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EVALUATION OF SALT AND DROUGHT TOLERANCES OF *POPULUS TALASSICA* × *POPULUS EUPHRATICA* SEEDLINGS USING LEAF ANATOMICAL STRUCTURES AND PHYSIOLOGICAL PROCESSES

YANG SUN[1,2#], YING LIU[1,2#], MENG XU SU[1,2#], ZHAN JIANG HAN[1,2*] AND JIAN YIN SHI[3]

[1]College of Life Science and Technology, Tarim University, Alar, Xinjiang 843300, China
[2]Xinjiang Production and Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Alar, Xinjiang 843300, China
[3]General Forestry Management Station of Xinjiang Production and Construction Corps, Urumqi, Xinjiang 830013, China
[#]These authors contributed equally
[*]Corresponding author's email: hanzhanjiang126@126com

Abstract

The salt- and drought-tolerance levels of potted *Populus talassica* × *Populus euphratica* seedlings were studied under different NaCl concentrations and simulated natural drought conditions, with *P. euphratica* (male parent) and *Populus pruinosa* seedlings as controls. Compared with those of the controls, the leaves of *P. talassica* × *P. euphratica* began to thicken at low and medium NaCl concentrations (200–300 mmol/L), the relative conductivity and of malondialdehyde (MDA) content increased less, the cell membrane damage to *P. talassica*× *P. euphratica* was less, and the soluble substance (free proline, soluble sugar and soluble protein) contents increased significantly. The reactive oxygen species free radicals were removed by increasing the superoxide dismutase, peroxidase and catalase activities. At high NaCl concentrations (350–450 mmol/L) compared with lower NaCl concentrations, the leaves of *P. talassica* × *P. euphratica* were small and thick, the relative conductivity and MDA content increased significantly, the cell membrane damage was more serious, and the three antioxidant enzyme activity levels decreased significantly. The osmotic regulatory capability was improved by increasing the contents of three soluble substances. Under drought conditions compared with normal conditions, the leaf thickness of *P. talassica* × *P. euphratica* was basically unchanged, and the relative conductivity and MDA content increased significantly. The seedlings adapted to and resisted the drought environment by increasing the superoxide dismutase, peroxidase and catalase activities, as well as the osmotic regulator, such as proline, soluble sugar and soluble protein, contents. In the control experiment, the leaf anatomical results for the three tree species were basically consistent with the changes in physiological and biochemical indexes caused by a high saline or arid environment. A comprehensive evaluation of the salt and drought tolerance levels of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* was performed using the fuzzy mathematics membership function method, which revealed that the salt and drought tolerance levels of the species were both: *P. euphratica* > *P. talassica* × *P. euphratica* > *P. pruinosa*. The *P. talassica* × *P. euphratica* seedlings showed strong salt and drought tolerance levels, making them suitable for planting in the saline and arid lands of the Tarim Basin.

Key words: *Populus talassica* × *Populus euphratica*, Anatomical structure, Physiological response, Salt tolerance, Drought tolerance.

Introduction

Drought conditions will become more frequent and severe in the future along with global climate change (Trenberth *et al*., 2014; Cook *et al*., 2015; Choat *et al*., 2018). Salinity is another severe abiotic stress factor that limits plant growth and production worldwide. In arid areas, evapotranspiration transports salt to the surface, which causes salt to accumulate to toxic concentrations. Additionally, irrigation water may dissolve salt from mineral stocks, and it may be transported back to the surface by water evaporation and plant uptake (Rengasamy, 2006). Therefore, unscientific irrigation occurring during climate change may result in further salinization (Polle & Chen, 2015). Xinjiang is in arid and semi-arid area, with a dry climate, low annual precipitation, strong surface evaporation and prominent water deficit. It has the widest distribution area, the most serious salt accumulation level, and is the most difficult area to improve in China. Owing to the geographical environment and human activities, the degree of soil secondary salinization in Xinjiang is still increasing, as is the saline land area. Improving and developing saline and arid lands are the primary tasks of agriculture and forestry in Xinjiang (Heng *et al*., 2019). *Populus talassica* × *Populus euphratica* was bred by the Xinjiang Academy of Forestry Sciences and Xinjiang Jimusar County Forest Improved Variety Experimental Station using *P. talassica* as the female parent and *P. euphratica* as the male parent. It passed the improved variety examination in 2009, and its strains were named *P. talassica* × *P. euphratica* 1 and *P. talassica* × *P. euphratica* 2 (Zhang *et al*., 2010). As a hybrid progeny of *P euphratica*, there are limited related research reports at present. Previous research included mainly cutting seedlings (Gong & Liu, 2015; Liu *et al*., 2015; Zhang & Liu, 2015), injury adaptation (Sun *et al*., 2019) and saline environmental responses (Sun *et al*., 2021; Tang *et al*., 2021; Liu *et al*., 2022). Previous studies revealed that *P. talassica* × *P. euphratica* has a stress-resistance advantage from *P. euphratica* (male parent) and the fast growth and strong-growth potential characteristics from *P. talassica* (female parent). Additionally, the asexual reproduction capability is significantly improved compared with *P. euphratica*, and it has good material quality, neat appearance and good landscape-effect characteristics. However, whether its stress resistance is better than that of *P. euphratica*, *P. pruinosa* or other tree species with strong stress-resistance levels needs to be further studied. Thus, the effects of different NaCl concentrations and different drought conditions on leaf anatomical structure and both physiological and biochemical indexes of *P. talassica* × *P. euphratica* were studied using a pot-resistance experiment. The physiological adaptability of *P. talassica* × *P. euphratica* to different NaCl concentrations and different drought environments was explored to scientifically evaluate whether the physiological adaptability and stress resistance of *P. talassica* × *P. euphratica* to saline alkali and drought environments are better than those of *P. euphratica* and *P. pruinosa*. This will provide a scientific basis for *P. talassica* × *P. euphratica* to be widely planted and scientifically managed.

Materials and Methods

Test materials: As test materials, 2-year-old *P. talassica* × *P. euphratica* 2 seedlings, with plant heights of 58–187 cm, ground diameters of 1–1.5 cm and crown widths of 50–60 cm, were selected. As controls, 2-year-old *P. euphratica*, with plant heights of 51–81cm, ground diameters of 0.9–1.5 cm and crown widths of 30–50 cm, and 2-year-old *P. pruinosa*, with plant heights of 44–97 cm, ground diameters of 1–1.5 cm and crown widths of 30–50 cm, were selected.

Test methods: The experiment was conducted at the seedling base of the 10th Regiment of the First Division of the Xinjiang Production and Construction Corps (81°15'32.64” E, 40°34'6.14” N, altitude 1,014 m) from May 16 to August 22, 2019. Each plastic pot was 35 cm in diameter at the top, 19 cm in diameter at the bottom, and 26 cm in height. Each contained 5,000 g of mature soil at pH 5.7.

Seedlings of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* were divided into seven groups with ten seedlings per group. A seven NaCl-concentration gradient was set: 0 (control), 200, 250, 300, 350, 400 and 450 mmol/L, and seedlings were treated every 5 days for a total of six times. The seedlings of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* were divided into four groups with five plants per group. Four different watering rates were set: CK (normal watering once every 3 days), mild drought (watering once every 5 days), moderate drought (watering once every 10 days) and severe drought (watering once every 15 days). The total drought treatment time was 15 days. After treatment, the growth of *P. talassica*× *P. euphratica* seedlings was observed regularly, and the related growth indexes and physiological and biochemical indexes were determined after maintaining the same growth conditions for 2 months.

Determination method of indicators: The leaves were fixed and preserved in Formaldehyde-acetic acid-ethanol Fixative (FAA) solution, and the paraffin section method was used to create permanent sections. The palisade tissue, spongy tissue and leaf thickness were observed and measured under a Leica microscope. Three visual fields were observed per slice, and ten values were observed per visual field. The average values of the anatomical structure parameters in three visual fields were used as the anatomical structure parameters of each leaf. In accordance with the following formulae, the palisade tissue to spongy tissue ratio, leaf tissue structural tightness and looseness were calculated:

Palisade tissue to spongy tissue ratio = palisade tissue thickness/spongy tissue thickness,

Leaf tissue structural tightness = (palisade tissue thickness/leaf thickness) × 100% and

Leaf tissue structural looseness = (sponge tissue thickness/leaf thickness) × 100%.

The relative conductivity was determined using a conductometer, the content of malondialdehyde (MDA) was determined using the microplate method. The superoxide dismutase (SOD) activity was determined using the WST-1 method, the peroxidase (POD) activity was determined using colorimetry and the catalase (CAT) activity was determined using the visible light method. The proline and soluble sugar contents were determined using colorimetry. The soluble protein content was determined using the Coomassie brilliant blue G-250 method. Each index was repeated three times. All the kits were purchased from the Nanjing Jiancheng Bioengineering Institute.

Comprehensive evaluation method for stress tolerance: For a comprehensive evaluation of the salt and drought tolerance levels of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa*, a fuzzy subordinate function that was determined either by the following formula (1):

$$\mathrm{X}(u) = \frac{X - X_{\min}}{X_{\max} - X_{\min}}$$

in which X represents the test value of a certain tolerance index and $X_{\max}$ and $X_{\min}$ represent the maximum and minimum values of the index, respectively, or by the following formula (2):

$$\mathrm{X}(u) = 1 - \frac{X - X_{\min}}{X_{\max} - X_{\min}}$$

if one of the indexes was negatively correlated to stress tolerance, was used First, the specific subordinate function values of each index for each of the ten species tested were calculated using Formula (1) or (2). Next, the weight for each index was calculated using a principal component analysis, and the weighted average values of the ten species were calculated to determine the salt and drought tolerance levels of each species (Qin and Liang, 2010; Han *et al*., 2017).

Statistical analysis

All the data were analyzed using SPSS ver. 22.0 (IBM Corp., Armonk, New York, USA) and expressed as means ± standard deviations. The least significant difference test was used for multiple comparisons to determine significant differences ($p<0.05$), if any, between individual treatments.

Results

Leaf anatomical structure under salt and drought conditions: Leaf anatomical structure after exposure to different NaCl treatments: The changes in the leaf anatomical structure parameters of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* under different NaCl concentrations are shown in (Table 1). As the NaCl concentration increased from 200 to 250 mmol/L, the palisade tissue thicknesses of the three tree species began to increase. Compared with the controls, the thickness of the sponge tissue began to decrease when the NaCl concentration was 250–350 mmol/L. Compared with the controls, the leaf thickness began to increase at the 200–250 mmol/L NaCl concentration. When the concentration of NaCl was 250–300 mmol/L, the palisade tissue to spongy tissue ratio began to increase compared with those of the controls. Compared with the controls, the leaf tissue structural tightness of *P. talassica* × *P. euphratica* and *P. pruinosa* showed no significant changes. When the NaCl concentration was 250 mmol/L, the leaf tissue structural tightness of *P. euphratica* began to decrease significantly. Compared with the control, the leaf tissue structural looseness began to decrease significantly when the NaCl concentration was 200 mmol/L..

Leaf anatomical structures after exposure to different drought treatments: As shown in (Table 2), compared with normal watering conditions, as the drought degree increased the palisade tissue thickness of *P. talassica* × *P. euphratica* showed no significant change under mild drought conditions, whereas it increased significantly under moderate and severe drought conditions. Compared with normal watering conditions, the palisade tissue thicknesses of *P. euphratica* and *P. pruinosa* increased significantly in drought environments. The sponge tissue thicknesses of *P. talassica* × *P. euphratica* and *P. pruinosa* decreased significantly under moderate and severe drought conditions compared with normal watering. Compared with normal watering conditions, there was no significant difference in the spongy tissue thickness of *P. euphratica.* Compared with normal watering conditions, as the drought degree increased, the leaf thickness of *P. talassica* × *P. euphratica* showed no significant change. However, the leaf thickness of *P. euphratica* increased significantly in moderate and severe drought environments, whereas the leaf thickness of *P. pruinosa* showed no significant change under moderate drought conditions but increased significantly under severe drought conditions. The palisade tissue to spongy tissue ratios of the three species increased significantly under moderate and severe drought conditions compared with normal watering conditions. Compared with under normal watering conditions, the leaf tissue structural tightness of *P. talassica* × *P. euphratica* leaves showed no significant difference, whereas the structural tightness of *P. euphratica* leaves increased significantly, under moderate and severe drought conditions. The leaf tissue structural tightness of *P. pruinosa* became significantly greater than under normal watering conditions as the drought severity increased. Compared with normal watering conditions, the leaf tissue structural looseness levels of *P. euphratica* and *P. pruinosa* decreased significantly as the drought degree increased, and the differences in leaf tissue structural looseness levels of *P. talassica* × *P. euphratica* were significant, except for under mild drought conditions.

Table 1. Characteristics of leaf anatomy in salt treatments.

Species	NaCl Concentration (mmol/L)	Palisade tissue thickness (μm)	Spongy tissue thickness (μm)	leaf thickness (μm)	Palisade tissue to spongy tissue ratio	Leaf tissue structure tightness	Leaf tissue structure looseness
P. talassica × *P. euphratica*	0	341.14±35.11f	329.03±21.07a	772.42±29.98f	1.05±0.15e	0.44±0.06a	0.43±0.01a
	200	394.22±11.08e	316.74±7.28ab	870.48±15.76e	1.30±0.07de	0.45±0.01a	0.37±0.02b
	250	493.28±8.09d	314.34±5.68ab	1100.52±14.97d	1.70±0.08cd	0.45±0.01a	0.28±0.01c
	300	534.14±16.79cd	295.16±19.79bc	1186.28±28.17c	2.09±0.25bc	0.46±0.03a	0.27±0.05c
	350	563.88±22.37c	272.08±16.61c	1218.84±28.88bc	2.43±0.04b	0.46±0.02a	0.22±0.02d
	400	608.61±8.97b	265.93±9.63c	1260.62±12.36b	2.53±0.11b	0.48±0.01a	0.21±0.01d
	450	722.93±18.10a	252.93±18.24c	1469.35±22.27a	3.63±0.44a	0.48±0.01a	0.18±0.01d
P. euphratica	0	197.71±11.07d	183.39±3.27a	487.59±10.45e	1.12±0.05d	0.42±0.01b	0.39±0.01a
	200	234.29±17.65d	177.40±14.64a	544.18±16.42d	1.37±0.20d	0.45±0.04ab	0.35±0.03b
	250	336.99±4.78c	170.80±3.91a	706.09±16.90c	2.11±0.07cd	0.49±0.02a	0.25±0.01c
	300	382.25±23.75b	159.21±11.85ab	804.18±15.74b	2.58±0.36bc	0.48±0.03a	0.20±0.02d
	350	563.88±22.37c	141.27±13.69bc	832.75±10.90b	3.24±0.54ab	0.49±0.01a	0.17±0.02d
	400	608.61±8.97b	126.35±20.54c	960.47±11.97a	4.08±0.86a	0.47±0.02a	0.13±0.02e
	450	722.93±18.10a	121.91±11.20c	966.82±26.37a	4.02±0.55a	0.49±0.02a	0.13±0.01e
P. pruinosa	0	252.12±0.48d	149.16±17.91a	550.81±29.46d	1.51±0.10e	0.38±0.03ab	0.27±0.04a
	200	295.61±0.34d	131.22±14.81ab	614.49±23.69c	1.94±0.25de	0.36±0.02b	0.22±0.02b
	250	334.09±0.46c	116.48±3.89b	631.12±15.63c	2.34±0.21d	0.42±0.01a	0.19±0.01bc
	300	384.69±0.54b	114.25±20.19bc	808.48±31.34b	3.14±0.58c	0.40±0.03ab	0.15±0.03cd
	350	444.96±0.33b	87.88±7.46cd	840.73±34.68b	3.92±0.26b	0.39±0.01ab	0.10±0.01de
	400	471.62±0.63a	86.00±7.75d	938.48±10.39a	4.69±0.22a	0.42±0.03a	0.09±0.01e
	450	471.62±0.60a	78.49±3.38d	945.14±7.11a	5.34±0.56a	0.43±0.03a	0.08±0.01e

Note: The data are mean ± standard error; Different letters of the same tree species showed significant differences in the same column of data (p<0.05). The same below

Table 2. Characteristics of leaf anatomy in drought treatments.

Species	Degree of drought	Palisade tissue thickness (μm)	Spongy tissue thickness (μm)	Leaf thickness (μm)	Palisade tissue to spongy tissue ratio	Leaf tissue structure tightness	Leaf tissue structure looseness
P. talassica × *P. euphratica*	Normal watering	297.50±2.88c	296.04±1.78a	588.21±0.68a	1.01±0.01d	0.51±0.01a	0.50±0.01a
	Mild drought	304.20±3.88bc	286.62±8.68a	593.35±3.88a	1.07±0.04dc	0.51±0.01a	0.48±0.02a
	Moderate drought	310.97±1.15ab	271.20±2.23b	596.09±8.56a	1.15±0.01b	0.52±0.01a	0.46±0.01b
	Severe drought	319.09±6.69a	252.94±4.15c	598.50±24.45a	1.27±0.03a	0.53±0.02a	0.42±0.01c
P. euphratica	Normal watering	257.37±22.37d	193.80±5.94a	782.76±11.51bc	1.37±0.08c	0.33±0.01c	0.25±0.01a
	Mild drought	308.86±8.19c	191.60±6.72a	871.44±7.59b	1.65±0.10bc	0.35±0.01bc	0.22±0.01b
	Moderate drought	363.61±15.73b	183.91±3.80a	931.13±46.86a	1.99±0.07b	0.39±0.04ab	0.20±0.01bc
	Severe drought	393.36±1.88a	170.45±17.35a	971.26±16.19a	2.39±0.28a	0.41±0.01a	0.18±0.02c
P. pruinosa	Normal watering	297.15±1.85d	181.12±10.53a	651.60±7.96b	1.68±0.11c	0.46±0.01c	0.28±0.02a
	Mild drought	308.92±3.09c	168.31±4.73a	658.65±10.48b	1.87±0.06c	0.47±0.01b	0.26±0.01b
	Moderate drought	327.28±3.99b	147.94±4.29b	673.92±13.10ab	2.25±0.04b	0.49±0.01a	0.22±0.01c
	Severe drought	342.07±2.29a	135.89±4.91b	688.87±5.28a	2.57±0.10a	0.50±0.01a	0.20±0.01c

Relative conductivity and MDA content under salt and drought conditions: Relative conductivity and MDA content after exposure to different NaCl treatments: As shown in (Fig. 1), as the NaCl concentration increased, the relative conductivity values of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* all showed increasing trends. Compared with the control, the relative conductivity of *P. talassica* × *P. euphratica* began to increase significantly when the NaCl concentration was 250 mmol/L, and the relative conductivity levels of *P. euphratica* and *P. pruinosa* began to increase significantly at the 200 mmol/L-NaCl concentration. As shown in (Fig. 2), as the NaCl concentration increased, the MDA contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* showed upward trends. When the NaCl concentration was 450 mmol/L, the MDA contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* were 107.69, 104.62 and 106.92 nmol/g, respectively. Compared with the control, the increases in the rates were 104.38%, 164.08% and 192.63%, respectively. The MDA contents of *P. euphratica* and *P. pruinosa* increased significantly compared with that of *P. talassica* × *P. euphratica* under the high NaCl concentration conditions.

Relative conductivity and MDA content after exposure to different drought treatments: As shown in (Fig. 3), as the drought degree increased, the change trends in the relative conductivity levels of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* remained basically the same. Compared with normal watering conditions, the relative conductivity of *P. talassica* × *P. euphratica* increased significantly under moderate and severe drought conditions. Compared with normal watering conditions, the relative conductivity levels of *P. euphratica* and *P. pruinosa* were significantly increased under all three drought conditions. As shown in (Fig. 4), under drought conditions, the change trends in the MDA contents and relative conductivity levels of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* remained basically the same. As the drought degree increased, the MDA content of *P. talassica* × *P. euphratica* increased significantly. Under moderate and severe drought conditions, the difference reached a significant level compared with under normal watering conditions. The MDA contents of *P. euphratica* and *P. pruinosa* under drought conditions were significantly different compared with under normal watering conditions.

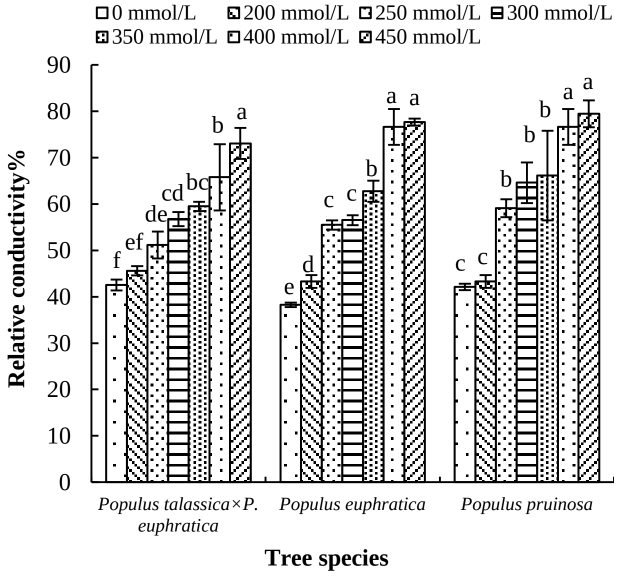

Fig. 1. Changes of relative conductivity in NaCl treatments.
Note: Different letters of the same tree species indicate significant differences among treatments (p<0.05). The same below.

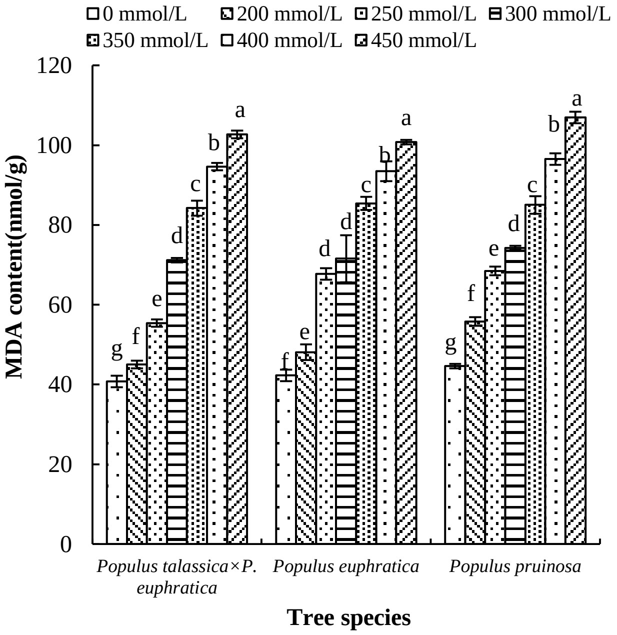

Fig. 2. Changes of MDA content in NaCl treatments.

Antioxidant enzyme (SOD, POD and CAT) activities under salt and drought conditions: SOD, POD and CAT activities after exposure to different NaCl treatments: The changes in the antioxidant enzyme activities (SOD, POD and CAT) of the three tree species under NaCl treatments were basically the same, and the overall trend first increased and then decreased (Fig. 5–7). The SOD activity of *P. talassica* × *P. euphratica* was not significantly different from that of the control at the 450 mmol/L-NaCl concentration, but it was significantly different from the control at other concentrations. Compared with the control, the SOD activity of *P. pruinosa* reached a significant level only when the NaCl concentration was 450 mmol/L, and the differences after other treatments did not reach significant levels. As the NaCl concentration (400–450 mmol/L) increased, the POD activity of *P. talassica* × *P. euphratica* decreased significantly. The change in the CAT activity of *P. talassica* × *P. euphratica* was consistent with those of SOD and POD, and it reached a significant level compared with the control. When the NaCl concentration was 300 mmol/L, the CAT activity of *P. euphratica* was significantly different from that of the control. The CAT activity of *P. pruinosa* was significantly lower than that of the control at the 450 mmol/L-NaCl concentration.

SOD, POD and CAT activities after exposure to different drought treatments: As shown in (Fig. 8), the SOD activity levels of *P. talassica* × *P. euphratica* and *P. euphratica* first increased and then decreased as the drought degree increased. Compared with under normal watering conditions, the SOD activity of *P. pruinosa* decreased significantly under moderate drought conditions, while the levels under other treatment conditions did not change significantly. As shown in Fig. 9, the POD activities of *P. euphratica* and *P. pruinosa* under drought conditions significantly increased compared with under normal watering conditions. Compared with under normal watering conditions, the POD activity of *P. talassica* × *P. euphratica* increased significantly under moderate drought conditions, while the levels under other treatment conditions did not change significantly. The CAT activity levels of the three tree species first increased and then decreased as the drought degree increased (Fig. 10). Compared with under normal watering conditions, the CAT activity of *P. talassica* × *P. euphratica* reached significant levels under mild and moderate drought conditions. Compared with under normal watering conditions, the CAT activity of *P. euphratica* reached significant levels under moderate and severe drought conditions. Compared with under normal watering conditions, the CAT activity of *P. pruinosa* reached significant levels under all three drought conditions.

Osmotic adjustment substance (proline, soluble sugar and soluble protein) contents under salt and drought conditions: Proline, soluble sugar and soluble protein contents after exposure to different NaCl treatments: As shown in (Figs. 11 and 12), as the NaCl concentration increased, the proline and soluble sugar contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* increased significantly compared with the control. Under increasing NaCl conditions, the proline content of *P. talassica* × *P. euphratica* increased sharply, and there was a significant difference compared with the control. The proline contents of *P. euphratica* and *P. pruinosa* increased when the NaCl concentration was 200–350 mmol/L and decreased slightly when the NaCl concentration was 400–450 mmol/L. When the NaCl concentration was 250 mmol/L, the soluble sugar contents of *P. talassica* × *P. euphratica and P. euphratica* began to increase significantly compared with those of the controls, while that of *P. pruinosa* began to increase significantly compared with the controls at the concentration of 200 mmol/L-NaCl concentration. The soluble protein contents of three tree species first increased and then decreased, and they reached significant levels compared with the controls (Fig. 13). When the NaCl concentration was 450 mmol/L, the soluble protein content of *P. talassica* × *P. euphratica* began to decrease significantly. When the NaCl concentration was 400 mmol/L, the soluble protein contents of *P. euphratica* and *P. pruinosa* began to decrease significantly.

Proline, soluble sugar and soluble protein contents after exposure to different drought treatments: As shown in (Figs. 14–16) as the drought degree increased, the change trends of the proline, soluble sugar and soluble protein contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* were basically similar, and they were significantly increased compared with under normal watering conditions. The soluble sugar contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* were 72.47, 66.60 and 44.39 mg/g, respectively, under normal watering conditions, and 123.74, 107.81 and 91.01 mg/g, respectively, under severe drought conditions, indicating increases of 70.76%, 61.87% and 105.24%, respectively. Compared with under normal watering conditions, the soluble protein contents of *P. talassica* × *P. euphratica* and *P. pruinosa* increased significantly under drought conditions. The soluble protein contents of *P. euphratica* under moderate and severe drought conditions were significantly greater than under normal watering conditions, but the difference was not significant under mild drought conditions.

Comprehensive evaluation of the salt and drought tolerance levels of the three species: In the descending order of their overall tolerance to NaCl on the basis of our comprehensive evaluation, the three species were ranked as follows: *P. euphratica*, *P. talassica* × *P. euphratica* and *P. pruinosa* (Table 3). Their overall tolerances to drought resulted in the same ranking, as follows: *P. euphratica*, *P. talassica* × *P. euphratica* and *P. pruinosa* (Table 4).

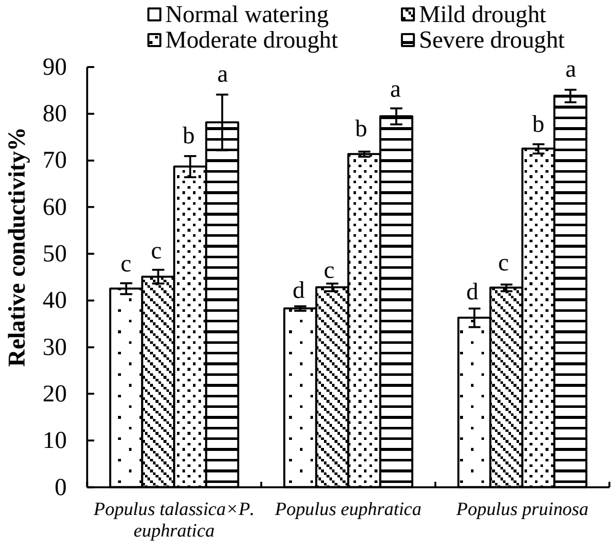

Fig. 3. Changes of relative conductivity in drought treatments.

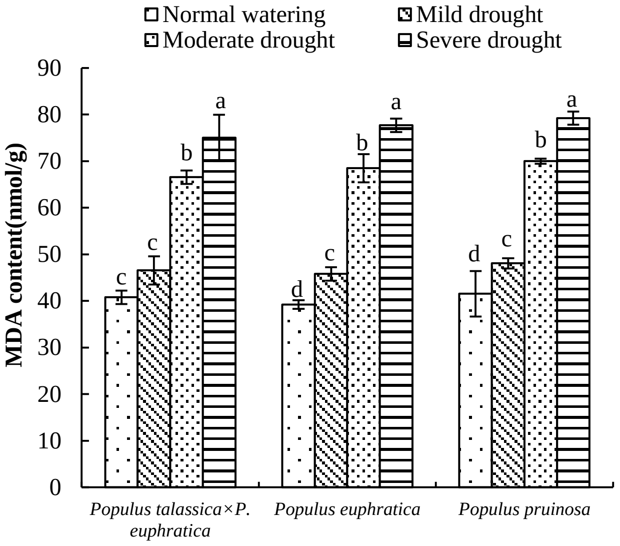

Fig. 4. Changes of MDA content in drought treatments.

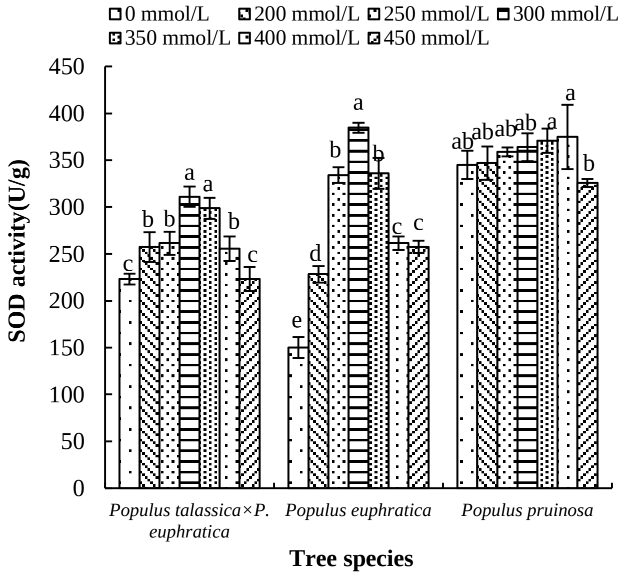

Fig. 5. Changes of SOD activity in NaCl treatments.

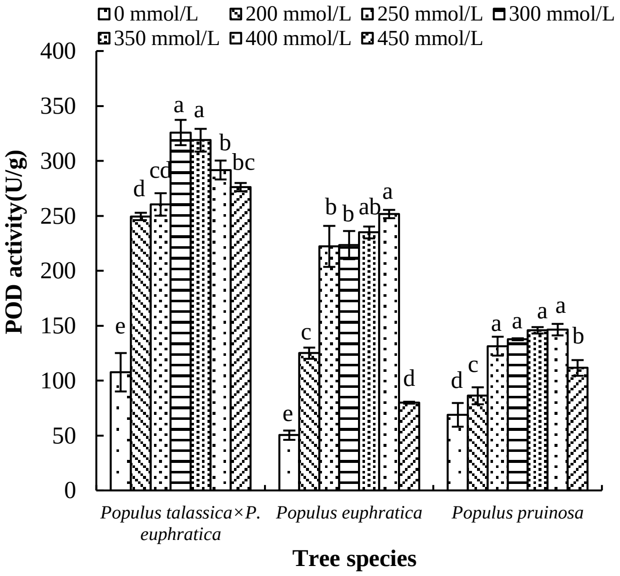

Fig. 6. Changes of POD activity in NaCl treatments.

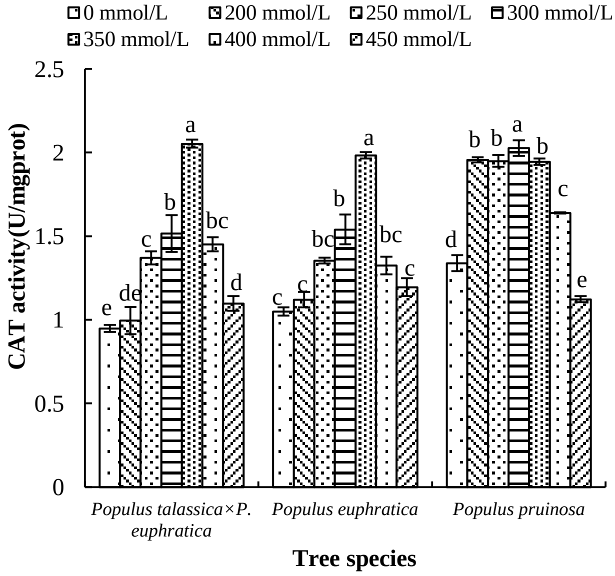

Fig. 7. Change of CAT activity in NaCl treatments.

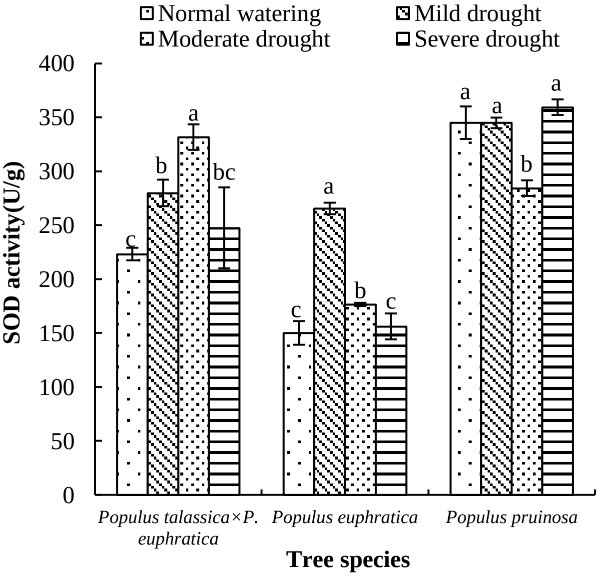

Fig. 8. Changes of SOD activity in drought treatments.

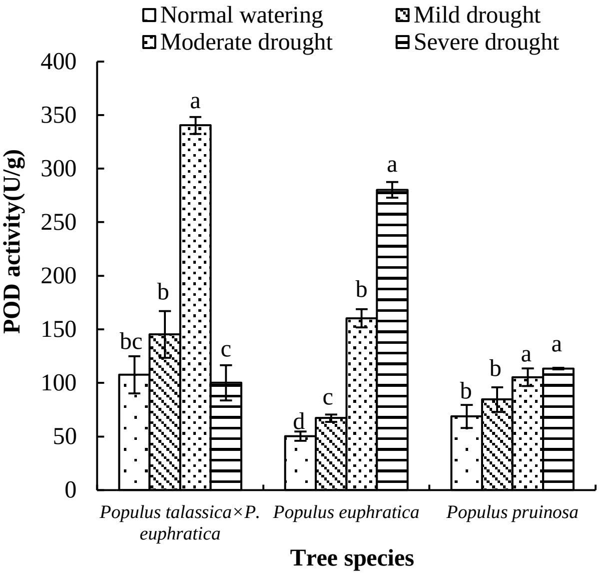

Fig. 9. Change of POD activity in drought treatments.

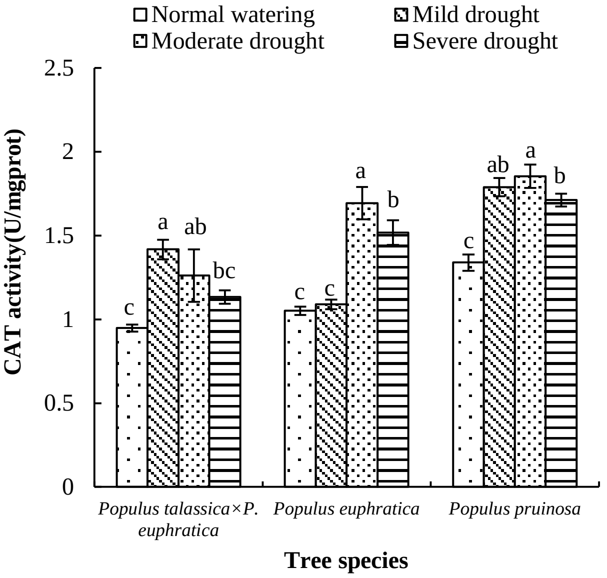

Fig. 10. Changes of CAT activity in drought treatments.

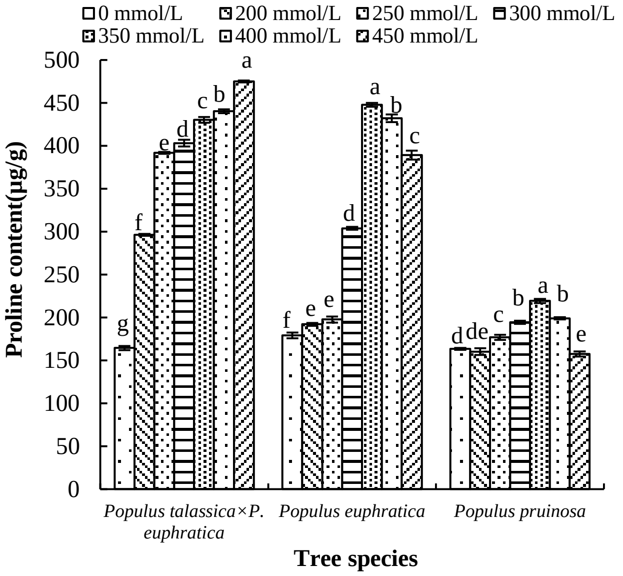

Fig. 11. Changes of proline content in NaCl treatments.

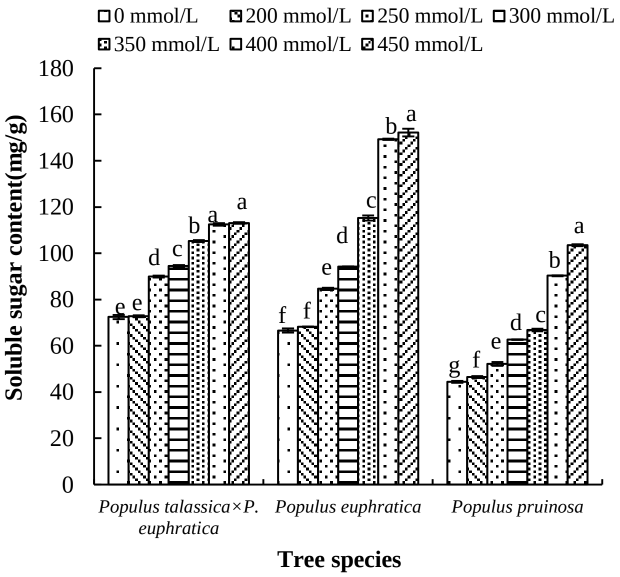

Fig. 12. Changes of soluble sugar content in NaCl treatments.

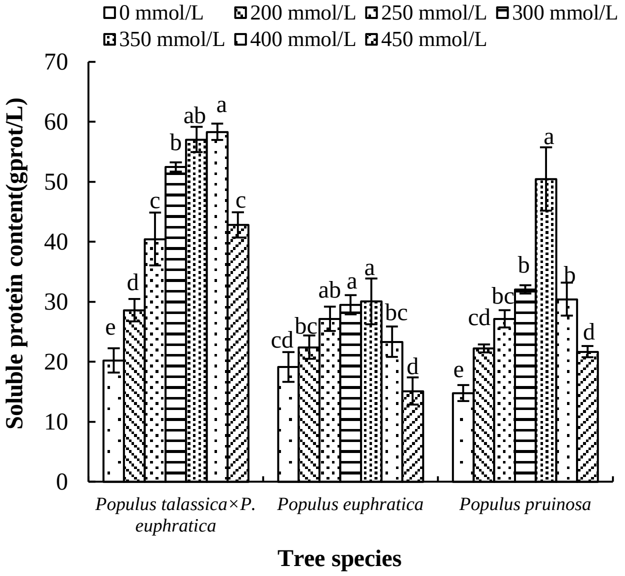

Fig. 13. Changes of soluble protein content in NaCl treatments.

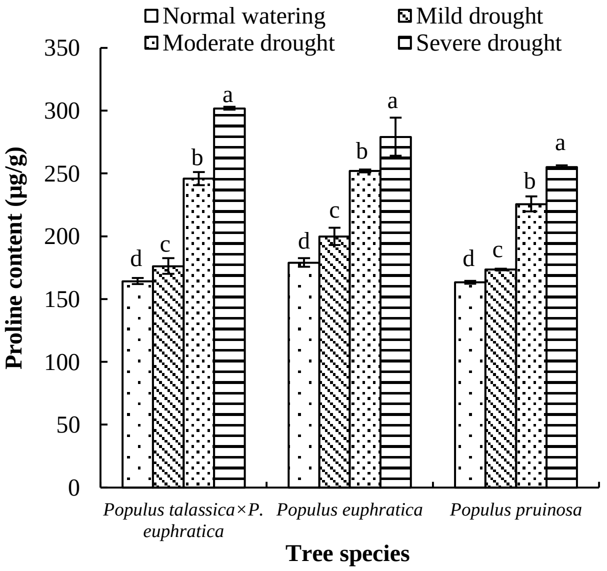

Fig. 14. Changes of proline content in drought treatments.

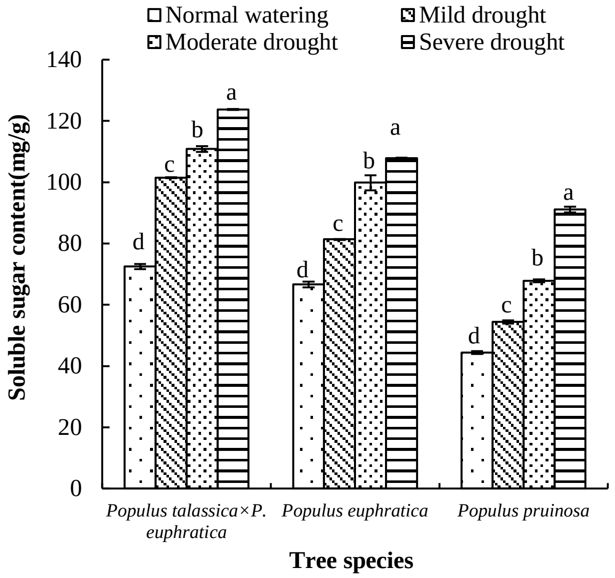

Fig. 15. Changes of soluble sugar content in drought treatments.

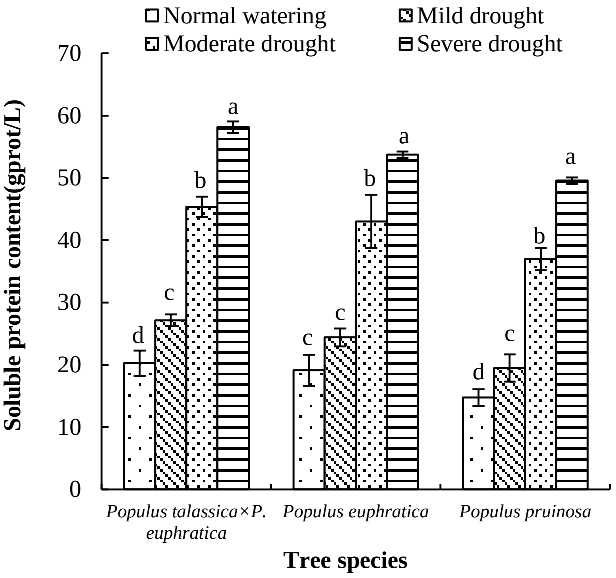

Fig. 16. Changes of soluble protein content in drought treatments.

Table 3. Comprehensive evaluation of salt tolerance in 3 tree species.

Index	**Membership function value and its weight**			
	P. talassica* × *P. euphratica	***P. euphratica***	***P. pruinosa***	**Index weight (%)**
Palisade tissue thickness	0.50	0.45	0.48	7.74
Spongy tissue thickness	0.64	0.58	0.55	5.04
Leaf thickness	0.51	0.58	0.46	8.79
Relative conductivity	0.51	0.51	0.44	11.24
MDA content	0.48	0.50	0.41	12.17
SOD activity	0.42	0.40	0.51	11.47
POD activity	0.43	0.49	0.49	8.26
CAT activity	0.46	0.48	0.44	12.81
Proline content	0.46	0.54	0.49	6.78
Soluble sugar content	0.58	0.51	0.52	4.99
Soluble protein content	0.53	0.52	0.51	10.70
Weighted average	0.49	0.50	0.48	
Ranking	2	1	3	

Table 4. Comprehensive evaluation of drought resistance of 3 tree species.

Index	**Membership function value and its weight**			
	P. talassica* × *P. euphratica	***P. euphratica***	***P. pruinosa***	**Index eight (%)**
Palisade tissue thickness	0.55	0.48	0.57	7.74
Spongy tissue thickness	0.54	0.45	0.50	5.04
Leaf thickness	0.54	0.50	0.48	8.79
Relative conductivity	0.57	0.52	0.50	11.24
MDA content	0.50	0.53	0.44	12.17
SOD activity	0.42	0.42	0.55	11.47
POD activity	0.45	0.43	0.45	8.26
CAT activity	0.40	0.51	0.44	12.81
Proline content	0.47	0.47	0.53	6.78
Soluble sugar content	0.60	0.53	0.38	4.99
Soluble protein content	0.47	0.57	0.48	10.70
Weighted average	0.49	0.50	0.48	
Ranking	2	1	3	

Discussion

Plant salt tolerance is related to leaf anatomical structures, which can be used as a screening index for plant salt and drought tolerance levels. Osmotic stress (drought or salt stress) has an impact on leaf parameters (Terletskaya & Kurmanbayeva, 2017). Plants growing in saline alkali environments often have fleshy roots and leaves. Salinity tolerance in the most tolerant population relies on thicker leaf and cortical regions. Structural modifications are crucial for attenuating undue water loss under physiological stress conditions caused by high amounts of soluble salts in the soil (Naz *et al*., 2014). In our study, the palisade tissue thicknesses and leaf thicknesses of the three tree species increased under salt-stress conditions compared with the controls. The spongy tissue thicknesses and the leaf tissue structural looseness levels decreased. There was no significant difference in leaf tissue structural tightness levels of *P. talassica* × *P. euphratica* and *P. pruinosa*, but that of *P. euphratica* decreased significantly. Previous studies found that the changes in leaf anatomical characteristics of different plants under salt- or drought-stress conditions are not completely consistent (Hassan *et al*., 2015; Rajput *et al*., 2015; Mehar-un-Nisa *et al*., 2016; Lie *et al*., 2018).

During salt or drought stress, a large number of reactive oxygen species (ROS) are produced in plant cells, resulting in membrane lipid bilayer peroxidation and excessive MDA production, which damage the cell membrane. A large number of water-soluble substances leak from the cells, resulting in an increase in the electrical conductivity of the plant cell fluid. The product of excessive ROS free radical peroxidation on the membrane lipid bilayer is MDA, which can thus be used as an intuitive indicator of the degree of plant cell membrane damage (Lei *et al*., 2006; Holasoo and Pourakbar, 2014; Najar *et al*., 2020). In this study, as the NaCl concentration increased, the relative conductivity levels and MDA contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* increased significantly, indicating that the cell membranes were seriously damaged, which was consistent with previous research results (Holasoo & Pourakbar, 2014; Yu *et al*., 2020).

As mentioned earlier, ROS in plants increases and accumulates rapidly in plant cells (Terletskaya & Kurmanbayeva, 2017). In addition, plants eliminate ROS through the synergistic action of intracellular antioxidant enzymes, resulting in a dynamic ROS balance and the maintenance of normal metabolic activities in plant cells. The POD activity increases along with the severity of NaCl stress, but the SOD activity varies at different salt levels (Rajput *et al*., 2015; Chen *et al*., 2016). Our results showed that the activities of three antioxidant enzymes in *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* increased significantly, first showing an increasing trend and then decreasing under salt-stress conditions, which was basically consistent with the results in *Poplar* and *Jatropha* (Jing *et al*., 2013; Hishida *et al*., 2014). Here, we found that under low NaCl concentrations, *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa*. The enzyme activity levels increased significantly. Thus, seedlings could eliminate ROS free radicals by increasing antioxidant enzyme activities. However, when the NaCl concentration exceeded 400 mmol/L, POD activity was inhibited, and the POD activity levels in *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* decreased significantly. We also found that the antioxidant enzyme activities of *P. talassica* × *P. euphratica* first increased and then decreased along with drought severity. The SOD and CAT activities of *P. euphratica* first increased and then decreased, whereas the POD activity increased. The SOD activity of *P. pruinosa* first decreased and then increased, the POD activity increased significantly, and the CAT activity first increased and then decreased.

Salt and drought tolerance levels depend greatly on osmotic adjustments (proline, soluble sugars and soluble proteins) (Muthulakshmi *et al*., 2015; Lie *et al*., 2018). Salinity tolerance in the most tolerant population relies on the accumulation of organic osmolytes, as well as thicker leaf and cortical regions (Naz *et al*., 2014). Here, the proline content of *P. talassica* × *P. euphratica* increased along with the NaCl concentration. The proline contents of *P. euphratica* and *P. pruinosa* increased at first and then decreased. The changes in the proline contents of *P. euphratica* and *P. pruinosa* in this study are consistent with previous research results (Wang *et al*., 2018). However, the proline content of *P. talassica* × *P. euphratica* in this experiment did not decrease significantly, indicating that the osmotic adjustment capability of *P. talassica* × *P. euphratica* was stronger than those of *P. euphratica* and *P. pruinosa.* Soluble sugars in plants have protective effects on cell membranes and protoplasts. Soluble proteins can enhance the water absorption capacities of plants. When plants are under salt- or drought-stress conditions, they can increase osmotic pressure inside and outside the cells by accumulating osmotic adjustment substances to improve the plant's adaptation to a stressful environment (Lie *et al*., 2018). We found that the soluble sugar and soluble protein contents of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* significantly increased under salt-stress conditions compared with normal conditions. Our results showed that the proline, soluble sugar, and soluble protein contents among the osmotic adjustment substances of *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* increased significantly under drought conditions, and the three tree species showed strong osmotic adjustment capabilities. Our findings were basically consistent with the results of previous studies on *P. euphratica* and *P. cathayana* seedlings (Xiao *et al*., 2008; Yang & Miao, 2010; Duan *et al*., 2020). Our results also showed that the accumulations of osmotic adjustment substances (proline, soluble sugar and soluble protein) in *P. talassica* × *P. euphratica*, *P. euphratica* and *P. pruinosa* in drought environments reduced the water potentials of tissues *In vivo* and maintained normal cell swelling pressure, providing resistance to the drought environment.

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