a slight salinization; level Ⅲ indicates moderate salinization; level Ⅳ presents severe salinization, and level V refers to heavy salinization.

Table 5 General guidelines for plant response to salinity (adapted from Abrol et al., 1988)

| Soil salinity class | Conductivity of the saturation extract (EC, ds/m) | Effects on crops |
|---|---|---|
| Non saline | 0~2 | Salinity effects negligible |
| Slightly saline | 2~4 | Growth of sensitive plants may be restricted |
| Moderately saline | 4~8 | Growth of many plants are restricted |
| Strongly saline | 8~16 | Only tolerant plants grow satisfactorily |
| Very strongly saline | > 16 | Only a few very tolerant plants grow satisfactorily |

The EC-threshold of Y/P in the present work was at 2.03 ds/m, which is lower than the tomato threshold EC of 3.2 ds/m (Magán et al., 2008). Because the cucumber is more sensitive to the SSS than that of tomato. When Y/P decreased by 10%, 30%, 50% and 70%, the soil EC was 2.03 ds/m, 3.67 ds/m, 5.32 ds/m and 6.97 ds/m, respectively. But when the soil EC reached the 6.15 ds/m (T4), the seedlings completely died. When the plant height decreased by 15%, 30% and 60%, the corresponding soil EC were 2.33 ds/m, 3.69 ds/m, 6.39 ds/m, respectively. Then, it is likely that the secondary salinization could be classified as follows, level Ⅰ: 0.89 ds/m < EC < 2.03 ds/m, level Ⅱ: 2.03 ds/m < EC < 3.67 ds/m, level Ⅲ: 3.67 ds/m < EC < 5.32 ds/m, level Ⅳ: 5.32 ds/m < EC < 6.15 ds/m, level Ⅴ: EC > 6.15 ds/m.

There are no criteria of salinity where plants fail to grow. As the salinity increases, growth decreases until plants become chlorotic and die. Plants differ widely in their ability to tolerate salts in the soil. Salt tolerance ratings of plants are based on yield reduction on salt-affected soils when compared with yields on similar non-saline soils.

The classification for $NO_3^-$ type SSS might be similar to the salt tolerance ratings of plants. Therefore, the classification criteria of $NO_3^-$ type SSS in cucumber-based greenhouse in China’s Yangtze River

Delta is proposed at Table 6.

Table 6 The classification criteria for the $NO_3^-$ type SSS in cucumber-based greenhouse in China's Yangtze River Delta

| Soil secondary salinity class | EC1:5, 25℃ (ds/m) | Effects on tomatoes |
|---|---|---|
| I | 0.89~2.03 | Salinity effects negligible or slightly improved with increased salinity |
| II | 2.03~3.76 | Plant growth decreased by 0%~15%, yield dropped by10~30%, |
| III | 3.76~5.32 | Plant growth decreased by 15%~30%, yield dropped by 30%~50%, |
| IV | 5.32~6.15 | Plant growth decreased by 30%~60%, yield dropped by 50%~70%, |
| V | > 6.15 | Plant growth decreased by > 60%, yield dropped by >70% |

## 3 Materials and Methods

### 3.1 Experimental design

The experiments were conducted with four replications during two successive cropping periods (from April to July, 2009 and from January to April, 2010) in Taizhou, Jiangsu Province (32.755°N, 119.891°E). The greenhouse (200 m × 25 m) was covered with polyethylene that is penetrable for sunshine. The experimental plots with 5 m × 3 m located at this greenhouse. The agrochemical properties of the experimental soil are presented as follow (Means, n = 4): the soil layer is from 0 cm to 8 cm; nitrate 98.92 mg/kg, available phosphorus 65.40 mg/kg, available potassium 117.54 mg/kg total nitrogen 2.479 g/kg; sulfate content 79.33 mg/kg; organic matter 2.253 g/kg; pH (-) 6.83 and the electric conductance 0.89 ds/m. Each experimental plot was equally divided into 5 rows and cucumber (*Cucumis sativus* L. *cv*. Dongfangmingzhu) was grown in the rows. To avoid the exchange of salty and water among plots, each row was isolated by polyethylene films (5.0 m × 0.8 m) that were buried at depth of 0.6 m on both sides of the plots.

The surface soils from 0 to 8 cm in the plots were treated by adding water soluble magnesium nitrate ($Mg(NO_3)_2$) and calcium nitrate ($Ca(NO_3)_2$), to make the initial concentrations of soil salinity at 1.0~3.0 g/kg for treatment 1 (T1), 3.0~4.0 g/kg for treatment 2 (T2), 4.0~5.0 g/kg for treatment 3 (T3), and 5.0-6.0 g/kg for treatment 4 (T4), respectively. No chemical was added into the control (ck).

Cucumber (*Cucumis sativus* L. *cv*. Dongfangmingzhu, mid-tolerant to salinity stress) was used in this research. The seeds were sanitized with sodium hypochlorite containing 5% active chloride for 5 min, and then washed five times prior to immersing in deionized water for 12 h. The soaked seeds were raised in well-washed quartz sand and irrigated with tap water. As long as the second true leaf of cucumber seedling appeared, the cucumber seedlings were transplanted to the treated plots in the greenhouse. During the growth stages the temperature in the greenhouse was maintained at 25~30℃ at daytime and 18~20℃ at night. All plots were irrigated five times with river water. The nitrate concentration in the river water was below 8.0 mg/L.

### 3.2 Sample and analyses

Soil samples were collected in triplicate on six occasions (April 14, June 25, July, 30, 2009 and January 10, February 25, April 2, 2010). Fresh soil (50 mg) was mixed with deionized water (10 mL). The soil nitrate concentration was determined by spectrophotometer using phenol disulfonic acid (Lu, 2000).

The rest of each fresh soil sample was air-dried and then sieved through a 1.0 mm mesh, homogenized and stored in plastic bags at room temperature (from 20℃ to 25℃) until they were required for analysis. Salinity as expressed by electrical conductivity (EC) was measured with a DDS-320 conductivity meter at 25℃ after the soil was mixed with water (the ratio of soil to water in weight is 1:5).

Initial soil available phosphorus (P) was extracted using 0.5 M $NaHCO_3$ (pH 8.5) according to the method of Olsen (Criquet et al., 2007) and the P concentrations in the extracts were determined using the molybdenum blue method (Lu, 2000). Soil available potassium (K) was extracted by 1 M

$NH_4OAc$ (pH 7) and K concentrations in the extracts were analyzed by flame photometry (Lu, 2000). Soil available sulfur (S) was extracted by Ca $(H_2PO_4)_2$-HOAc and analyzed by spectrophotometer (Zhao et al., 2006). Organic matter was determined using the wet digestion tube method using potassium dichromate as oxidant (Bu et al., 2010). The total nitrogen content was measured by the Kjeldahl method (Domene et al., 2010). The pH was measured as a soil - water slurry (the weight ratio of soil to water is 1:2.5).

To assess the effects of different levels of SSS on the cucumber vegetative growth, the plant height was investigated at different days after transplanting at the first $7^{th}$ days, $14^{th}$ day, $21^{th}$ day, $28^{th}$ day, and $35^{th}$ day, and the stem diameter was measured at the $35^{th}$ day after the planting.

In order to estimate the effects of different SSS on the cucumber fruit yield, TP (total yield per treatment), Y/P (fruit yield per plant), No (Number of fruits per plant), WSF (weight of single fruit) were determined at the harvesting time.

To assess the organoleptic fruit quality, 6 mature fruits from each plot were randomly selected every 2 weeks over the course of the experimental period. The vitamin C, total soluble sugars (TSC) and total soluble proteins (TSP), and crude fiber (CF) were determined according to previously described methods (Nagao et al., 2005; Massot et al., 2010; Tewari et al., 2008).

### 3.3 Statistical Analysis

The data were statistically analyzed by the SPSS software (version17.0) and Origin (verision8.0). All data sets were tested for normal distribution and variance homogeneity. In case of homogenous sample variances, calculated means were compared by Duncan (D) and in the case of non-homogenous variances by Tamhane (T) test. The level of statistical significance was accepted when $p < 0.05$.

**Authors' contributions**

In this paper, ZQD is responsible for overall investigation. Experiments and original data collecting were done by JJZ and XL. Data analysis and manuscript preparation was completed by JJZ. Authors sincerely thank to two anonymous reviewers for their critical comments and revising advice to this paper.

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