Front. For. China 2009, 4(2): 227–235
DOI 10.1007/s11461-009-0023-5

RESEARCH ARTICLE

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Investigation of anti-salt stress on tetraploid *Robinia pseudoacacia*

Abstract Tetraploid *Robinia pseudoacacia* was used as a main test material and diploid *R. pseudoacacia* was used as the control. The indices of shape, physiology and biochemistry, photosynthesis and anatomic structure of the young plants were investigated under salt stress (NaCl and Na_2SO_4). The treatment time was 30 d with an interval time of 7 d. Before and after treatment, the indices were measured. Results show that: 1) the growth of diploid *R. pseudoacacia* inhibited an evident symptom of salt damage and the leaf moisture content was lower under salt stress than that of control. But the tetraploid *R. pseudoacacia* was contrary. 2) The relative electric conductivity and proline (Pro) of tetraploid *R. pseudoacacia* increased slightly and had no significant difference compared with its control, which was contrary to diploid *R. pseudoacacia*. At the same time, three protective enzymes including perocidase (POD), superoxide (SOD) and catalase (CAT) kept higher activities at a post stage of salt stress to tetraploid *R. pseudoacacia*, which enhanced its anti-salt characteristics. Diploid *R. pseudoacacia* was sensitive to salt and had contrary information. 3) Salt stress had little influence to photosynthesis of tetraploid *R. pseudoacacia*. The net photosynthetic rate (P_n) and intercellular CO_2 concentration (C_i) had no significant changes, but those of diploid *R. pseudoacacia* decreased singificantly. 4) After salt stress, the anatomic structure of tetraploid *R. pseudoacacia* had a positive reaction, including the palisade parenchyma of diachyma, was prolonged and arranged more tightly. The spongy parenchyma was shrunk and was arranged tightly, which was contrary with diploid *R. pseudoacacia*. These data demonstrate that tetraploid *R. pseudoacacia* had superior anti-salt performance.

Keywords tetraploid *Robinia pseudoacacia*, diploid *Robinia pseudoacacia*, salt stress, salt resistance

Translated from *Journal of Plant Ecology*, 2008, 32(3): 654–663 [译自: 植物生态学报]

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1 Introduction

When dissoluble salts are accumulated to a certain degree, the saline soil will be formed and can destroy agricultural ecosystem and affect environment. From the global perspective, any climatope is influenced by the saline soil to some extent, although the soil concentrates at arid and semi-arid regions (Hong and Eduardo, 2001; Pichu, 2006). The saline soil is distributed in an area of near 10^9 hectares and 100 countries and regions (Hong and Eduardo, 2001; Pichu, 2006). Especially in China, the proportion is higher than the average level in the world. Its area of saline soil is 1.7×10^8 hectares and 1/3 of irrigation soil had salination problems (Shi, 2004). Land source is increasingly deficient today, and saline soil, as a potential land source, is highly thought of by the public, governments and scientists. The plants that grow well in the saline soil are expected for excavation and then for achieving the purpose of soil amendment, development and utilization.

Tetraploid *Robinia pseudoacacia* (also named multiploid *R. pseudoacacia*) originated from the Republic of Korea and was introduced to China by the Beijing Forestry University in 1997 as a good tree species with large leaves, powerful photosynthetic capability, multivitamins and mineral matters, quick growth, salt resistance, fume and hypothermic resistances, high capability of absorbing harmful gas and rejecting pest (Ren, 2003; Tian and Li, 2003; Lu, 2004). At present, there was a trial planting in Henan, Hebei, Qinghai, Xizang, Ningxia, Gansu provinces and Qiqihaer of Heilongjiang Province, and had reached a certain range (Xu and Yang, 2006).

There are some research reports on tetraploid *R. pseudoacacia* concerning three aspects. The first is the introduction and cultivation. The parameters include adaptability, biomass and nutrition content of its leaves, which affirm its value of introduction. The second is the common and micro-breeding techniques, which have become mature and reached the request of scale production. The media to induce calli, differentiation and suitable transplanting style have been found. The third is genetic transformation of anti-salt genes. The gene of betaine-aldehyde dehydrogenase (BADH) has been transferred to the tetraploid *R. pseudoacacia* successfully and the transformed plants have been obtained (Li and Jiang, 2006), but the anti-salt mechanism has not been reported. Tetraploid *R. pseudoacacia* was used as a main test material and diploid *R. pseudoacacia* was used as the control. The indices of shape, physiology and biochemistry, photosynthesis and anatomic structure of their young plants were investigated under salt stress (NaCl and Na_2SO_4), which revealed the anti-salt mechanism from many points of view, provided a theoretical basis for the generalization and application of tetraploid *R. pseudoacacia* anddrew a reference for the study of anti-salt stress in plants.

2 Materials and methods

2.1 Experimental materials and treatments

The two-year-old tetraploid and diploid *R. pseudoacacia* were from the Beijing Forestry University, which were all planted in the same enviroment and whose shape, growth and size were basically uniform. They were managed using a common method during the growing period. The experiment was carried out in the greenhouse of the Northeast Forestry University and the time of field planting was on the 5th of March in 2006. The plastic culture pan was 21 cm × 21 cm and the medium of seeding soil was sandy soil, the percentage of which is 2∶1 of soil to sand. NaCl (300 mmol/L) and Na_2SO_4 (300 mmol/L) were prepared using Hoagland's growth-promoting medium. The period of salt treatment was 28 d. The salt stress was done after field planting of 30 d per 7 d and the process was replicated four times. The treatment combinations were tetraploid *R. pseudoacacia* under NaCl stress(SL), tetraploid *R. pseudoacacia* under Na_2SO_4 stress (SS), diploid *R. pseudoacacia* under NaCl stress (EL), diploid *R. pseudoacacia* under Na_2SO_4 stress (ES), and the controls were diploid *R. pseudoacacia* (CK1) and tetraploid *R. pseudoacacia* (CK2). The amount of sprinkling solution was 250 mL and 30 plants were used as one treatment and the arrangement was random arranged indoors.

2.2 Determination of items and methods

2.2.1 Determination of shape index

The height, width, ground diameter and lateral-branch diamter were determined per 7 d before and after salt stress, which usde the measuring-tape and sliding caliper, respectively. The mean of four replicates was used as the measured value. The amplification was the mean of the difference sum of the back and the front.

On Day 28, the chlorophyll content, green weight (FW), dry weight (DW) and leaf moisture content (MC) were determined. Determination of chlorophyll content employed the acetone method (Zhang and Zhai, 2003). Determination of FW and DW used a puncher of leaf at same region. The FW was measured immediately after collection. The leaves were removed green at 105°C for 15 min and dried at 60°C to a constant weight. MC = (FW–DW) / FW × 100%.

2.2.2 Determination of physiological and biochemical indicators

The physiological and biochemical indicators of each treatment were determined before and after salt stress per 7 d. The determination of relative electric conductivity (REC) used the conductimeter instrument (Zhang and Zhai, 2003), and dissociative pyrrolidinecarboxylic acid was measured using the ninhydrin method. Superoxide dismutase (SOD), peroxydase (POD) and catalase (CAT) were determined using the nitroblue tetrazolium (NBT) photoreduction method, the guaiacol method and ultraviolet spectrophotometry, respectively (Shanghai Plant Physiology Research Institute of Chinese Academy of Sciences and Shanghai Society for Plant Physiology, 2004).

2.2.3 Determination of photosynthetic indices

The net photosynthetic rate (P_n), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) of each treatment were determined before and after salt stress per 7 d at 9:00 using CIRAS-2 portable photosynthesis systems. The two or three functional leaves in the sunny side were collected and determined *in vivo* from horizontal angles. The density of light quantum is 900 μmol/($m^2 \cdot s$), the density of CO_2 is 360 μmol/mol and the temparature is 25±2°C.

2.2.4 Observation of anatomic structure on leaves

The observation of anatomic structure on leaves refered to the paraffin sectioning technique according to Zheng (1979).

2.2.5 Data processing

Data entry used EXCEL software and the correlation and gradual regressive analyses used SPSS12.0 software. The significance analysis used Denkens multiple comparison. The data of diagram were the mean of three repeats.

3 Results and analysis

3.1 Effects of different salt stresses on plant shape

3.1.1 Effects on the increasing amplitude of height, width, ground diameter and lateral-branch diamter

After different salt stresses, the shape index of two *R. pseudoacacia* had changes. Compared to CK1 and CK2, the increasing amplitude of height, width, ground diameter and lateral-branch diameter on the diploid *R. pseudoacacia* shows a descending tendency and reached a significant level, which desceded by 49.89%, 40.00%, 33.33% and 41.67% compared to CK1 and by 49.36%, 34.69%, 36.51% and 41.67% compared to CK2 after NaCl stress. They decreaseed to 48.89%, 56.52%, 58.33% and 47.22% compared to CK1 and by 68.42%, 60.0%, 60.31% and 47.22% compared to CK2 after Na_2SO_4 stress. From the above data and Fig. 1, the values of height, width and ground diameter achieved significant levels except for that of ateral-branch diamter, which indicated that the stress of Na_2SO_4 inhibited the plant growth more seriously than that of NaCl.

The plant growth of diploid *R. pseudoacacia* was inhibited seriously after salt stress, which had little influence to tetraploid *R. pseudoacacia*. The increasing amplitude of height, width, ground diameter and lateral-branch diameter of the tetraploid *R. pseudoacacia* had no significant difference under NaCl stress compared to CK1 and CK2, but the increasing amplitude of ground diameter and lateral-branch diameter under Na_2SO_4 stress show a descending tendency and had no significant difference compared to that of diploid *R. pseudoacaciay*. They decreased to 11.67% and 16.67% compared to CK1 and to 15.87% and 16.67% compared to CK2, respectively. All indices reached significant levels except for the increasing amplitude of lateral-branch diamter under two salt stresses, which indicated that different salt treatments had different impacts on the tetraploid *R. pseudoacacia*.

3.1.2 Effects on the chlorophyll content, FW, DW and MC of leaves

At Day 28, total chlorophyll content, FW, DW and MC of leaves were determined. From Table 1, every index of CK1 and CK2 had no significant difference. The total chlorophyll content of tetraploid *R. pseudoacacia* under two salt stresses had no significant difference compared to CK1 and CK2, which was contrary to that of diploid *R. pseudoacacia*. The significant difference existed between two salt stresses. Compared to CK1, CK2, the FW of two locusts' leaves under NaCl had no significant difference, which was contrary to tetraploid *R. pseudoacacia* under two salt stresses. That of diploid *R. pseudoacacia* had significant difference under two salt stresses. The DW of leaves had no significant difference in all treatments. The MC of leaves had no significant difference between CK1 and CK2. The MC of leaves of tetraploid *R. pseudoacacia* had no significant difference compared to CK1 and CK2, which was contrary to that of diploid *R. pseudoacacia*.

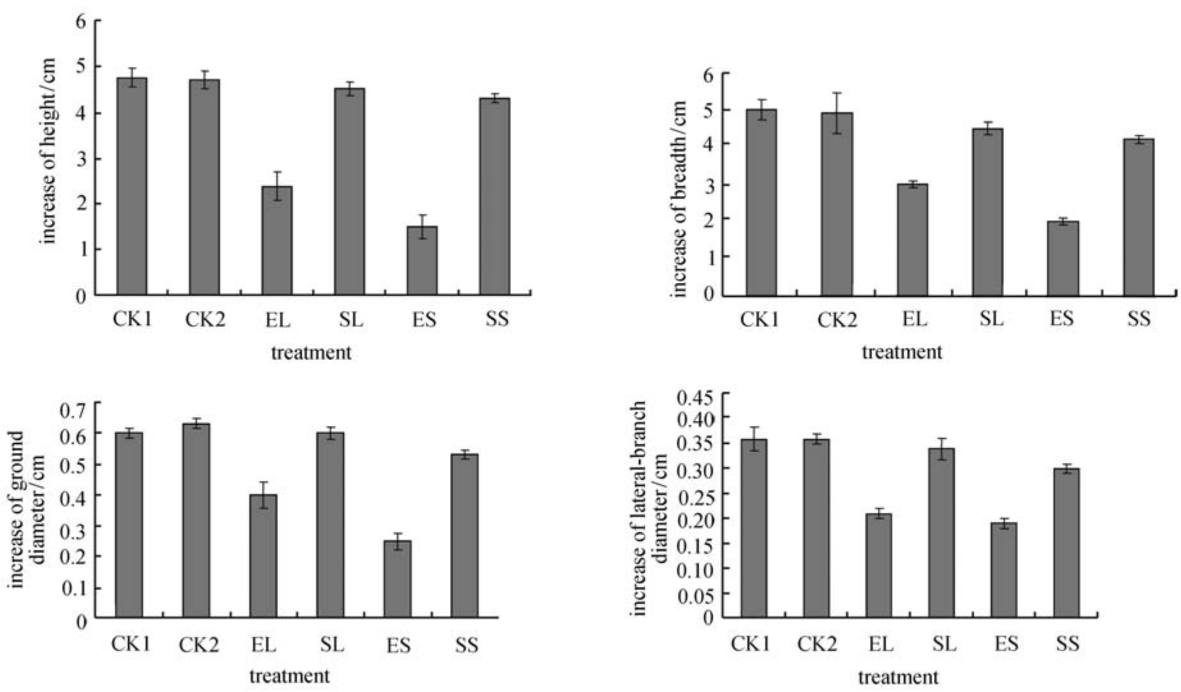

Fig. 1 Effects of salt stress on increasing amplitude of plant shape indices

Table 1 Comparison of tatal chlorophyll content, FW, DW and MC of leaves under salt stress

different treat	total chlorophyll content/(mg·g^{-1} FW)	FW/mg	DW/mg	MC/%
CK1	0.78±0.08a	0.26±0.01a	0.03±0.001a	88.46±0.02a
CK2	0.80±0.03a	0.22±0.01a	0.03±0.005a	86.36±0.06a
SS	0.72±0.05a	0.14±0.03b	0.02±0.002a	85.71±0.03a
SL	0.83±0.06a	0.18±0.02ab	0.03±0.001a	83.33±0.01a
EL	0.58±0.02b	0.25±0.04a	0.05±0.001a	80.00±0.04c
ES	0.35±0.02c	0.13±0.02b	0.03±0.002a	76.92±0.05b

Note: the value is a mean value±standard deviation. Different letters indicate different significance at $p < 0.05$.

3.1.3 Changes of plant shape under salt stress

After salt stress at Day 28, the leaves of the diploid *R. pseudoacacia* appeared etiolated, withered, curling, chlorotic, transparent and deciduous. At the same time, parts of the branches had fallen off. The degree of injury of its leaves under Na_2SO_4 stress was more serious. These symptoms did not appeared in tetraploid *R. pseudoacacia* under salt stress. Its leaf color appeared emerald green except that the apices of parts turned yellow, and had no symptoms of salt damage (Fig. 2).

3.2 Effects on physiological and biochemical indicators

3.2.1 Effects on cell membrane permeability

The REC of two loci show a gradually increasing tendency after NaCl and Na_2SO_4 stresses (Fig. 3). The REC of diploid *R. pseudoacacia* increased significantly at Day 14 and achieved the highest at Day 28, which was elevated by 95.65% and 87.50% significantly compared to CK1 and CK2 under Na_2SO_4 stress. The REC of diploid *R. pseudoacacia* increased significantly at Day 21 and also achieved the highest at Day 28, which improved significantly by 94.30% and 45.45% compared to CK1 and CK2 under NaCl stress. The REC of tetraploid *R. pseudoacacia* kept stable during treatmeat and did not achieve the level of significance compared to the control. The sequence of the REC at Day 28 d was ES > EL > SS > SL > CK2 and CK2 from big to small. The REC of CK1 and CK2 was kept stable during the process.

The content of Pro on two locusts indicated a continuously increasing tendency under salt stress, but that of diploid *R. pseudoacacia* rose rapidly and that of tetraploid *R. pseudoacacia* rose slowly. That of diploid *R. pseudoacacia* under Na_2SO_4 stress was higher than that under NaCl stress, and the difference was significant. It significantly increased at Day 7, and increased by 40.46%

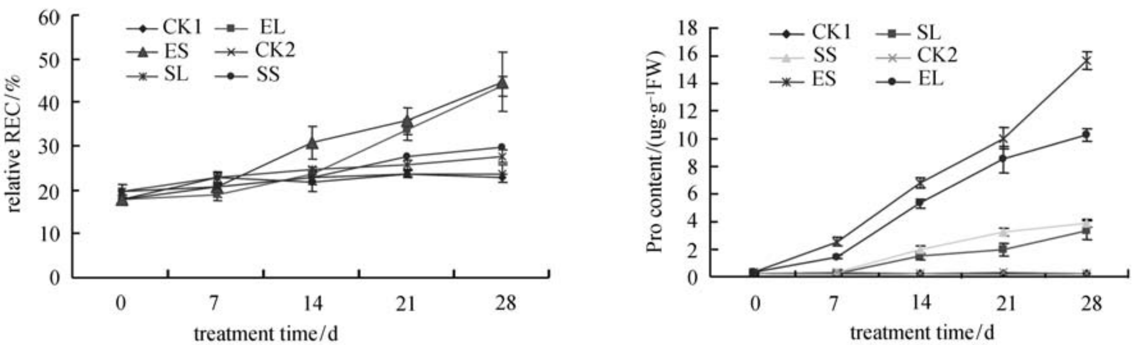

Fig. 2 Effects of salt stress on REC and Pro content of leaves

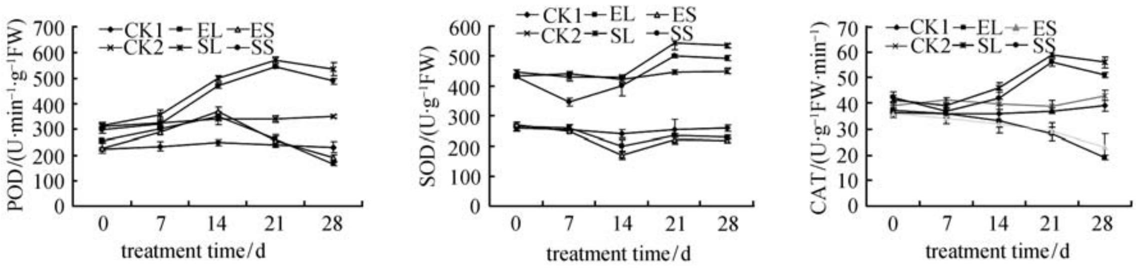

Fig. 3 Effects of POD, SOD, CAT activities on leaves under salt stress

and 27.11% compared to CK1 and CK2 at Day 28. The content of Pro on tetraploid *R. pseudoacacia* increased under two salt stresses, but the initiation time was delayed. It started to increase at Day 14 and its extent was little. It was 9.67% and 11.19% compared to CK1 and CK2, which kept stable (Fig. 3).

3.2.2 Effects on protective enzymes of leaves

During the treatment, the activity of POD on CK2 was significantly higher than that of CK1, which indicated that tetraploid *R. pseudoacacia* had a powerful system of protective enzymes. Two controls kept stable. Two loci offered the tendency of upgrading first and then descending with the extension of salt stress, which indicated that the salt stress could influence the metabolism of the plant. The activity of POD on tetraploid *R. pseudoacacia* increased significantly, which elevated by 136.51% and 125.31% compared to CK1, and by 67.64% and 59.71% compared to CK2 under two salt stresses at Day 21. The activity of POD on diploid *R. pseudoacacia* achieved the highest value at Day 14, and elevated by 29.34% and 41.53% compared to CK1, by 3.23% and 8.53% compared to CK2, whose extent was smaller than that of tetraploid *R. pseudoacacia*. After Day 14, it had a descending tendency and decreased by 28.57% and 17.75% compared to CK1, and by 52.57% and 39.71% compared to CK2. The analysis of variance indicated that POD had high activity compared to that before treatment, which achieved the level of significance. Diploid *R. pseudoacacia* had contrary results (Fig. 4).

The activity of SOD on CK2 was higher than that of CK1, which indicated that tetraploid *R. pseudoacacia* had a powerful protective enzyme system. The two controls were kept stable. Two loci first increased and then decreased. The activity of SOD of tetraploid *R. pseudoacacia* achieved the lowest at Day 7, descending by 2.27% and 21.25% compared to CK2 under Na_2SO_4 and NaCl. Under Na_2SO_4, it achieved the lowest compared to the controls, and then increased rapidly and reached to the highest at Day 21, elevating significantly by 12.35% and 21.79% compared to CK2, and decreased slowly at Day 28. Under NaCl stress, compared to CK1 the activity on SOD of diploid *R. pseudoacacia*, it achieved the lowest at Day 14 and the highest at Day 21, elevating significantly by 31.22% and 22.52%, and decreasing by 7.69% and 15.38% at 28 d compared to CK1. The impacts of different salts to SOD indicated that the two loci had the characteristics that the decreased extent under Na_2SO_4 was larger than that under NaCl, which may be the reason that different salts had different toxic mechanism and induced different extents of toxicity to the plant (Fig. 4).

The activity of CAT on CK2 was higher than that of CK1 significantly, which also indicated that tetraploid *R. pseudoacacia* had a powerful protective enzyme system. The two controls were kept stable. Under two salt stresses, the activity of CAT on tetraploid *R. pseudoacacia* offered a decreasing tendency at Day 7 (initial stage), then increased

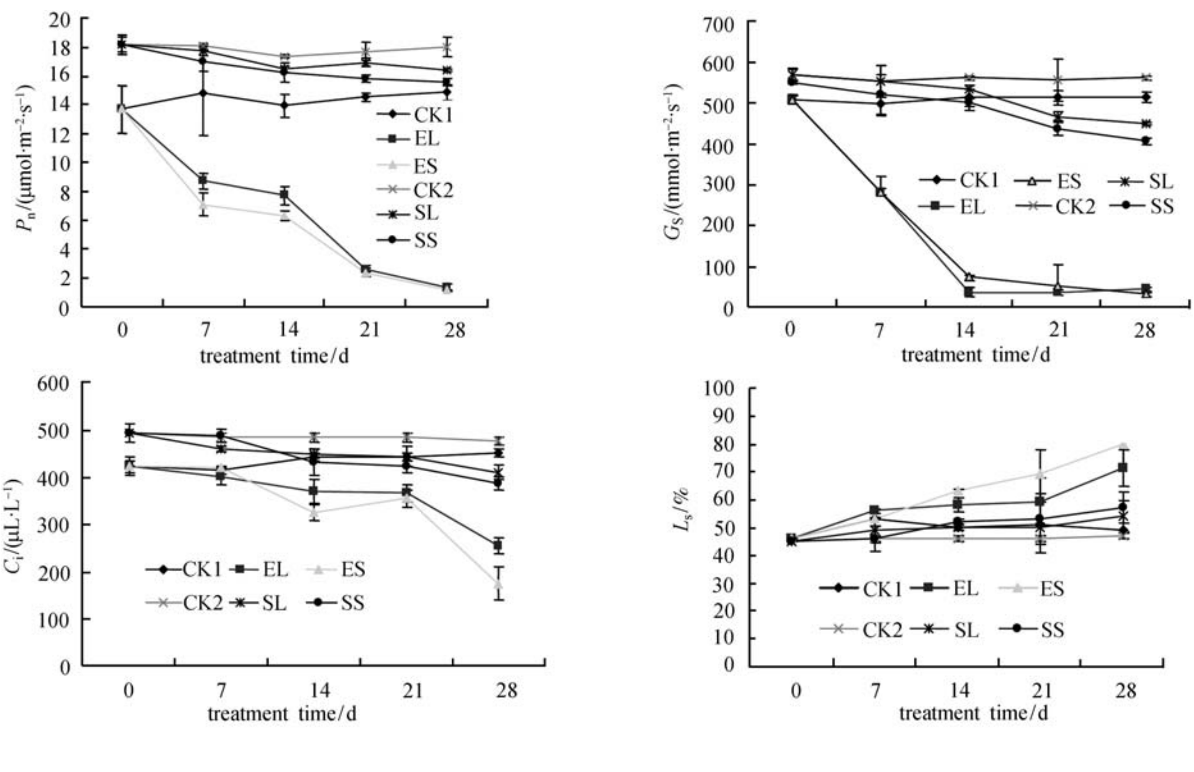

Fig. 4 Effects of P_n, G_i, G_s and L_s under salt stress

suddenly and reached the highest at Day 21. It descended at Day 28 and elevated significantly by 30.23% and 18.60% compared to CK2. Compared to the tetraploid *R. pseudoacacia*, the activity of CAT on the diploid *R. pseudoacacia* offered a decreasing tendency and reached the lowest at Day 28, decreasing by 51.28% and 41.03% compared to CK1, and by 55.81% and 46.51% compared to CK2, whose difference was significant. The upgraded extent of the activity of CAT under NaCl was higher than that under Na_2SO_4 to tetraploid *R. pseudoacacia*, in which the damage of Na_2SO_4 to tetraploid *R. pseudoacacia* was more serious than that of NaCl. Diploid *R. pseudoacacia* had the contrary tendency, which indicated that the damage extent of salt to plant was involved with salt and plant variety (Fig. 4).

3.3 Effects on photosynthetic characteristics

Under salt stress, the value of P_n and G_s of leaves on diploid *R. pseudoacacia* show a decreasing tendency. At Day 28, the value of P_n achieved the lowest under NaCl and Na_2SO_4, decreasing by 90.82% and 92.19% compared to CK1 and by 93.56% and 98.00% compared to CK2. The value of G_s decreased by 91.40% and 93.76% compared to CK1, and by 92.14% and 94.29% compared to CK2. CK1 and CK2 kept stable. The value of P_n and G_s of leaves on tetraploid *Robinia pseudoacacia* decreased compared to CK2 and their amplitude was not large but higher than CK2. At Day 28, under two salts stresses, the value of P_n decreased by 10.36% and 4.51% compared to CK1 and by 8.89% and 13.72% compared to CK2, the value of G_s decreased by 12.67% and 21.05% compared to CK1, and by 20.00% and 27.68% compared to CK2. The above analysis indicates that salt stress induced the decrease of the value of P_n and G_s, which had little impact on tetraploid *R. pseudoacacia*. At the same time, different salt stresses had different effects on the plant. The stress of Na_2SO_4 was more serious than that of NaCl. Under salt stress, except for the control, the value of C_i for every treatment shows a decreasing-descending-decreasing tendency. The value of L_s had a contrary tendency. The value of C_i descended at Day 14 at first, then decreased at Day 28 again, but the value of L_s had the contrary tendency. The descended extent of C_i was the largest under salt stress and the increased extent of L_s was the largest. The extent of C_i and L_s had changed little compared to the control.

3.4 Effects on anatomic structure of leaves

The anatomic structure of leaves on the locus includes epidermis, diachyma and leaf vein. The epidermis has stomas. Diachyma includes palisade parenchyma and spongy parenchyma. Under NaCl stress, the diploid *R. pseudoacacia* had such characteristics as: the fibrosis appeared in the spongy parenchyma, no integrated structure, increasing blank, shorten and puff palisade parenchyma (Fig. 5b), the percentage of palisade parenchyma diminished compared to CK1. Under Na_2SO_4 stress, the palisade parenchyma was shortened markedly and a puffy disposition appeared. The spongy parenchyma was damaged seriously and an increasing blank and fuzzy cellular structure appeared. Under NaCl stress, the tetraploid *R. pseudoacacia* had contrary results compared to the diploid *R. pseudoacacia* (Fig. 5a). Under Na_2SO_4 stress, the palisade parenchyma of tetraploid *R. pseudoacacia* was not prolonged and was arranged tightly, and the spongy parenchyma of which had cells that were fairly little comapred to the diploid *R. pseudoacacia*. We

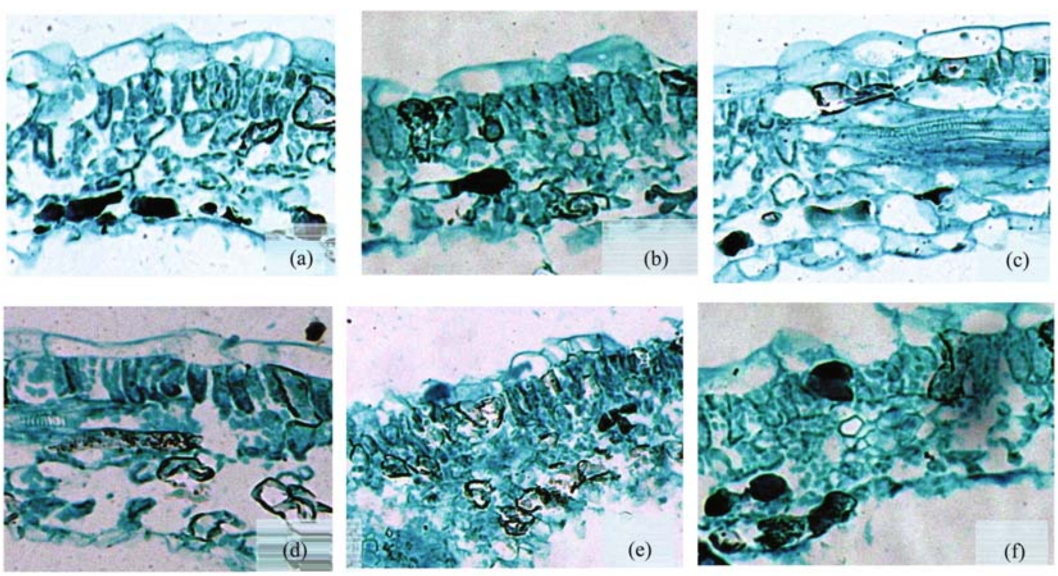

Fig. 5 Anatomic structure of leaf on CK1 (a), CK2 (b), EL (d), ES (c), SL (e), SS (f) (20×)

considered that little and more layers of diachyma, and the prolonged and close palisade parenchyma are beneficial to elevate the adaptability of the locus to adversity.

4 Discussion

4.1 Effects of different salts on plant

Different salts have different effects on the change of the physio-biochemical response and anatomic structure for the same plant, which was reported in *Prunus persica* (Hou et al., 2005), *Agropyron elongatum* (Huang et al., 2007), *Artemisia halodendron* (Shi et al., 2007), *Suaeda salsa* and *Atriplex centralasiatiallijin* (Duan et al., 2003; Li et al., 2005). In this study, the locus had serious damage under Na_2SO_4 stress, especially for diploid *R. pseudoacacia*, which was mainly manifested by the membrane permeability of cells. Parts of protective enzyme, photosynthetic characteristics and anatomic structure of leaves also had significant changes. So, mere consideration of single salt-stress effect is not enough and the effects of multi-salts are needed to identify the salt resistance of the plant. The main saline components in saline soil are Na_2SO_4 and NaCl (Wang et al., 2005). Their effects should be comprehensively considered. Which salt has more serious damage had not be reported before. In this study, the damage of Na_2SO_4 was more serious, which is consistent with study of peach and but contrary to some studies of *Artemisia halodendron*, *Suaeda salsa* and *Atriplex centralasiatiallijin* (Duan et al., 2003; Li et al., 2005; Huang et al., 2007).

At present, the reseach of anti-salt stress on forests was confined to some tree species, including *Poplus euphratica* and *P. tomentosa* (Lin et al., 2002; Wang et al., 2005; Dai et al., 2006) and little has been done on other tree species. NaCl was used in most salt stress studies, which limits the evaluation of salts to anti-salt tree species.

4.2 Effects of salt stress on plant morphology

Salt stress had significant effects on the morphogenesis of plants, featured by inhibiting the growth and differentiation of organs of plant and delaying the growth process (Qiu et al., 2006). Therefore, shape indices were important to determine salt resistance of plants. These indices included height, width, dry and fresh weight of leaves, dry weight of the whole plant, chlorophyll content, leaf shape and leaf color (Asada et al., 1997; Guo et al., 2003; Li et al., 2007; Wang et al., 2007). Chlorophyll, where photosynthesis happens, is the main substance absorbing and transmitting energy in the photosynthetic process. Low chlorophyll content will result in serious damage to plants by weakening the capability of photosynthesis. With the increase of salinity, the water content of leaves will decrease under salt stress, which indicted that the cells of leaves will lose water and will then wilt, influencing the physiological functions of leaves. So the water content of leaves reflected not only the water regime but also the resisting capability (Li et al., 2007). In this study, under NaCl and Na_2SO_4, the total chlorophyll content of tetraploid *R. pseudoacacia* had significant differences compared to the control, which was contrary to that of diploid *R. pseudoacacia*. According to the water content of the leaves, we found that tetraploid *R. pseudoacacia* had salt resistance. The dry weight and the fresh weight cannot measure whether tetraploid *R. pseudoacacia* had salt resistance. The reason needs further research to be clarified.

The main objectives of intensive studies on tetraploid *R. pseudoacacia* are to improve large areas of saline land and make it a fine animal-feeding species, which requires tetraploid *R. pseudoacacia* to be not only salt-resistant, but fast-growing as well. The changes of morphological indices including height, width, ground diameter and lateral-branch diamter of tetraploid *R. pseudoacacia* were investigated. The results show that the growth of diploid *R. pseudoacacia* was inhibted under salt, which was contrary to that of tetraploid *R. pseudoacacia*. So, tetraploid *R. pseudoacacia* had higher anti-salt ability and could keep vigorous life in enviroment of salt stress.

4.3 Changes of different physio-biocehmical indicators

Active oxygen (ROS) is the generic name of matters having oxygen which has active chemical properties and oxidizing capability. They include superoxide free radical (O_2^-), hydrogen dioxide hydrogen dioxide (H_2O_2) and hydroxy radical (^-OH). The level of ROS determines the level of lipid peroxidation (Asada, 1997; Sairam et al., 2002; Munns, 2002; Mittler, 2002). In normal conditions, the level of ROS has a low dynamic balance. It will accumulate when confronting adverse stresses, and then oxygenation happens between ROS and unsaturated fatty acids, which produce lipid peroxidation and damage integrity of membrane in structure and function. ROS can damage almost all living molecules including proteins, DNA and chlorophylls (Alscher et al., 1997; McCord, 2000). The accumulation of ROS and its peroxidation are the main mechanism of salt stress (Fadzilla et al., 1997). SOD, POD and CAT as important antioxidases have joint action with reduced glutathione (GSH), anti-ascorbic acid, α-tocopherol and carotenoid, which had the task of quenching ROS. SOD transforms O_2^- to H_2O_2 with dismutation. H_2O_2 can be formed to H_2O with enzymatic reaction of POD and CAT (Garratt et al., 2002). So many researches have made clear that the level of protective enzymes had high dependability to the characteristics of anti-salt characteristics on plants (Dionisio et al., 1998; Meneguzzo et al., 1999).

The activity of POD of diploid *R. pseudoacacia* decreased at Day 14 (initial stage), which can relieve the damage of ROS to some extent. The quick decay rate of

SOD explained that the protective enzyme was unstable. So, the peroxidation of membrane was serious, which induced ion exosmosis and then the higher level of REC and Pro. At Day 28 (later stage), POD decreased rapidly and achieved the lowest level. SOD increased a little, which could not eliminate the accumulation of ROS and induced more serious results. The decreasing tendency of CAT activity in diploid *R. pseudoacacia* induced the damage to the membranes. Compared to diploid *R. pseudoacacia*, the activity of POD increased significantly and had significant difference with its control, expecting that it had little decrease at Day 7. The activities of SOD and CAT were significantly higher than the control, which explained that the joint action of POD, SOD and CAT limited effictively the increase of ROS level and kept the balance of active oxygen in the cells which avoided the damage to the membrane and elevated the anti-adversity ability of diploid *R. pseudoacacia*.

The activity of CAT, as one of ROS, presented a decreasing-increasing-decreasing tendency, similar to that of POD and SOD of tetraploid *R. pseudoacacia* under NaCl and Na_2SO_4. The activity of CAT of diploid *R. pseudoacacia* decreased rapidly, which was diferent from that of POD and SOD. CAT, POD and SOD can eliminate the damage of the ROS to membrance, but the time and regularity are different, which is related to their generation pathway. Therefore, the changes of the three protective enzymes should be considered when evaluating the characteristics of the anti-salt ability of plants.

4.4 Changes of photosynthetic characteristics

The reason for decreasing photosynthesis under salt stress has not been clarified. Generally, the diosmosis stress is one of the reasons inhibiting photosynthesis, which can cause the loss of stomatal conductance, limit the access of CO_2 to photosynthetic organs and inhibit photosynthesis (Zhu et al., 1999).The stoma-related or stoma-irrelevant factors led to the decrease of photosynthetic rate of leaves according to the change of C_i and L_s. In the decrease of C_i and the increase of L_s, the stomas are the main factor, but in the increase of C_i and the decrease of L_s stomas are not the main factor (Farquhar and Sharkey, 1982). In this study, the value of P_n and G_s displayed a descending tendency and C_i first increased and then decreased, which was contrary to L_s with the extension of salt stress, indicating that stomas were the factor limiting the photosynthetic rate of leaves of diploid *R. pseudoacacia*. Every index of tetraploid *R. pseudoacacia* had significant changes, so it kept the powerful photosynthetic capability and salt resistance.

4.5 Changes of anatomic structure on leaves

Plants under salt stress can change their shape and internal structure to adapt to the stress environment. Some of plants can change their organ structure to adapt to the environment, but the structure of organs of plants which are sensitive to salt will be damaged (Xia et al., 2002; Li et al., 2003; Dong et al., 2005; Zuo et al., 2006). For example, the autoplast, mitochondrion and arrangement of cell will be influenced (Li et al., 2004; Wei et al., 2006). In this study, the NaCl stress influenced the palisade parenchyma and spongy parenchyma and the Na_2SO_4 stress influenced the arrangement of cells of spongy parenchyma to diploid *R. pseudoacacia*. The two salts had different damage extents to tetraploid *R. pseudoacacia*, which indicated that different salt stresses had different damage to the plants. This difference caused by Na^+, density of Cl^- or SO_4^{2-} needed further study.

Acknowledgements This work was supported by the Research Award Fund for Outstanding Young Teachers in the Northeast Forestry University (No. 602026) and the Heilongjiang Postdoctoral Sustrntation Fund, China (No. LBH-Z06146).

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