

Effect of salt stress on the physiological and photosynthetic characteristics of *Weigela florida*

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Abstract Effects of salt stress on the physiological and photosynthetic characteristics of *Weigela florida* were studied. The results showed that the leaf area of *Weigela florida* was enhanced at 0.1% and 0.2% salt concentration, but decreased obviously when salt concentration was higher than 0.3%. The symptom of the salt injury was not significant when the salt concentration was lower than 0.3%, but was significant when higher than 0.4%. The water content decreased gradually but the electrolyte leakage increased gradually with the increasing of salt concentration and time. The total content of chlorophyll was rising while salt concentration was lower than 0.3%, but decreased while salt concentration was higher than 0.4%. The proline contents increased gradually while salt concentration was lower than 0.5%, but decreased at 0.5%. The soluble sugar content increased gradually with the increasing of salt concentration and prolonging of treatment, but decreased at 0.4% salt concentration 15 days after salt treatment. To the photosynthetic characteristics, the salt concentrations of 0.1% and 0.2% did not affect the photosynthetic characteristics of *Weigela florida*, when salt concentration was higher than 0.3%, the salinity significantly reduced Pn, Gs and Tr, but enhanced intercellular CO₂ concentrations at the salt concentrations of 0.3% and 0.4%. It was indicated that 0.1% and 0.2% salt concentrations had little influence on *Weigela florida*. The growth of *Weigela florida* was slightly decreased at the salt concentration of 0.3% without effect on its normal growth. However, the salt concentration of 0.4% affected the growth of *Weigela florida* obviously. *Weigela florida* died 7 days after treatment and it could not normally grow when salt concentration is higher than 0.5%.

Keywords *Weigela florida*, salt stress, physiological characteristics, photosynthetic characteristics

Introduction

Soil salinization is a global issue. There are more than 1.3 million hectares of saline-alkali soil in China, which poses a great threat on the increasingly decreased arable lands and increasingly fragile ecological environment. Hence, the improvement and treatment of saline-alkali soil have become an imperative task (Wang et al., 2001). Currently, there are two major measures for saline-alkali treatment, one is engineering-based and the other is of biological. The engineering-based measure costs too much and the lands treated are apt to restore to saline lands, while biologic measure requires lower cost and can form beautiful landscape and improve the ecological environment. Hence, the selection

of some saline-tolerance garden tree species is crucial for the improving of saline-alkali soil and the changing better of ecological environment.

There are many studies on the saline tolerance of plants currently. Nevertheless, most of them revolve around the saline tolerance of cash crops, some fruit trees and various lawn grasses instead of garden tree species. The selected material in this study is right a flower shrub commonly seen in greening named *Weigela florida*, a deciduous shrub of Caprifoliaceae with an as long as 2 months florescence. *Weigela florida* is more adaptable and to some extent resistant to hydrogen fluoride and hydrogen chloride. To date, few data concerning *Weigela florida* have been reported and all the existed reports focus on its propagation, while no study on its saline tolerance has been reported. In this paper, the cutting seedlings of annual *Weigela florida* were studied and their physiological and photosynthetic characteristics under salt stress were analyzed through potted plant salt-controlling experiment to probe into the saline resistance of *Weigela*

Received March 2, 2011; accepted August 24, 2011

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florida in order to provide theoretical basis for the application and generalization of *Weigela florida* in alkaline lands.

Materials and methods

Experimental materials

The cutting seedlings of annual *Weigela florida* were selected as the experimental materials of potted plant. The cutting was picked from Wuling Mountain of Hebei Province in March, 2010 and was shot for reproduction in the Specimen Garden of Agricultural University of Hebei. Robust seedlings of equal size were selected for potted plantation in June. Then, salt treatment and test began in mid-July.

Experimental method

Test and treatment

Potted plantation was adopted for this test, which was carried out in the Specimen Garden of Agricultural University of Hebei. The ground substances were garden mellow soil and sand with the proportion of 3:1, with the mixed dry weight of 3 kg. The container for potted plantation was mud pot with such 20 cm upper caliber, 15 cm minor caliber and 15 cm height. Randomized block experiment was adopted with 6 blocks including 0.0% (CK), 0.1% (A), 0.2% (B), 0.3% (C), 0.4% (D) and 0.5% (E) (percentage of NaCl in dry soil) arranged with 10 replicates each block. The soil water content was controlled around 75%–80% of the field capacity with weighing method at every night. A pallet was placed beneath the mud pot to get the water leaked after weighting and water replenishment at every night back to the mud to prevent the salinity in the mud pot from fleeing.

Measurement of indices

1) Growth index

(1) Observation of growth and survival rate

The salt injury of all the plants was investigated respectively 5, 10 and 15 days after salt stress to determine its degree of salt injury. The salt injury was classified as Class 0 with no salt stress symptom, Class 1 with a little portion of leaf apex, leaf margin and vein turning yellow, Class 2 with approximately half of the leaf apex and leaf margin withering, Class 3 with most of leaves with withering of leaf apex and leaf margin defoliating commonly, Class 4 with the branches withering and leaves defoliating until the plant withered completely.

The survival rate was investigated after one month of salt stress and calculated as follows:

Survival rate = number of plant survived/total number of plant × 100%.

(2) Measurement of leaf area

Unexpanded leaves on the top of the plants were selected as test leaves after salt stress and were measured with leaf area

meter after 30 days of salt stress.

2) Measurement of physiological index

The index was measured 5, 10 and 15 days after salt treatment by leaf sampling. Relative water content was measured by saturated weighing method (Zou, 2000). Chlorophyll content was measured by leaching 0.1 g fresh leaf with 20 mL 95% alcohol (Zhang, 1990). Proline content was measured by acid ninhydrin colorimetric method (Li, 2000). Soluble sugar content was measured by anthrone colorimetric method (Li, 2000). Cell membrane permeability was measured using Li Hesheng's method (Li, 2000).

3) Measurement of photosynthetic characteristics

The index was measured with portable photosynthesis system during 9:00–10:00 a.m. of the 8th day after salt treatment. The following photosynthetically active radiations (PARs) were provided with red and blue light sources, including 2000, 1500, 1000, 600, 300, 200, 100, 50, 30, 10 and 0 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$.

Data statistics and analysis

Analysis of variance of the experimental data was performed by SPSS software.

Results

Effect of salt stress on the growth of *Weigela florida*

Salt injury symptom and survival rate of Weigela florida under salt stress

According to Table 1, when salt contents were 0.1% and 0.2%, there was no obvious injury symptom for *Weigela florida* and the survival rates were both 100% as opposed to the control group. When salt content reached 0.3%, there was no obvious salt injury symptom for *Weigela florida* in the first 10 days of salt stress and a little leaf apex, leaf margin and vein turned yellow after 15 days of salt stress with 90% survival rate, indicating that the 0.3% salt content posed little impact on *Weigela florida*. When salt content reached 0.4%, a little leaf apex, leaf margin and vein turned yellow after 5 days of salt stress, approximately half of the leaf apex and leaf margin withered after 10 days of salt stress. Defoliation of laminae with withering of leaf apex and leaf margin was common for most leaves after 15 days of salt stress, which

Table 1 The symptom of salt injury for *Weigela florida*

Salt treatments	Description of salt injury degree of leaves			Survival rate (%)
	5 days	10 days	15 days	
CK	0	0	0	100
A	0	0	0	100
B	0	0	0	100
C	0	0	1	90
D	1	2	3	65
E	3	4	4	0

indicated that as the time of salt stress was lengthened, the salt injury degree of *Weigela florida* was worsened obviously and the survival rate was 65%. When salt content reached 0.5%, defoliation of leaf blades with withering of leaf apex and leaf margin was common for most leaves after 5 days of salt stress. Branches of most *Weigela florida* were withered and leaves defoliated after 10 days of salt treatment until the plant withered completely and the survival rate was 0.

Effect of salt stress on the leaf area of *Weigela florida*

According to Fig. 1, the leaf area of *Weigela florida* generally increased first and then decreased gradually under salt stress. Compared with the control group, the leaf area of *Weigela florida* increased by 48.9% when salt content was 0.1% and reached the conspicuous level ($P < 0.05$) upon analysis of variance. When salt content was 0.2%, the leaf area increased by 8.7% and differed little from the control group. When salt content was 0.3%, the leaf area decreased by 29.4% and differed little from the control group upon analysis of variance. When salt content was 0.4%, the leaf area decreased by 68.1% and significantly differed from the control group ($P < 0.05$).

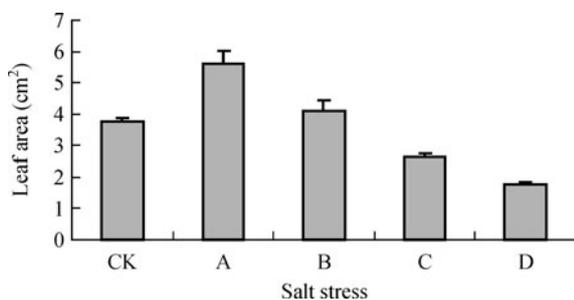


Figure 1 Effect of salt stress on the leaf area of *Weigela florida*.

Effect of salt stress on the physiology of cutting seedlings of *Weigela florida*

Effect of salt stress on relative water content in *Weigela florida* leaves

Salt stress leads to osmotic stress on plants, to difficulty in plant water absorption and finally to the physiological drought. According to Fig. 2, 5 days after salt stress, the relative water content of leaves decreased gradually with the increase of salt content. As apposed of the control group, when the salt content was 0.1%, the relative water content decreased by 5.4%, and at 0.2% by 12.3%, neither of which reached the conspicuous level. However, when the salt contents were 0.3%, 0.4% and 0.5%, the relative water contents of leaves decreased by 26.3%, 46.8% and 67.3% respectively and all reached the conspicuous level. With the same salt content, the water contents of leaves all decreased gradually and none reached the conspicuous level upon analysis of variance.

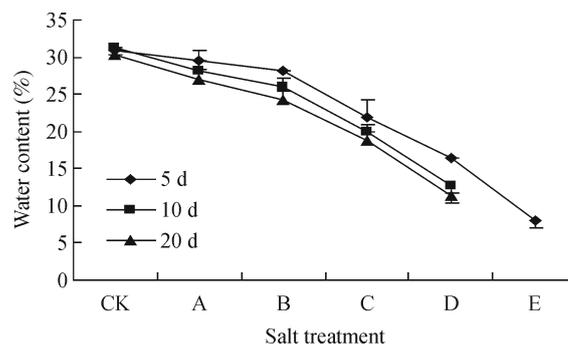


Figure 2 Effect of salt stress on water content in *Weigela florida*.

Effect of salt stress on proline content in *Weigela florida* leaves

Proline is an important osmotic adjustment substance in frond (Zhao, 1989). As shown in Fig. 3, 5 days after salt stress, proline content in the leaves of *Weigela florida* increased gradually when at lower than 0.5% salt content. As apposed to the control group, there was no significant difference when the salt contents were 0.1%, 0.2% and 0.3%, while the difference reached the conspicuous level when at 0.4% and the proline content decreased to some extent when the salt content reached 0.5%. As time went by, proline content increased slowly in leaves with low salt content, while the accumulative rate was quickened in leaves with high salt content. Yet, its increase dwindled away in leaves with different contents. Besides, significant difference was shown on time gradient upon analysis of variance.

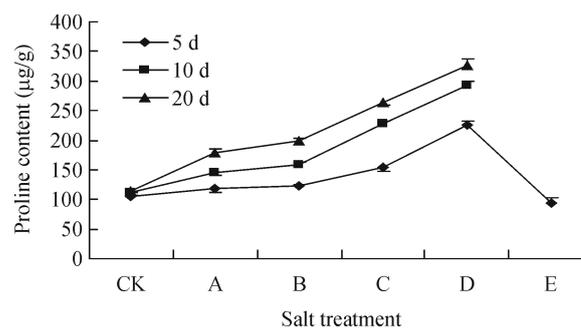


Figure 3 Effect of salt stress on proline content in *Weigela florida*.

Effect of salt stress on soluble sugar in *Weigela florida* leaves

Soluble sugar is also an important osmotic adjustment substance in frond (Zhai, 1989). As shown in Fig. 4, the changing trend of soluble sugar is roughly consistent with that of proline. Five days after salt treatment, soluble sugar content increased gradually along with the increase of salt content and decreased slightly when salt content reached 0.5%. As time went by, soluble sugar content tended to increase in leaves with different salt contents. Besides, significant difference was shown on time gradient upon analysis of variance.

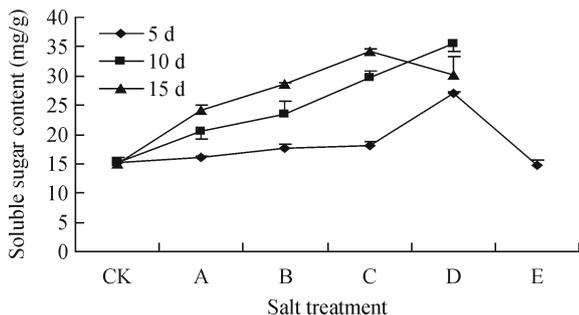


Figure 4 Effect of salt stress on soluble sugar content in *Weigela florida*.

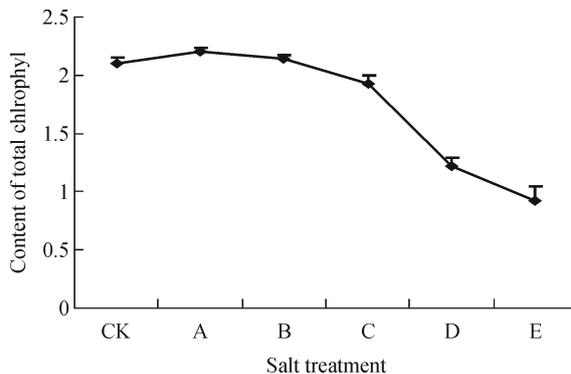


Figure 6 Effect of salt stress on content of total chlorophyll of *Weigela florida*.

Effect of salt stress on cell membrane permeability in Weigela florida leaves

Membrane system is the major part of salt injury on plants and cell membrane permeability is an important index reflecting plant injury extent (Leopold and Willing, 1989). As shown in Fig. 5, with the increase of salt content, the cell membrane permeability increased. After 5 days of salt stress, the cell membrane permeability under salt treatments increased by 12.4%, 18.9%, 51.4%, 91.9% and 368%. The cell membrane permeability differed little from the control group when the salt contents were 0.1% and 0.2% upon analysis of variance. The index differed significantly from the control group when salt contents reached 0.3%, 0.4% and 0.5%; at the level of 0.5% the increase reached 368%. As time went by, the cell membrane permeability of leaves increased gradually with the same salt content.

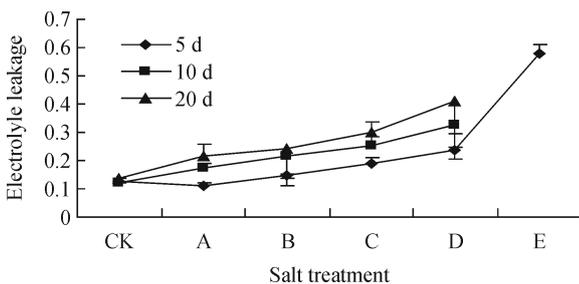


Figure 5 Effect of salt stress on electrolyte leakage in *Weigela florida*.

Effect of salt stress on the photosynthetic characteristics

Effect of salt stress on chlorophyll in Weigela florida leaves
Chlorophyll is indispensable for plant photosynthesis and is comprised of chlorophyll a and chlorophyll b, the content of which is closely linked with the intensity of plant photosynthesis. As shown in Fig. 6, with the increase of salt content, the chlorophyll content and chlorophyll a/b content at salt contents of 0.1% and 0.2% increased slightly. Both decreased only when salt content exceeded 0.2%.

Effect of different light intensities on net photosynthetic rate of Weigela florida under salt stress

According to Fig. 7, the changing trends of net photosynthetic rates under different salt treatments were consistent along with the increasing of light intensity. That is, the net photosynthetic rate increased along with the increasing of light intensity within certain scope. The net photosynthetic rate of *Weigela florida* increased rapidly when the salt contents in soil were 0.1% and 0.2%, while it changed slowly at 0.3% and 0.4% salt contents and increased along with the increasing of light intensity and finally tended to be stable. It indicates that the increase of light intensity under low-salt stress can significantly increase the net photosynthetic rate of plants, while the increase of light intensity under high-salt stress will not influence the net photosynthetic rate of *Weigela florida* significantly. In addition, most studies suggest that salt stress reduces the net photosynthetic rate of plants. According to Fig. 7, the net photosynthetic rate of *Weigela florida* increased slightly when salt contents were 0.1% and 0.2% and decreased significantly only at 0.3% and 0.4%.

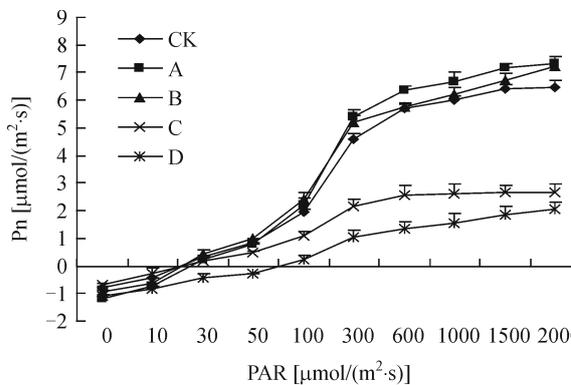


Figure 7 Effect of salt stress on Pn of *Weigela florida*.

Effect of different light intensities on the stomatal conductance of Weigela florida under salt stress
Stoma is the important channel for plants to exchange air with

the outside and the size of stoma is critical to plant photosynthesis (Zheng and Shangguan, 2006). According to Fig. 8, with the increasing of salt content in soil, the stomatal conductance of *Weigela florida* tended to decrease. As opposed to the control group, it decreased significantly under high-salt stress ($\geq 0.2\%$). It indicates that salt content of 0.1% poses little impact on stomatal conductance and salt contents of 0.2%, 0.3% and 0.4% have posed great impact on stomatal conductance. In addition, the Fig. 8 shows that with the increase of light intensity, the stomatal conductance of *Weigela florida* under various stresses will increase and more increase will be gained at the salt contents of 0.1% and 0.2% than at 0.3% and 0.4%. It indicates that hard light can increase the stomatal conductance of plants and such increase is significant under low-salt stress.

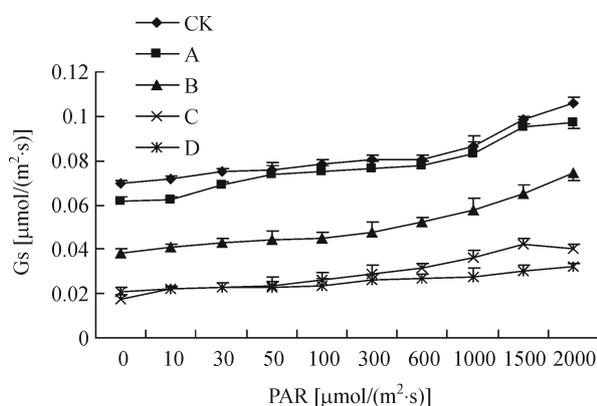


Figure 8 Effect of salt stress on Gs of *Weigela florida*.

Effect of different light intensities on intercellular CO₂ of Weigela florida under salt stress

Intercellular CO₂ of plant is an important material foundation (Chen, 2002) for photosynthesis. Concluded from Fig. 9, under different light intensities, the intercellular CO₂ has almost no change at 0.1% salt content, the main reason is that 0.1% salt content influences little upon the Gs of *Weigela florida*. While the intercellular CO₂ decreases significantly at 0.2% salt content, mainly because that with the increasing of salt stress, the Gs decreases significantly. Compared with the

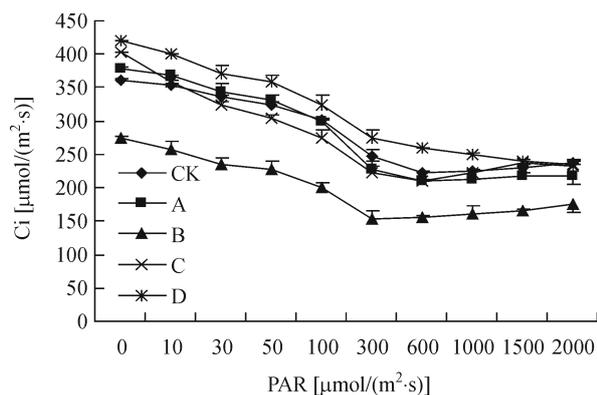


Figure 9 Effect of salt stress on Ci of *Weigela florida*.

condition of 0.2% salt content, the intercellular CO₂ increases significantly both at 0.3% and 0.4% salt content, moreover, the intercellular CO₂ at 0.4% salt content is higher than the contrast case.

Effect of different light intensities on Tr of Weigela florida seedling under salt stress

The decrease of Tr is a self-protective reaction of plant under salt stress. The plant under salt stress will close its stoma on leaves to reduce the transpiration of water, and keep a relative high water potential, and accordingly reduce the absorption of water and salt ions (Zhang et al., 2003) by roots. Drawn from Fig. 10, salt stress decreases the Tr of plant, whose change trend is similar to that of Gs. Compared with the contrast case, the decrease range at 0.1% and 0.2% salt content is small, however, significant at 0.3% and 0.4%. With the increase of light intensity, the Tr under each salt treatment shows an ascending trend.

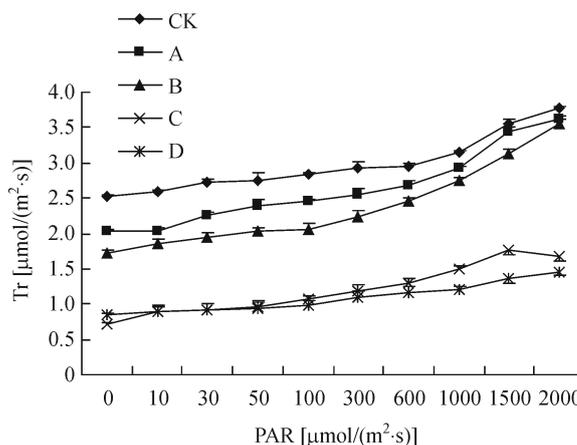


Figure 10 Effect of salt stress on Tr of *Weigela florida*.

Effect of different light intensities on Ls of Weigela florida seedling under salt stress

Figure 11 indicates that with the increase of light intensity, the Ls decreases gradually. Under different light intensities, the Ls at 0.1% and 0.2% increase significantly, while the Ls at 0.3% and 0.4% decrease significantly.

Discussion

Effect of salt stress on the growth of *Weigela florida*

Researches have shown that salt stress significantly inhibits the growth of plants, and reduces the Biomass. This study indicated that low salt stress under 0.1% and 0.2% salt contents promoted the growth of *Weigela florida* to some extent. The *Weigela florida* under that salt content showed no obvious symptom of salt injury, and the seedlings grew normally, with the leaf area expanded. When the salt content increased to 0.3%, the salt injury symptom increased, and its

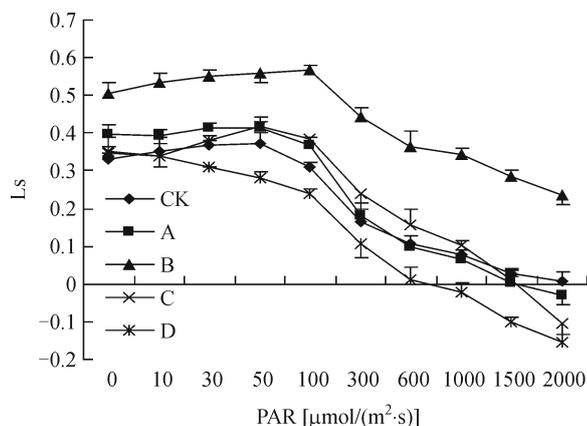


Figure 11 Effect of salt stress on Ls of *Weigela florida*.

harm to *Weigela florida* seedling aggravated, with some leaf apex, margin and vein yellowing. As a result, the salt stress at this content affected the growth of *Weigela florida*. The symptom of salt injury at 0.4% salt content became obvious, which did a significant harm to the growth of *Weigela florida*. As is shown, half leaf apex and leaf margin frizzled, and the growth of *Weigela florida* seedling was seriously affected. Under the salt content of 0.5%, all the *Weigela florida* die after one week of salt treatment, indicating that 0.5% salt content was the salt-tolerance limit of *Weigela florida*.

Effect of salt stress on the physiological characteristics of *Weigela florida*

The relative water content of plant leaves under salt stress is one of the important indicators of salt stress degree. The result of this experiment showed that only when the salt content was more than 0.3%, the water content of *Weigela florida*'s leaves was affected significantly. Under the same salt content, with the time of salt treatment prolongs, the relative water content always decreased, accompanied by gradual reduce of the decrease rate, which shows that with the time prolonging, the *Weigela florida* begins to gradually adapt itself to the salt stress environment by itself adjusting system.

The capacity of cell membrane permeability reflects the degree of plant damage, the greater the capacity, the larger the damage (Li et al., 2002). The result of this experiment indicated that with the increase of salt content in soil, and the time prolongs, the cell membrane permeability of *Weigela florida* was always increasing, but the increase rate was reducing with time prolonging, which means that as time prolongs, the *Weigela florida* begins to be gradually adapted to the salt stress environment. Permeability adjustment is one of the basic features of plant's adaptation to salt stress. Proline and soluble sugar are two important permeability adjustment substances in plants. Among them, proline has a good water-solubility, which can reduce the water potential, improve the water absorbing capacity of plants, and keep the water status in cytoplasm (Xiao et al., 2000). The result of this experiment

also indicated that the permeability adjustment of *Weigela florida* at a low salt content (i.e. 0.1% and 0.2%) was insignificant, while at a high salt content (i.e. 0.3% and 0.4%), the *Weigela florida* showed a significant permeability adjustment. When the salt content exceeded a certain limit (i.e. 0.5%), the permeability adjustment system of *Weigela florida* was completely lost, which consequently brought about a devastating effect upon *Weigela florida*. Seen from time gradient, the permeability capacity increased significantly in the initial stage of salt stress, which is shown by a significant increase of permeability adjustment substances; however, as time prolonged, the plant adapted itself to the salt stress environment gradually, and the permeability adjustment capacity of *Weigela florida* decreased gradually as a result.

Effect of salt stress on the photosynthetic characteristics of *Weigela florida*

It is hold by most researches that the salt stress reduces the chlorophyll content of plants. As an example, "Studies on Photosynthetic Physiological Properties and Saline-tolerance of *Lycium barbarum* under Salt Stress Condition in the Ningxia Drought Area by Hui et al. (2002)" shows that the salt stress reduces the chlorophyll content of *Lycium barbarum*. The result of this experiment indicated that within a certain range, low salt stress increased the chlorophyll content of plants. When at 0.1% and 0.2% salt contents, the chlorophyll a and the total amount of chlorophyll content increased to some extent, while the chlorophyll content decreased significantly only at 0.3%, 0.4% and 0.5% salt content, which is consistent with some researches by others. For instance, Hao et al. (2009) found that when the NaCl content was lower than 0.3%, the chlorophyll a and chlorophyll b and their content ration in leaves of *Campotheca acuminata* seedling increased to various degrees. Hao suggested that the increase of chlorophyll at low salt stress was resulted from the loose combination between chlorophyll and phyllochlorin, leading to the easy extraction of chlorophyll and the significant increase of chlorophyll. This study showed that the increase of chlorophyll at low salt content may be resulted from the low salt stress that excited the self-adjustment system, consequently promoted the synthesis of chlorophyll and drove the photosynthesis of plants, and accordingly accumulated photosynthate and facilitated energy conversion to against adverse environment. This was in compliance with the fact that the Pn increased to some extent at low salt content. With the salt content growing to a certain amount, exceeding the self-adjustment of *Weigela florida*, the activity of chlorophyllase increased, promoting the degradation of chlorophyll, and resulting in the decrease of chlorophyll content.

Photosynthesis is an important way for plants to accumulate substances and promote the growth. and Pn reflects the rate of photosynthate accumulation. Some researches held

that the salt stress decreases the photosynthesis of plants and consequently affects the growth of plants (Berry and Downton, 1982). This study suggested that low salt stress (i.e. at 0.1% and 0.2% salt content) promoted the photosynthesis of *Weigela florida* and improved the Pn. Only when the salt content reached some certain limit (i.e. at 0.3% and 0.4% salt content), the Pn of *Weigela florida* decreased with the increasing of salt content, which was consistent with the conclusion drawn by Ke and Zhou (2009) from “Effects of Salt Stress on Photosynthetic Characteristics of Mulberry Seedlings”, that low salt (i.e. at 0.1% salt content) could promote the photosynthesis of mulberry seedlings and when the salt content in soil reached a certain amount (i.e. more than 0.1% salt content), the higher the salt content, the lower the Pn of mulberry. Under low salt content, the increase of Pn of *Weigela florida* seedling may be an indication of its self-adjustment to be adapted to the adverse environment. Under salt stress, plants have to accumulate substances through improving their net photosynthesis, promote energy conversion and accordingly restore damaged tissues, keep the completeness of cell membrane, and reject the entrance of ions. With the increasing of salt content, a large amount of ions enter the cells, and inhibit the activity of photosystem (Zhu and Bie, 2007) as a result, besides both the increase of cell membrane permeability capacity and decrease of chlorophyll are important causes of the reduction of Pn.

It is generally believed that the stomatal factor and non-stomatal factor (Xu, 1999) are two major factors affecting the photosynthesis of plants. Ren and He (2008) states in “Effects of NaCl Stress on Growth and Photosynthetic Characteristics of lettuce (*Lactuca sativa* L.) Seedlings” that whether the photosynthesis of plants is affected by stomatal factor and non-stomatal factor cannot be generalized, because it closely relates to the type of researched plants, as well as the salt stress degree and time. This experiment showed that at 0.1% and 0.2% salt content, the Gs and Ci of *Weigela florida* decreased, but Ls increased, while at 0.3% and 0.4% salt content, the Gs and Ls of *Weigela florida* decreased, but Ci increased. According to Farquhar and Sharkey (1982), it is the stomatal factor that affects the decrease of Gs and Ci, and the increase of Ls; while it is the non-stomatal factor that affects the decrease of Gs and Ls and the increase of Ci. It can be seen that at 0.1% and 0.2% salt content, the stomatal factor dominated, while at 0.3% and 0.4% salt content, the non-stomatal factor dominated.

From the analysis of the growth, various physiological and photosynthetic indicators of *Weigela florida* cutting seedlings under salt stress, it can be concluded that *Weigela florida* cutting seedlings have a certain salt-tolerance ability, when the NaCl content in soil is less than 0.2%, the growth of *Weigela florida* is promoted, while the NaCl content reaches 0.4%, its growth is inhibited significantly, and when the NaCl content increases to 0.4%, all *Weigela florida* dies in 7 days, which means it cannot normally grow under 0.5% or more salt content in soil.

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