***Pak. J. Bot.*, 53(5): 1539-1544, 2021.** **DOI: http://dx.doi.org/10.30848/PJB2021-5(27)**

PHYSIOLOGICAL ALTERATIONS OF *EUCOMMIA ULMOIDES* OLIV. SEEDLINGS UNDER SALINE WATER STRESS AND NITROGEN FERTILIZER APPLICATION

YA SU, YI PEI[*], AKBAR TOWSON, YONGJIE HE, JIANGLI NIE AND BAIMING WANG

College of Horticulture and Landscape Architecture, Tianjin Agricultural University, Tianjin 300384, China
[*]Corresponding author's email: peiyee@126.com

Abstract

Adequate management of nitrogen fertilizer application in agriculture can contribute substantially to expanding *Eucommia ulmoides* Oliv. cultivation in the saline-alkali region of Tianjin. The aim of this study was to explore the changes of chlorophyll and osmotic adjustment substance content in *Eucommia ulmoides* Oliv. cultivated with different irrigation water salinity and nitrogen concentration. The experiment was carried out under the condition of pot experiment in the greenhouse of Tianjin Agriculture University. The randomized block design with 4×3 factor was adopted for the experiment, with four doses of N (0.3, 0.5, 5.0, 10.0 mmol·L^{-1}) and three levels of irrigation water salinity (CK,150,300 mmol·L^{-1}). In this experiment, each treatment was repeated 3 times, with a total of 36 experimental units and 3 plants in each pot. Growth and contents of chlorophyll, carotenoids, soluble sugar and soluble protein were quantified at 60 days after transplantation. With increasing irrigation water salinity, the content of chlorophyll a, chlorophyll b and carotenoids reduced and the content of soluble sugar and soluble protein increased. With the increase of nitrogen concentration, the content of chloroplast pigments first increased and then decreased. Except for the control, soluble sugar and soluble protein content gradually decreased. In a word, increments in N dose attenuate the deleterious effects of irrigation water salinity, and the optimum nitrogen concentration for the growth of *Eucommia ulmoides* Oliv. seedlings is 5.0 mmol·L^{-1}.

Key words: *Eucommia ulmoides* Oliv., Saline stress, Nitrogen level, Photosynthetic pigment, Osmotic adjustment substance.

Introduction

Eucommia ulmoides Oliv., also known as gum tree and kapoku, is a dioecious plant, which is a rare relict tree and a medicinal plant endemic to China. It belongs to one family and one genus and is distributed in many provinces and regions of China (Du, 2014).*Eucommia ulmoides* Oliv. is also the most widely used gum source plant in the world. Its bark, fruits and leaves contain gutta-percha, an isomer of natural rubber, which can be used in aerospace, military and rubber industry. Because *E. ulmoides* may replace rubber trees to produce rubber in the future, it is called "Chinese rubber tree". The bark of *E. ulmoides* was first used in medicine in the Han dynasty, and its has been reported to be able to notify liver and kidney, reinforce muscles and strengthening spleen, treat backache, act as a diuretic, prevent fetal leakage, lower blood pressure and provide a range of other benefits (Wang *et al*., 2001). However, the annual production rate of Eucommiae cortex is very low, which is not suitable for large-scale industrial production. In recent years, scholars have proved that Eucommia folium and bark have very similar chemical constituents and pharmacological activities. Eucommia folium is a renewable resource with huge yield (Yu *et al*., 1992). If it is processed and utilized, it can not only make up for the shortage of medicinal resources of *E. ulmoides*, but also change the situation that 80% of natural rubber relies on import in China.

Along with the further comprehensive development and utilization of *E. ulmoi*des, the natural growth of *E. ulmoides* can hardly meet the needs of market and production practice. The production and cultivation of *E. ulmoides* has also changed from extensive management to intensive management. However, with the increase of soil dryness and salinization, how to exploit and utilize this potential land resource is an important topic for long-term research today and even in the future.

Salinity can have deleterious effects on plants, directly or indirectly affecting chlorophyll content by reducing the permeability of soil solutions, destroying the membrane structure associated with photosynthesis, and inhibiting the synthesis of 5-aminolevulinate acid, which is the precursor molecule of chlorophyll (Freire *et al*., 2011). Among a series of processes affected by salt stress, it is well known that a large number of sodium ions gather in plant roots, leading to depolarization of cell membrane, thus inhibiting the absorption of nitrogen by plants and inhibiting the growth of plants. To adapt plants to adverse environmental conditions, osmoprotectants are synthesized to increase cytoplasmic osmotic pressure for the purpose of stabilizing proteins and membranes (Yancey, 1994).

It is the main direction of modern agricultural development to improve the salt tolerance of plants and comprehensively control soil salinization through rational irrigation and fertilization, use of chemical modifiers and biological and ecological measures, among which the rational application of nitrogen fertilizer is a more critical link (Sui *et al*., 2018). As the chemical element most needed by plants, nitrogen is also one of the basic elements that constitute plants, and it is the key limiting factor for plants (Attiwill & Adams, 1993).

It is shown that the response to saline stress varies with plants that supply different nitrogen concentrations. Xie *et al.,* (2018) found that the growth index and photosynthetic parameters of *Elaeagnus angustifolia* seedlings were significantly decreased under 100 mmol·L^{-1}NaCl treatment, while the damage of *Elaeagnus angustifolia* seedlings caused by salt stress could be alleviated by applying 2 mmol·L-1 nitrogen. Li *et al*., (2018) found that compared with salt-tolerant sweet potato, the salt-sensitive sweet potato (S19) showed a more significant salt-resistant effect after the salt-nitrogen interaction treatment in hydroponic nutrient solution increased with the nitrate nitrogen supply. Besides, the mitigation effects of different nitrogen forms on salt stress are also different. Mixed nitrogen supply can reduce the harm of salt stress to *Catharanthus roseus* (L.) G. Don (Zhu *et al*., 2015). Studies have shown that an appropriate increase in nitrogen concentration under saline stress can improve the yield and quality of *Zizyphus jujuba* Mill. cv. *Lingwuchangzao* (Cao *et al*., 2012), *Festuca elata* Keng ex E. Alexeev (Liu *et al*., 2013), *Suaeda salsa* (L.) Pall. (Wang *et al*., 2015), cotton (Zou *et al*., 2015), *Helianthus annuus* L. (Dang *et al*., 2017). Such an effect can be attributed to the functions of the nitrogen in plants, since it has structural functions and is involved in the synthesis of various organic compounds which are essential to plants, such as amino acids, proteins and proline. In addition, studies conducted by Lacerda *et al.,* (2003) and Silva *et al.,* (2008) have shown that plants can increase osmotic regulation ability by accumulating these organic solutes, thus increasing the resistance to water and saline stress. Accordingly, adequate management of nitrogen fertilizer application can be an alternative to mitigate the effects of salinity on plants.

It is vital to study the salt tolerance mechanism of *E. ulmoides* for the production of high-yield and high-quality medicinal materials in saline areas. However, researches on *E. ulmoides* mainly focus on breeding, economic value development, pharmacology and other aspects, only few reports on salt tolerance of *E. ulmoides* (Zhang, 2000; Wu, 2015). Data on physiological change of *E. ulmoides* under saline stress with nitrogen is still unknown. In this context, this experiment was conducted determine the effect of salinity levels and different N doses on the content of soluble protein, soluble sugar and chloroplast pigment in *E. ulmoides* seedlings, and preliminarily analyze whether saline stress produced a mitigating effect at different nitrogen levels. In addition, the optimum nitrogen concentration corresponding to different salt concentration was discussed, so as to provide theoretical basis for rational application of nitrogen fertilizer in saline stress environment.

Materials and Methods

Experimental site: The experiment was conducted from October to December 2017 in the greenhouse of Tianjin Agriculture University, Tianjin, China, at the coordinates 39°05′32.68″N and 117°06′2.12″E and 4m of altitude, in pots adapted as lysimeters under field conditions.

Experimental design: The randomized block design with 4×3 factor was adopted for the experiment. Each treatment was repeated 3 times. Treatments corresponded to four doses of N (0.3, 0.5, 5.0, 10.0 mmol/L) and three levels of irrigation water salinity (CK, 150, 300 mmol/L).

Germination: The collected *Eucommia ulmoides* Oliv., seeds are subjected to peeling treatment to remove seeds with virus infection and aphids, and seeds with full grain size and uniform size are selected for germination treatment. Its seedlings were obtained by sowing in plastic containers with the volume of 210 cm^3, containing substrate composed of vermiculite, perlite and peat and soil at 1:1:1 proportion, respectively. When the seedlings of *E. ulmoides* had 6-8 fully expanded true leaves, they were transplanted to pots perforated at the bottom for free drainage. The bottom of pot was involved by a nonwoven geotextile (Bidim OP 30), connected to a hose to the plastic bottle for monitoring the exudate volume and estimating the water consumption of the crop. The chemical and physical properties of the soil were determined (Table 1).

Salt treatment: Before transplanting, water content of potted soil was increased to field capacity, using the water from the local supply system.Saline water irrigation began 10 days after transplantation (DAT).The irrigation amount was determined based on the plant water requirement , which was calculated by the difference between the volume applied and the volume discharged in the previous irrigation. In the case of saline stress, in order to avoid initial salinity shock in the plants, salinity was carried out by stepwise incremental treatment of NaCl (Sui *et al.*, 2018), that is, daily increments of 50 mmol·L^{-1} NaCl, until the targeted salt concentration were achieved. At 30 DAT, the leaching fraction of 0.15 was adpoted in all treatments to minimize the accumulation of salt in the soil according to the previous irrigation amount.

Nitrogen treatment: Nitrogen fertilization treatment began at 25 DAT. The nitrogen in the experiment was provided by NH_4NO_3, and other large amounts of elements and trace elements required for plant growth were supplied by Hoagland nutrient solution. The nutrient solution was applied at 5-day intervals, which lasted 30 days. The formulation of nutrient solution is shown in Table 2, and the indexes were measured at 60 DAT.

Table 1. Physical and chemical properties of the soil used in the experiment.

Textural class	Apparent (Bulk) density (kg· dm^{-3})	Total porosity (%)	Aerate porosity	Water holding porosity	Size pore ratio
Clay loam	0.96	51.29	7.90	43.38	0.22
pH	Hydrolysable nitrogen	Available P	Available K	Organic matter	Salt content
	mg·kg^{-1}			%	
7.91	185.30	108.5005	357.5004	1.61	0.06

Table 2. Contents of nitrogen and other components in nutrient solution.

Nutrient solution composition	The contents of various components in nutrient solutions containing different nitrogen concentrations(mmol·L^{-1})			
	0.1N	0.5N	5.0N	10.0N
NH_4NO_3	0.1	0.5	5.0	10.0
$MgSO_4$	2.0	2.0	2.0	2.0
KH_2PO_4	1.0	1.0	1.0	1.0
K_2SO_4	2.5	2.5	2.5	2.5
$CaCl_2$	5.0	5.0	5.0	5.0

Note: Reference to Hoagland nutrition solution for iron salt and trace element

Determination of physiological indexes: The chlorophyll was extracted by 95% ethanol in dark. The absorbance of the resulting solution was read at 665,649 and 470nm with a UH 5300 Spectrophotometer (HITACHI, Japan), respectively. The concentrations of photosynthetic pigments were calculated (mg·L^{-1}) as follows:

$$C_a = 13.95D_{665} - 6.88D_{649} \quad (1)$$

$$C_b = 24.96D_{649} - 7.32D_{665} \quad (2)$$

$$C_{x.c} = (1000D_{470} - 2.05C_a - 114.8C_b)/245 \quad (3)$$

where Ca, Cb, Cx. c stand for the concentrations of chlorophyll a, chlorophyll b and carotenoids, respectively, and D represents the absorbance at the corresponding wavelength (Li, 2000).

The content of pigments was calculated according to the obtained concentration and its value was expressed as mg pigment per gram fresh weight of leave. The soluble sugar was quantitatively determined at 620nm by the anthrone-sulfuric acid colorimetric method with a spectrophotometer (Yemm & Willis, 1954). The soluble protein was bound to coomassie brilliant blue G-250 and its content was determined at 595nm by ultraviolet-visible spectrophotometer (Li, 2000).

Statistical analysis

Studies were carried out following randomized design with three replications. The data of each index were collected and analyzed by IBM SPSS Statistics 19.0 software (SPSS Inc., United States) and Excel 2007 software (Excel Technology, China). The data were analyzed by ANOVA for analysis of variance. Comparison of means were performed using Duncan's multiple range test ($p \leq 0.05$).

Result

Effects of different nitrogen levels on chlorophyll content of *Eucommia ulmoides* seedlings under saline stress: The change trend of the mean leaf pigment contents of chlorophyll a, chlorophyll b and carotenoid was basically the same, showing a trend of first increasing and then decreasing under three different degrees of saline stress. According to the analysis of variance, the chlorophyll a content in leaves treated with 5.0 mmol·L^{-1} nitrogen concentration was higher than the other three levels, and there was no significant difference between the chlorophyll a content and 10.0 mmol·L^{-1} nitrogen concentration ($p<0.05$), but it was significantly higher than that of 0.1 mmol·L^{-1} and 0.5 mmol·L^{-1} (Fig. 1). On the other hand, the chlorophyll a content was inversely proportional to NaCl concentration at any nitrogen level. The chlorophyll a content was the highest when the concentration of nitrogen was 5.0 mmol·L^{-1} under the control condition, up to 17.689 mg· g^{-1}, while the content of chlorophyll a was the lowest when NaCl concentration was 300 mmol·L^{-1} and the concentration of nitrogen was 0.1 mmol·L^{-1}, which was only 11.578 mg·g^{-1}.

The chlorophyll b content in leaves was the lowest under 0.1 mmol·L^{-1} nitrogen concentration under different saline stress (Fig. 2). It increased significantly under 0.5 mmol·L^{-1} and 5.0 mmol·L^{-1} nitrogen treatments. The chlorophyll b content reached the highest at 5.0 mmol·L^{-1}, and then began to decrease. Irrigation water salinity decreased chlorophyll b contents in the *E. ulmoides* seedlings, resulting in 17.09%, 18.67%, 33.59% and 31.69% reduction of chlorophyll content corresponding to the four nitrogen concentration treatments when salinity increased to 300 mmol·L^{-1}, respectively. In addition, the values of chlorophyll b content was the highest when the concentration of nitrogen was 5.0 mmol·L^{-1} under the control condition, which was as high as 8.347 mg· g^{-1}, and was the lowest under the condition of 300 mmol·L^{-1} NaCl concentration and 0.1 mmol·L^{-1} nitrogen concentration, only 4.345 mg g^{-1}.

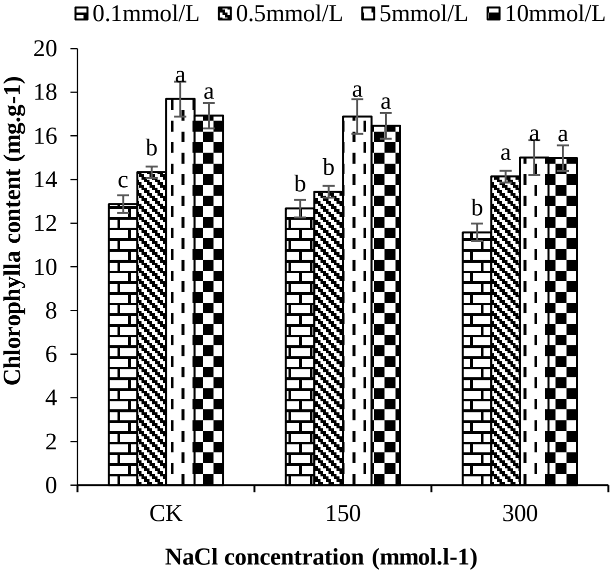

Fig. 1. Effects of different nitrogen levels on chlorophyll a content of *Eucommia ulmoides* Oliv. seedlings under saline stress.

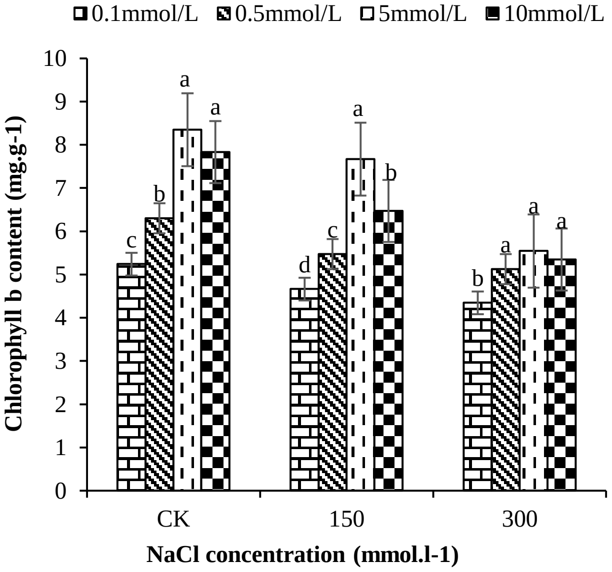

Fig. 2. Effects of different nitrogen levels on chlorophyll b content of *Eucommia ulmoides* Oliv. seedlings under saline stress.

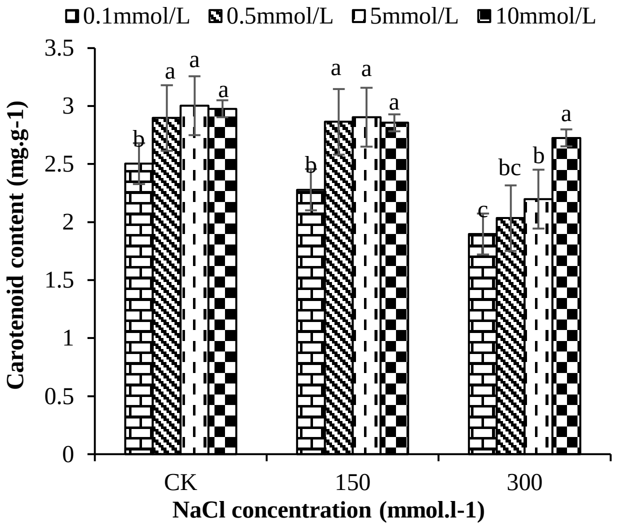

Fig. 3. Effects of different nitrogen levels on carotenoid content of *Eucommia ulmoides* Oliv. seedlings under saline stress.

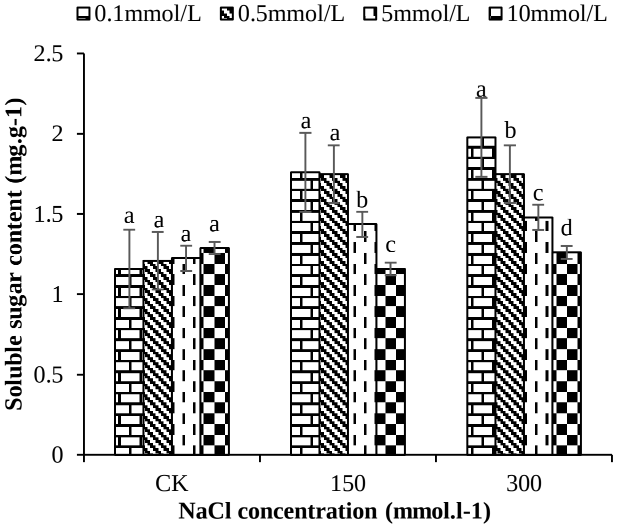

Fig. 4. Effects of different nitrogen levels on soluble sugar content of *Eucommia ulmoides* Oliv. seedlings under saline stress.

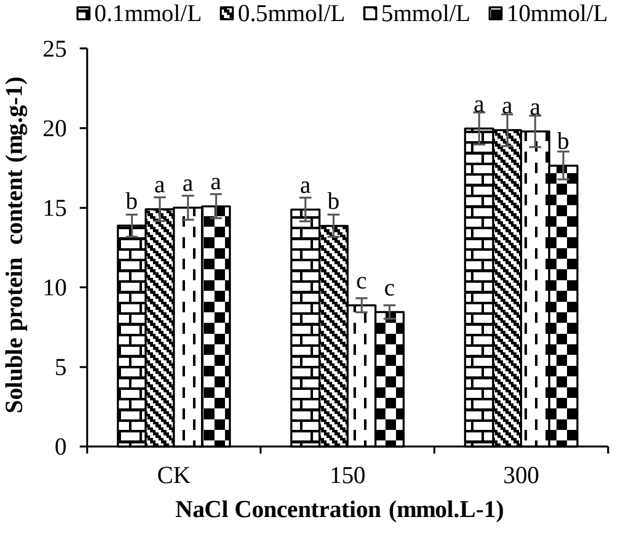

Fig. 5. Effects of different nitrogen levels on soluble protein content of *Eucommia ulmoides* Oliv. seedlings under saline stress.

Leaves of *E. ulmoides* seedlings treated with nitrogen concentration of 5.0 mmol·L^{-1} under different saline stress had the highest carotenoid content, with no significant difference between 0.5 mmol·L^{-1} and 10.0 mmol·L^{-1} nitrogen levels ($p<0.05$), but both were significantly higher than 0.1 mmol·L^{-1} nitrogen levels (Fig. 3). When NaCl concentration was 300 mmol·L^{-1}, the carotenoid content gradually increased with increasing nitrogen concentration. Regardless of the nitrogen level, the concentration of carotenoids was inversely proportional to NaCl concentration at different nitrogen levels. Among them, under the control condition with nitrogen concentration of 5.0 mmol·L^{-1}, the content of carotenoids was the highest, up to 3.003mgg^{-1}. Under the condition of 300 mmol·L^{-1} NaCl concentration and 0.1 mmol·L^{-1} nitrogen concentration, the content of carotenoids in *E. ulmoides* seedlings was the lowest, only 1.897 mg·g^{-1}.

Effects of different nitrogen levels on soluble sugar content of *Eucommia ulmoides* Oliv. seedlings under saline stress: Under the control conditions, the soluble sugar content in leaves of *E. ulmoides* seedlings was basically the same (Fig. 4). Under saline stress of 150 mmol·L^{-1} and 300 mmol·L^{-1}, the soluble sugar content gradually declined with increasing nitrogen concentration. Except for the control, the soluble sugar content with nitrogen concentration of 0.1 mmol·L^{-1} was the highest under saline stress, which was significantly higher than the other three levels ($p<0.05$). Among them, the soluble sugar content was the highest when NaCl concentration was 300 mmol·L^{-1} and nitrogen concentration was 0.1 mmol·L^{-1}, which was as high as 1.978 mg· g^{-1}. Under the control condition, the soluble sugar content was the lowest when nitrogen concentration was 0.1 mmol·L^{-1}, which was only 1.257 mg· g^{-1}. At any nitrogen level, the soluble sugar content in *Eucommia folium* was proportional to NaCl concentration.

Effects of different nitrogen levels on soluble protein content of *Eucommia ulmoides* Oliv. seedlings under saline stress: As shown in Fig. 5, under the control conditions, the change trend of soluble protein content in *E. ulmoides* seedlings leaves was in direct proportion to nitrogen concentration. The soluble protein content at 0.5 mmol·L^{-1}, 5.0 mmol·L^{-1} and 10mmol·L^{-1} nitrogen levels had little difference ($p<0.05$), but it was significantly different from that of 0.1 mmol·L^{-1}.When NaCl concentration was 150 mmol·L^{-1} and 300 mmol·L^{-1}, the soluble protein content decreased gradually with increasing of nitrogen concentration. Among them, the content of soluble protein in *E. ulmoides* seedlings was the highest at NaCl concentration of 300 mmol·L^{-1} and nitrogen concentration of 0.1 mmol·L^{-1}, reaching 19.98 mg· g^{-1}, and there was little difference between the soluble protein content corresponding to 0.5 mmol·L^{-1} nitrogen concentration and 5.0 mmol·L^{-1} nitrogen concentration under the same concentration of saline stress. The soluble protein content in *Eucommia folium* was the lowest when NaCl concentration was 150 mmol·L^{-1} and nitrogen concentration was 10 mmol·L^{-1}, which was only 8.457 mg·g^{-1}.

Discussion

Plant photosynthesis is the power source of life activities. Chlorophyll, as the main pigment of plant photosynthesis, can indirectly reflect the strength of photosynthesis. According to Figures 1-3, the content of chlorophyll in the *Eucommia folium* after saline stress treatment decreased, which is consistent with the conclusion of Qin Jing *et al.* (2009). Under salt stress, the significant decrease of chlorophyll content in leaves of *Eucommia ulmoides* seedlings is closely related to the increase of salt ions, which is mainly due to the loosening of chlorophyll-chloroplast protein binding and the destruction of more chlorophyll, leading to the decrease of photosynthesis (Zhao, 1993).Especially after excessive Cl-infiltration into cells, protoplasm agglomerates and chlorophyll is destroyed (Xu *et al.*, 2002).

Improving the photosynthetic capacity of plants is one of the effective ways to alleviate the growth inhibition of plants under saline stress. As an important component of chlorophyll, nitrogen content is affected by nitrogen (Amini *et al.*, 2019). Appropriate application of nitrogen is conducive to improving the chlorophyll content in leaves (Duan *et al.*, 2015), which in turn affects the photosynthesis of plants. Previous studies have shown a significant positive correlation between leaf nitrogen content and leaf photosynthetic capacity. The application of nitrogen fertilizer increased the chlorophyll content and accelerated the photoreaction (Pan & Dong, 1995). The results showed that chlorophyll content in *E. ulmoides* seedlings decreased after increasing as nitrogen concentration increasing under saline stress. This indicates that applying a certain concentration of nitrogen can alleviate the saline stress of *E. ulmoides* seedlings, but excessive application of nitrogen is not conducive to the accumulation of chlorophyll in *Eucommia folium*, which is aligned with the results of Ning, (2005). In the process of saline stress, Na^+ and Cl^- absorbed by *E. ulmoides* seedlings may exchange with applied nitrogen (including NH_4^+ and NO_3^-), thereby reducing the toxic effects of Na^+ and Cl^- in plants. If the nitrogen concentration is too high, the excess nitrogen will accumulate in the plant, and may react with other substances in the matrix to form ammonia salt. On the contrary, it will aggravate the adverse environment's influence on *E. ulmoides* seedlings and reduce the chlorophyll content.

In summary, chlorophyll content in *Eucommia folium* were reduced to varying degrees under saline stress, while the application of a certain concentration of nitrogen would alleviate stress symptoms. The results of this study showed that under saline stress, when the nitrogen concentration was increased to 5.0 $mmol \cdot L^{-1}$, the chlorophyll content in the *Eucommia folium* increased significantly compared with that under low nitrogen (0.1 $mmol \cdot L^{-1}$), indicating that increasing the nitrogen concentration under saline stress could promote the growth of *E. ulmoides*.

With the gradual aggravation of stress, the concentration of solution in the matrix will increase, which will eventually exceed the concentration of cellsap in *E. ulmoides* seedlings, leading to cell dehydration and death. In order to alleviate this situation, under saline stress, some organic compounds are often synthesized in plant cytoplasm to increase the osmotic pressure of vacuole membranes. Soluble sugars and soluble proteins are important osmotic regulators in plants，and are the carbon scaffolds and energy sources for the synthesis of other organic solutes (Zhang & Zhao, 1998). They also stabilize cell membranes and protoplasmic colloids, and protect enzymes when the content of inorganic ions in cells is high (Chen *et al.*, 2001). In addition, the increase of soluble sugar content plays an important role in improving cellsap concentration, reducing cell water potential and enhancing water absorption function under adverse conditions. According to Fig. 4 and Fig. 5, with the increase of NaCl concentration, soluble sugar and soluble protein content in *Eucommia folium* increased, which indicated that the osmotic regulation substances might accumulate rapidly after saline stress to coordinate osmotic pressure between cells and the outside world, to protect the surface of cell membranes, and thus resist salt damage, which was in Skeff. Ingeon and others have basically the same view (Skeffingeon & Jeffrey, 1998). Therefore, the higher the salt concentration, the higher soluble sugar and soluble protein content in *Eucommia folium*. Under the control conditions, soluble sugar and soluble protein contents increased with the increase of nitrogen concentration. Although not significant, it further indicated that ammonia salt produced by nitrogen accumulation exerted a slight stress effect on *E. ulmoides* seedlings when nitrogen was applied alone. Under saline stress of 150 $mmol \cdot L^{-1}$ and 300 $mmol \cdot L^{-1}$, soluble sugar and soluble protein contents in *Eucommia folium* decreased gradually with the increase of nitrogen concentration. This indicated that the increase of nitrogen could significantly reduce the toxicity of saline stress on *E. ulmoides* seedlings.

Conclusion

The increment in soil salinity slowed the growth and contents of chlorophyll a, chlorophyll b and carotenoids, and increased the content of osmotic adjustment substance. However, appropriate increments in N dose attenuate the deleterious effects of salt on *Eucommia ulmoides* Oliv.. In the case of nitrogen alone, the ammonia salt produced by nitrogen accumulation has a slight stress effect on *Eucommia ulmoides* Oliv. seedlings.

Acknowledgments

This study was funded by Tianjing Science and Technology Project (17JCYBJC29800 and 16PTZS TG00020), Tianjin Jinnan District Science and Technology Project (201802055) and Tianjin Binhai New District Science and Technology Project (BHXQ KJXM-SF-2018-33).

References

Amini, S., M. Kafi and M. Parsa. 2019. Effects of macronutrient supplementation on physiological, qualitative and quantitative traits of *Salvia leriifolia* Benth. *Pak. J. Bot.*, 51(6): 2053-2058.

Attiwill, P.M. and M.A. Adams.1993. Nutrient cycling in forests, *New Phytol.*, 124: 561-582.

Cao, B., J. Wang and Q.X. Ji. 2012. Effect of salt stress on seedling growth of *Lingwu Long* Jujube under different nitrogen applying levels. *J. Northwest Forest. Uni.*, 27(2): 34-38.

Chen, S.L., K.J. Li, W.F. Bi and S.S. Wang. 2001. Genotypic variation in accumulation of salt ions, betaine and sugars in poplar under conditions of salt stress. *Chinese Bull. Bot.*, 5: 587-596.

Dang, K.K., J. Zhang, W.T. He, Z. Cao and X.X. Zhao. 2017. Effects of fertilization on nutrient absorption, yield and quality of oil sunflower in saline soil. *Jiangsu Agri. Sci.*, 45(10): 70-76.

Du, H.Y. 2014. *China Eucommia Pictorial.* China Forestry Publishing House, Beijing.

Duan, Y.J., K.C. Wang, L.H. Niu, K. Li and Y.Y. Su. 2015. Effect of different nitrogen forms and ratio on growth and active ingredient content of *Platycodon grandiflorum. China J. Chinese Mat. Med.,* 40(19): 3754-3759.

Freire, J.L.de O. 2011. Clorofila e carotenoides em maracujazeiro-amarelo irrigado com águas salinas no solo com biofertilizante bovino. *Revista Brasileira de Fruticultura*, 33: 699-705.

Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz. 2003. Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress, *Braz. J. Plant Physiol.*, 15: 113-118.

Li, H.S. 2000. *Plant Physiology and Biochemistry Experiments: Principle and Technology.* Higher Education Press, Beijing.

Li, W.W., X.D. Ding, Q. Liu, D.L. Zhang, L.Y. Li and S.R. Zhang. 2018. Effect of nitrate on growth and osmotic regulation of sweet potato under salt stress. *Chinese J. Trop. Crops*, 39(1): 6-12.

Liu, A.R., Y.B. Zhang, J.F. Wang, M. Jiao and X.M. Liu. 2013. Appropriate nitrogen fertilizer strengthens growth and antioxidative ability of *Festuca arundinacea* under salt stress. *Transactions of the Chinese Society of Agricultural Engineering*, 29(15): 126-135.

Ning, J.F. 2005. Effects of nitrogen on growth and physiological characteristics of *Aloe Vera* under salt stress. Nanjing: Nanjing Agricultural University, 29-32.

Pan, R.Z. and Y.D. Dong. 1995. *Plant Physiology*. Vol: 3. Higher Education Press, Beijing,

Qin, J., W.Y. Dong, K.N. He, J. Chen, Y. Yu and Z.L. Wang. 2009. Effects of salt stress on growth and photosynthetic physiological features of *Hippophae rhamnoides* seedlings, *Ecol. & Environ. Sci.*, 18(3): 1031-1036.

Silva, E.C., R.J. Nogueira and F.P. Raujo. 2008. Physiological responses to salt stress in young umbu plants. *Environ. & Exp. Bot.,* 63: 147-157.

Skeffingeon, M.J.S. and D.W. Jeffrey. 1998. Response of America maritime (MILL) Willd and *Plantago martima* L. from an Irish salt marsh to nitrogen and salinity. *N. Phytol.*, 110: 3391.

Sui, L., J.N. Yi, K.C. Wang and Y.Q. Li. 2018. Effects of different forms and ratios of nitrogen on physiological characteristics of *Perilla frutescens* (L.) Britt under salt stress. *Chinese J. Ecol.,* 37(11): 3277-3283.

Wang, J.H., L.Y. Du and X.G. Lu. 2001. lndustrialization of the high tech products of *Eucommia ulmoides* Oliv., *Non-wood Forest Res.*, 19(2): 18-21.

Wang, Q., L. Xiao, X.Y. Tang, Q. Xu, H.P. Yi and H.F. Tian. 2015. Effects of saline alkali stress and nitrogen supply on germination and seedling growth of *Suaeda salsa. Acta Prataculturae Sinica*, 24(9): 216-222.

Wu, J.B. 2015. Effects of fertilization and salt stress on growth and physiology of *Eucommia ulmoides* seedlings. Changsha: Central South University of Forestry and Technology, 38-44.

Xie, X.H., L.X. Li and M. Chen. 2018. Effects of different nitrogen levels on salt tolerance of *Elaeagnus angustifolia* seedlings. *J. Shandong Normal Uni. (Natural Science),* 33(3): 309-315.

Xu, X., S.H. Li, H.X. Hui and H.L. Mi. 2002. Effect of NaCl stress on growth, chlorophyll content and K^+, Na^+ absorption of spring wheat seedlings. *Acta Botanica Boreali-Occidentalia Sinica*, 2: 70-76.

Yancey, P.H. 1994. *Compatible and Counteracting Solutes*. In: Cellular and Molecular Physiology of Cell. Volume. CRC Press: Boca Raton, FL.

Yemm, E.W. and A. J. Willis. 1954. The estimation of carbohydrate in plant extracts by anthrone. *Biochem. J.*, 57: 508-514.

Yu, X.L., R.Y. Zhu and X.M. Sun. 1992. Analysis of nutritional components of *Eucommia ulmoides* bark and leaf. *Chinese Trad. & Herbal Drugs*, 23(3): 161.

Zhang, H.Y. and K.F. Zhao. 1998. Effects of salt and water stress on osmotic regulation of Suaeda salsa seedlings. *Acta Botanica Sinica*, 1:61-66.

Zhang, M.Y. 2000. Study on the response of *Eucommia ulmoides* to salt stress. Lanzhou: Gansu Agricultural University.

Zhao, K.F. 1993. *Salt-tolerant Physiology of Plants.* Science and Technology of China Press, Beijing.

Zhu, H. and X.R. Guo. 2015. Effects of exogenously supplied nitrogen on growth and nitrogen metabolism of Periwinkle (*Catharanthus roseus* (L.) G. Don) under salt stress. *J. Northeast Forest. Uni.*, 43(7): 59-62.

Zou, F.G., W.Q. Guo, Y.H. Wang, W.Q. Zhao and Z.G. Zhou. 2015. Effects of nitrogen application rate on the nitrogen uptake and utilization of cotton grown in coastal saline fields of Yangtze River Valley. *J. Plant Nutr. & Fert.*, 21(5): 1150-115.

(Received for publication 8 August 2019)