

Drought stress tolerance analysis of *Populus ussuriensis* clones with different ploidies

Jianqiu Xu¹ · Jiaojiao Jin¹ · Hui Zhao² · Kailong Li¹

Received: 19 August 2017 / Accepted: 26 November 2017 / Published online: 25 June 2018
© Northeast Forestry University and Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract The selection of drought-tolerant plants is an important aspect of plant breeding. We studied physiological and biochemical mechanisms of different ploidies of *Populus ussuriensis* Kom. that relate to drought stress tolerance. We used a 5% (v/v) polyethylene glycol (PEG-6000) solution to simulate drought stress. We recorded leaf phenotypes including color, dry area and curl degree. We evaluated sequential variations in some drought stress tolerance-related physiological and biochemical indices and compared these among diploid clones (CK), triploid clones (T12) and tetraploid clones (F20). T12 leaves exhibited slightly more drought stress damage than CK and F20 leaves. CK leaves suffered the most severe drought stress damage. The physiological and biochemical indices of the different ploidies differed significantly 12 days after drought stress treatment. The activities of superoxide dismutase, peroxidase, catalase and proline in the triploid (T12) leaves were the highest. The relative electric conductivity and malondialdehyde content of T12 leaves were the lowest. The index values of F20 were between those of

the diploid and triploid. In consideration of these results, the drought resistance of the three different ploidies of *P. ussuriensis* can be ranked as T12 > F20 > CK. We speculate that the gene expression patterns of polyploid clones of poplar will change after genome doubling and that some of the drought stress tolerance-related physiological and biochemical indices will be improved, resulting in greater drought tolerance of polyploid clones.

Keywords *Populus ussuriensis* · Polyploidy · Drought stress · Tolerance

Introduction

When the number of chromosomes in body cells increases three or more times, the organism is characterized by polyploidy (Storchova and Pellman 2004; Stebbins 1970). As the chromosomes in polyploid plants double, they exhibit larger cell volume and contain higher nutrient content. Sometimes, the increased chromosomes result in giant organs (such as leaves, flowers and stems), deep color, and strong stress tolerance/resistance (Randolph 1941; Otto and Whitton 2000). For example, the cell volume of nine-year-old triploid *Populus tomentosa* was 2–3 times that of diploids under the same growth conditions (Zhu and Kang 1998; Fang et al. 2001). Triploid or tetraploid Japanese black pine contained higher superoxide dismutase (SOD, which can prevent the formation of lipid peroxides and oxidative damage) than did diploid Japanese black pine (Niwa and Sasaki 2003). Triploid *Populus tremula* not only exhibited stronger drought/barrenness stress tolerance but also displayed stronger disease resistance than diploids and was more suitable for mountain planting under poor natural conditions. Triploid birch

Project funding: This work was supported by the National Key R&D Program of China (Grant No. 2016YFD0600404).

The online version is available at <http://www.springerlink.com>

Corresponding editor: Zhu Hong.

✉ Kailong Li
yzlk1123@126.com

¹ State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin 150040, People's Republic of China

² College of Forestry, Henan Agricultural University, Zhengzhou 450002, People's Republic of China

showed enhanced resistance to rust (Johnsson 1944; Ramsey 2011; Eifler 1960; Sita et al. 1980). Taken together, the increase in gene dose played an important role in plant stress resistance/tolerance (Panchuk et al. 2002).

Populus ussuriensis has excellent characteristics, such as strong adaptability, rapid growth and high yield (Li et al. 2005). In northern China, drought stress is one of the most severe abiotic limiting factors affecting the growth and development of *P. ussuriensis* (Anjum et al. 2011; DaMatta 2004). Therefore, drought tolerance is one of the most important objectives of polyploid *P. ussuriensis* breeding (Li et al. 2006; Flowers 2004).

Osmond et al. (1980) demonstrated a series of physiological changes in plants in response to water stress. First, the cytoplasmic membrane structure was damaged, and leaves were then dehydrated, resulting in intracellular fluid extravasation and increased contents of malondialdehyde (MDA) and reactive oxygen species (ROS) (Abuduwaili et al. 2015; Martin et al. 2008). Second, the activities of SOD and other antioxidant enzymes increased to maintain low levels of ROS to protect plant cells from oxidative stress damage (Gill and Tuteja 2010; Alscher et al. 2002; Michalak 2006). Then, proline (PRO) osmolytes accumulated to maintain cell turgor and allowed cells to suffer less damage from drought stress (Pérez-Pérez et al. 2007; Fan et al. 2012; He et al. 2011; Tsugane et al. 1999). Changes in physiological and biochemical indices (e.g., SOD, POD, and MDA) can reflect the degree of drought stress damage, and they are therefore useful for evaluating drought tolerance of plants (Zhang and Kirkham 1996; Liu et al. 2013; He et al. 2014; Hasheminasab et al. 2012).

We evaluated drought stress tolerance of diploid (CK), triploid (T12) and tetraploid (F20) *P. ussuriensis* by treating plants of the three ploidies with polyethylene glycol 6000 (PEG-6000). Then, the relative conductivities, the contents of MDA and PRO, and the activities of SOD, POD and CAT of the three different ploidies of *P. ussuriensis* were investigated. Our study objective was to identify the physiobiochemical mechanisms responsible for response to drought stress by *P. ussuriensis* polyploids. Our goal was to provide guidance for the practice of poplar polyploid breeding and the selection and popularization of forest resources.

Materials and methods

Plant materials and drought stress treatment

The plant materials included three polyploid lines of *P. ussuriensis*: diploid (CK), triploid (T12) and tetraploid (F20). On 20 April 2016, twigs of CK, T12 and F20 were prepared for cutting propagation by planting in plastic pots

(21 cm diameter × 21 cm depth) containing soil, sand and peat (v/v/v = 2/3/5) at the Northeast Forestry University nursery shed. Plant culture conditions were: relative humidity, 50–65%; temperature (day/night), 26–30 °C/22–25 °C; and photoperiod, natural light conditions. After 60 days, 35 healthy plants (approximately 100 cm in height) from each line were selected for subsequent drought stress treatment.

The drought stress treatment was performed with 5% (v/v) PEG-6000 solution. The third to fifth functional leaves were harvested just before drought stress treatment (i.e., 0 day samples) and 3, 6, 9 and 12 days after drought stress treatment at 8:30 am. Meanwhile, 6 healthy plantlets (approximately 100 cm in height) from each line were set as the control group and were irrigated with water. All leaf samples were harvested with three biological replicates. Each plant was sampled at one time point and only once to avoid effects caused by mechanical injury. Then, the harvested leaves were directly frozen in liquid nitrogen for subsequent experiments.

Phenotype investigation

To perform phenotype evaluation, the leaf phenotypes including color, dry area and curl degree were recorded using a Canon camera just before commencement of the drought stress treatment (i.e., 0 day) and at 3, 6, 9, and 12 days after commencement of the drought stress treatment.

MDA and PRO content detection and antioxidant enzyme (SOD, POD and CAT) activity detection

The contents of MDA and PRO and the activities of SOD, POD and CAT were detected using kits purchased from Suzhou Keming Biotechnology Company (Suzhou, China). Detection procedures were conducted according to the manufacturer's instructions in the product manual by utilizing a Lambda 25 UV/VIS spectrophotometer (Perkin Elmer, Illinois, USA). Detection for all the samples was performed with three independent repeats.

Relative electrolyte leakage content detection

For each sample, approximately 0.3 g leaf tissue was taken by using a puncher and avoiding main veins. These samples were then transferred into a syringe containing 20 mL deionized water. Gas was continuously pumped into the syringe until the leaf tissues completely sank into the deionized water. We then measured the conductivity (R_1) of these leaf tissues with a conductivity meter (Five Easy Plus FE38, Mettler Toledo, Zurich, Switzerland). The detection procedure was performed according to the manufacturer's instructions. We then heated the leaf samples in

boiling water for 30 min, cooled them to room temperature, and again recorded their conductivity (R_2). Detection for all the samples was performed with three independent repeats. The relative electrolytic leakage of each sample was calculated as follows:

$$R_L = \left(R_1/R_2 \right) \times 100\% \quad (1)$$

where R_L is relative electrolyte leakage, R_1 is the first detected conductivity value of each leaf sample and R_2 is the total conductivity value of each leaf sample.

Statistical analysis

The experimental data were analyzed using SPSS 19.0 statistical software with variance analysis and multiple comparisons. The following linear models were used for ANOVA:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (2)$$

where μ is the general mean, α_i is the effect of the ploidy lines, and ε_{ij} is the random error term.

Results

Phenotype investigation of different ploidy lines during drought stress treatment

The CK, T12 and F20 lines showed normal and healthy growth before drought stress treatment. With increasing duration of drought stress, the symptoms of damage displayed by each became apparent. The leaf color around veins turned yellow, the yellow area gradually expanded, and the leaf finally withered and curled.

Three days after the onset of drought stress treatment, yellow stripes appeared on the CK leaves. The F20 leaves also exhibited drought stress injury but to a lesser degree than CK. The T12 leaves showed no effect of drought stress at 3 days. At 6 days after drought stress treatment, brown stripes appeared on the CK and F20 leaves. Drought injury to leaves was more severe in the CK leaves than in the F20 leaves. At 6 days of treatment, the T12 leaves began to display slight symptoms of drought stress injury. At 9 days of drought stress treatment, most of the CK leaves had withered, and the symptoms of injury were severe. The degree of injury of the F20 leaves was less than that recorded for the CK leaves. Only the edges of the T12 leaves turned yellow and withered. At 12 days of drought stress treatment, the CK plants exhibited more severe drought stress injury. The leaves had withered, and the leaf edges showed evidence of necrosis and rolled up. The T12 leaves turned yellow and withered, and the edges of the

F20 leaves turned scorched. Throughout the process of drought stress treatment, the drought stress symptoms of the T12 leaves were less pronounced than those of the CK and F20 leaves. The CK leaves displayed the most severe drought stress damage (Fig. 1).

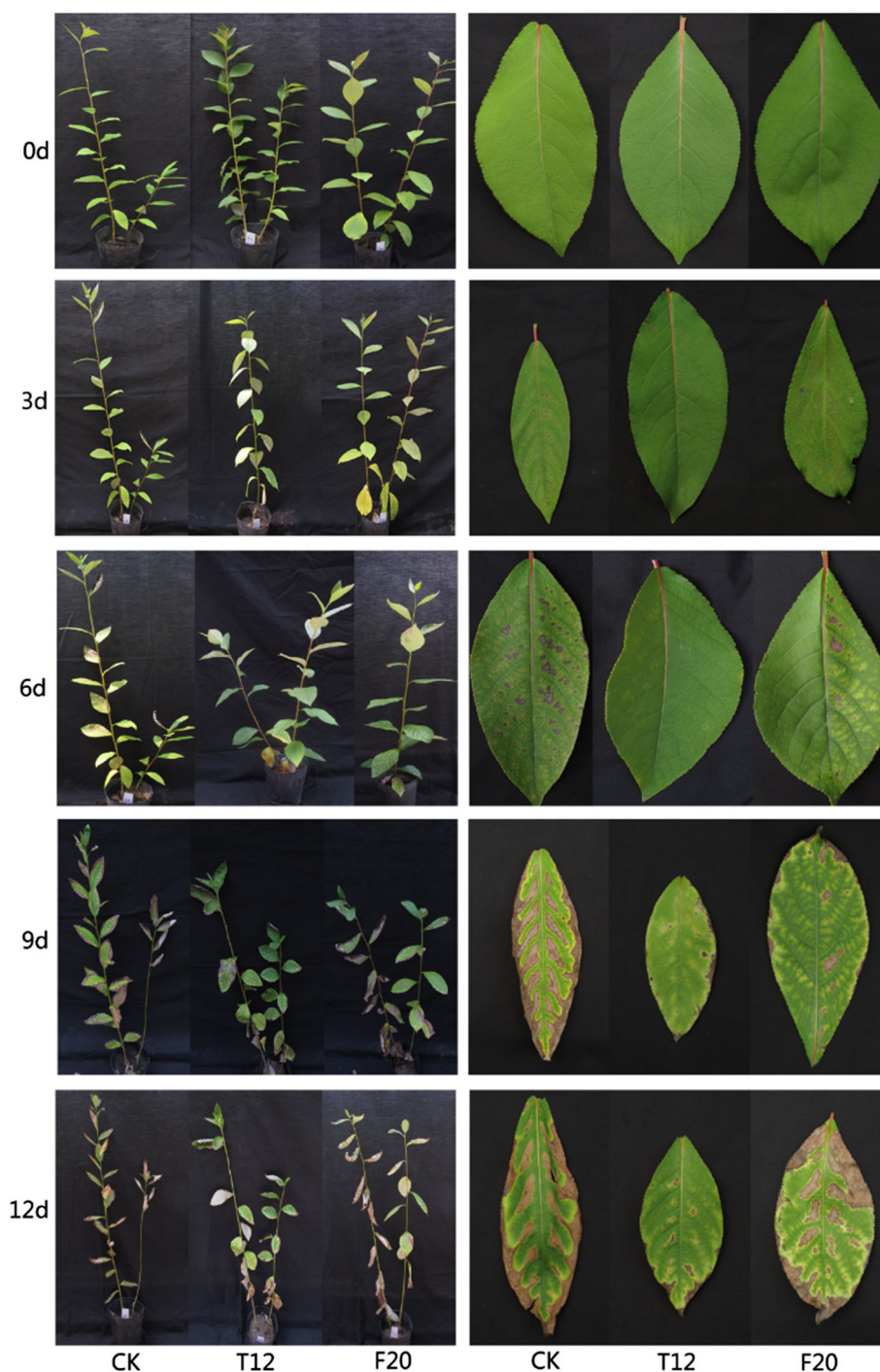
Cell membrane permeability of leaves of the different ploidy lines under drought stress

Relative electrical conductivity (REC) reflects the osmotic regulation capacity of the plasma membrane under drought stress. The prestress (0 day) REC values were set as the comparison values for the different ploidy lines. Under normal water conditions, there was no obvious REC difference among leaves from the three different ploidy lines. Throughout the drought stress treatment, the REC values of the leaves of the three different ploidy lines showed an increasing trend, but their increasing ranges differed from one another. At 3 days of drought stress, the REC values increased by 11.2% for CK and 11.1% for F20 leaves. The REC of T12 leaves was 7.3%, which was lower than the REC values for CK and F20 leaves ($P < 0.01$). At 12 days of drought stress, the CK leaves exhibited the maximum recorded REC values, while the T12 leaves exhibited the minimum REC values, with a lower mean REC for T12 leaves ($P < 0.01$), (Fig. 2).

MDA contents of leaves of the different ploidy lines under drought stress

MDA is the final product of membrane lipid peroxidation, which can combine with the membrane protein, the enzyme and the cross-linker to inactivate and destruct the structure and function of the biofilm. The initial (0 day) MDA contents were set as the comparison values for the different ploidy lines. The MDA contents of the leaves from the three different ploidy lines increased with stress as time went on. Under normal water conditions, the MDA contents of the leaves from the three different ploidy lines were not identical, while the CK leaves contained the highest MDA contents. On the 3rd day after drought stress, the MDA contents of CK leaves had significantly increased by 39.0% compared with T12 and F20 (increased by 9.3 and 37.8%, respectively) ($P < 0.01$). On the 9th day after drought stress, the MDA contents of CK leaves had increased by 43.3%, while those of F20 leaves had increased by 32.2% and those of T12 leaves had increased by 19.8% ($P < 0.01$). On the 12th day after drought stress, the CK leaves contained 30.03 nmol g⁻¹ MDA, the F20 leaves contained 24.76 nmol g⁻¹ MDA, and the T12 leaves contained 20.02 nmol g⁻¹ MDA ($P < 0.05$) (Fig. 3). The results show that under drought stress, the CK leaves

Fig. 1 Dynamic morphological observation of different polyploidy lines of *P. ussuriensis* under drought stress conditions. [The third functional leaves of the CK, T12 and F20 lines were dynamically observed at 0, 3, 6, 9 and 12 days after drought stress. The leaves in this figure presented the average degree of drought stress injury of each detected line (a CK, b T12, c F20)]



suffered the most severe membrane damage and exhibited the weakest oxidation resistance.

The PRO contents of leaves of the different ploidy lines under drought stress

Proline is an osmotic regulator whose content is affected by the availability of water, and when soil moisture is deficient, the free proline content increases. The initial (0 day)

PRO contents were set as the comparison values for the different ploidy lines. Under normal water conditions, the PRO contents of leaves of the three different ploidy lines did not exhibit significant differences, but the T12 leaves contained the highest PRO content (Fig. 4). Under drought stress, the PRO contents of CK, T12 and F20 leaves first increased and then decreased, but their peak time points and increasing ranges were different. On the 3rd day after drought stress, the PRO content of CK reached its peak

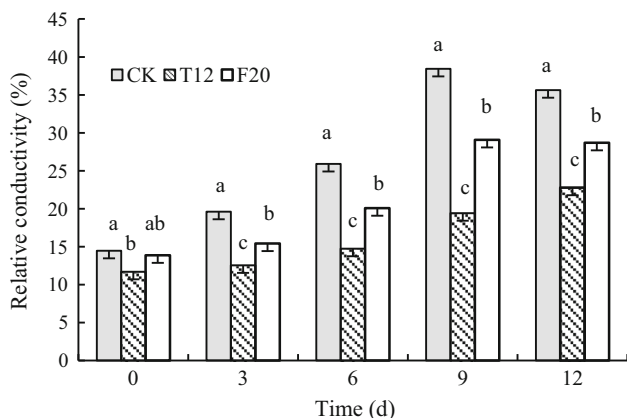


Fig. 2 The relative electrical conductivity of leaves of different ploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in relative electrical conductivity (REC) (unit is nmol g^{-1} FW) in CK, T12 and F20 leaves during drought stress treatment. Data are mean \pm SD ($n = 3$), ($P < 0.05$)]

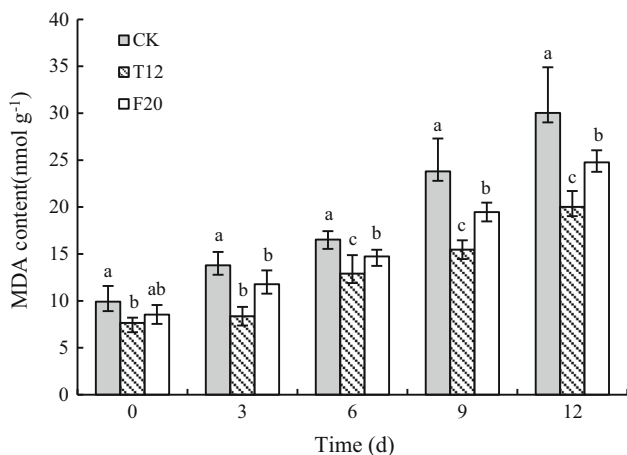


Fig. 3 The MDA contents of leaves of different ploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in MDA content (unit is nmol g^{-1} FW) in CK, T12 and F20 leaves during drought stress treatment. Data are mean \pm SD ($n = 3$), ($P < 0.05$)]

level ($31.16 \mu\text{g g}^{-1}$); the PRO content of T12 was $36.302 \mu\text{g g}^{-1}$; and the PRO content of F20 was $29.93 \mu\text{g g}^{-1}$, indicating that T12 was the most sensitive to drought stress ($P < 0.05$). On the 6th day after drought stress, the PRO contents of the triploid T12 and the tetraploid F20 reached their peak, but their peak values, which were 43.31 and $31.14 \mu\text{g g}^{-1}$ ($P < 0.01$), respectively, were significantly different. On the 12th day after drought stress, the PRO contents of CK leaves decreased by 19.7%, while the PRO contents of T12 and F20 decreased by 9.3 and 13.7%, respectively ($P < 0.01$).

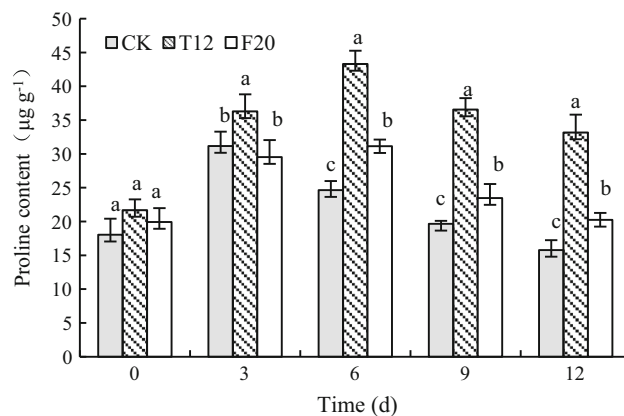


Fig. 4 The PRO contents of leaves of different ploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in proline content (unit is $\mu\text{g g}^{-1}$ FW) in CK, T12 and F20 leaves during drought stress treatment. Data are mean \pm SD ($n = 3$), ($P < 0.05$)]

The SOD activities of leaves from the three different ploidy lines under drought stress

The SOD enzyme activity reflects the ability of plants to clear active oxygen. The initial (0 day) SOD activities were set as the comparison values for the different ploidy lines. Under normal water conditions, the SOD activities of T12 leaves were higher than those of CK and F20 (Fig. 5). After drought stress, the SOD activities of CK, T12 and F20 leaves first increased and then decreased with stress as time went on. On the 3rd day after drought stress, the SOD activity increased to its maximum level, and T12 displayed the highest increasing range, while CK exhibited the lowest increasing range. Moreover, the SOD activities of T12 leaves increased rapidly, their average peak value reached up to 263.97 U g^{-1} , and their increasing range was 85.2%;

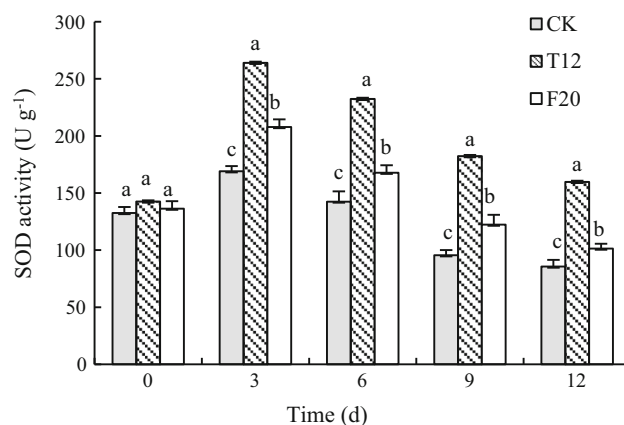


Fig. 5 The SOD activities of leaves of different ploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in SOD activity (unit is U g^{-1} FW) in CK, T12 and F20 leaves during drought stress. Values labeled with different letters differ significantly among different lines; data are mean \pm SD ($n = 3$), ($P < 0.05$)]

the SOD activity increasing range of CK leaves was at its minimum, 27.6%, and its peak value was 169.06 U g^{-1} ; the SOD activity increasing range and the peak value of F20 leaves were 52.4% and 207.79 U g^{-1} , respectively ($P < 0.01$). During the entire stress treatment, T12 exhibited the highest SOD activity, while CK exhibited the lowest SOD activity.

The POD activities of leaves of the three different ploidy lines under drought stress

Peroxidase mainly catalyzes the oxidation of hydrogen peroxide on various organic and inorganic substances. In the absence of hydrogen peroxide, oxygen molecules are oxidized as hydrogen receptors. The initial (0 day) POD activities were set as the comparison values for the different ploidy lines. The POD activity of T12 was higher than that of CK and F20 under normal water conditions (Fig. 6). Under drought stress, the POD activities of CK, T12 and F20 initially increased and then decreased, but their peak time points and increasing ranges were different. Specifically, on the 3rd day after drought stress, the POD activity of CK reached its peak value (298.284 U g^{-1}), with an increase of 23.9%; the POD activity and the increase of F20 leaves were 304.48 U g^{-1} and 23.2%, respectively; and the POD activity (336.51 U g^{-1}) and the increasing range (32.9%) of T12 were the highest. On the 6th day after drought stress, the POD activity of T12 (363.24 U g^{-1}) and F20 (325.66 U g^{-1}) leaves reached their peak levels, and their increasing ranges were 7.9 and 7.0%, respectively; the POD activity of CK leaves decreased by 9.8%. On the 9th day after drought stress, the POD activities of CK, T12 and

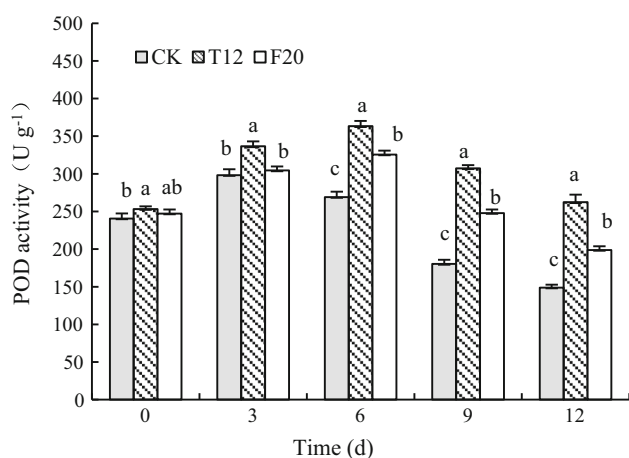


Fig. 6 The POD activities of leaves of different polyploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in POD activity (unit is $\text{U g}^{-1} \text{ FW}$) in CK, T12 and F20 leaves during drought stress. Values labeled with different letters differ significantly among different lines; data are mean \pm SD ($n = 3$), ($P < 0.05$)]

F20 leaves decreased by 33.0, 15.4 and 23.9%, respectively.

The CAT activities of leaves of the three different ploidy lines under drought stress

Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen. The initial (0 d) CAT activities were set as the comparison values for the different ploidy lines. Under normal water conditions, the CAT activity of T12 was higher than that of F20 and CK (Fig. 7). The CAT activities of CK, T12 and F20 reached their peak levels on the 3rd day after drought stress treatment. The increasing range of T12 leaves was 65.8%, and the activity value was $236.94 \text{ U g}^{-1} \text{ min}^{-1}$ ($P < 0.01$). The CAT activity of CK (51.3%) increased more obviously than that of F20 (48.7%), but the enzyme activity value of F20 ($203.19 \text{ U g}^{-1} \text{ min}^{-1}$) was higher than that of CK ($189.80 \text{ U g}^{-1} \text{ min}^{-1}$). The CAT activities of CK, T12 and F20 reached their lowest values on the 12th day after drought stress treatment; the values were 78.63, 118.49 and $88.38 \text{ U g}^{-1} \text{ min}^{-1}$, respectively, and their decreasing ranges were 24.0, 19.6 and 23.9%, respectively ($P < 0.01$).

Discussion

Drought tolerance of plants involves complex physiological regulation mechanisms that combine various physiological and biochemical factors, and are related to the plant's own genetics and the interaction between plants and

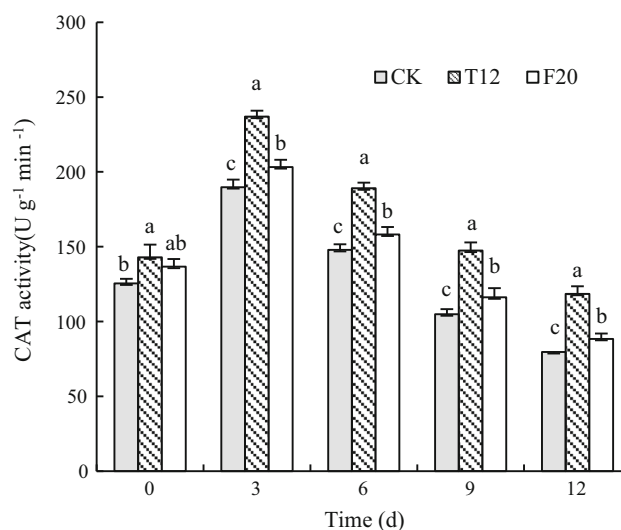


Fig. 7 The CAT activities of leaves of different polyploidy *P. ussuriensis* lines during drought stress. [The dynamic changes in CAT activity (unit is $\text{U g}^{-1} \text{ min}^{-1} \text{ FW}$) in CK, T12 and F20 leaves during drought stress. Values labeled with different letters differ significantly among different lines; data are mean \pm SD ($n = 3$), ($P < 0.05$)]

the external environment (Gupta and Huang 2014; Xiong and Zhu 2003). Generally, we cannot directly judge the drought tolerance characteristics of plants by only one index. In recent years, environmental adaptability research on polyploid plants has made significant progress. Ramsey's survey found that hexaploid yarrow was distributed in arid dunes, while tetraploid yarrow was mostly distributed in moist grasses; moreover, the hexaploid proved more adaptable to drought than the tetraploid. Several published reports proposed that the effects of the antioxidant enzymes in tetraploid and hexaploid *Cenchrus* on the removal of ROS were greater than those in diploids under drought stress; similarly, tetraploid melon and tobacco showed strong drought tolerance (Sivakumaran, et al. 1980; Sutka et al. 1995). The results achieved in the present study also showed that there were differences in the drought tolerance-related traits between the diploid *P. ussuriensis* and polyploid *P. ussuriensis*, and these results were in agreement with previously published research. Based on the determinations of drought tolerance-related physiological and biochemical indicators, the performance of leaves of different ploidy lines of *P. ussuriensis* was different. Under drought stress, plant growth was weakened, specific leaf area was damaged, and leaf number and biomass significantly decreased (Bréda et al. 2006; Xu and Zhou 2005). The morphological observations after drought stress showed that the leaves of the polyploid strains (T12 and F20) exhibited less injury than those of the diploid, and the CK plants suffered the most severe drought stress damage.

Increased cell membrane permeability is the most direct and obvious injury to the cell membrane by water stress (Bajji et al. 2002). As the stability of the cell membrane decreases under drought stress, the selective permeability of the cell membrane changes or is completely lost, and thus, the cells are damaged due to a large amount of intracellular ion osmosis (Pembrey et al. 1999). In the process of drought stress treatment, the increasing range of REC of T12 leaves was the smallest, and its peak value was the lowest, while CK leaves exhibited the maximum REC increasing range and the highest peak value. The results indicated that the stability of the triploid cell membrane was better than that of the diploid under drought stress conditions. MDA is an important product of membrane lipid peroxidation, and its content can reflect the relative permeability of the plasma membrane, which is one of the indices of the degree of plant injury (Dhindsa et al. 1981; Yan et al. 1996). Generally speaking, with an increase in the degree of water stress, MDA content will increase rapidly. The MDA peak value (Sairam and Srivastava 2001) and increasing range of T12 leaves were both lower than those of CK and F20 leaves in the process of drought stress, indicating the triploid leaves suffered less drought stress damage. Under osmotic stress, plants can reduce

water potential by accumulating a certain amount of solutes to maintain the water balance and ensure normal growth (Morgan 1984; Hsiao 1973; Anjum et al. 2011). Among these solutes, proline is the most widely distributed and the most effective. When the plant is confronted with drought stress, proline can balance cellular metabolism, and thus, the antireversion force of plants will be enhanced (Weimberg et al. 1984; Lisar et al. 2012). Our results showed that the PRO content of the triploid T12 was significantly increased on the 6th day after drought stress. The PRO contents of T12 and F20 on the 12th day after drought stress treatment increased slightly compared to untreated plants (0 day). SOD, POD and CAT are important components of the antioxidant enzyme system (Anjum et al. 2011) and play important roles in the removal of superoxide radicals, hydrogen peroxide and peroxides, and in the prevention of or reduction in the formation of radicals (Pan et al. 2006; Gong et al. 2005). They are scavengers of oxygen free radicals. Under drought stress, the balance of free radical production and the cell removal system are destroyed, and excess free radicals can damage the cell membrane systems (Jung 2004; Toivonen and Sweeney 1998). According to the present study, the SOD/POD/CAT activity of T12 leaves was higher than that of CK and F20 leaves during the whole drought stress treatment, indicating that the triploid strain contains a stronger antioxidant defense system than the diploid CK and the tetraploid F20 (T12 > F20 > CK). In summary, the results obtained in the present study suggest that the triploid *P. ussuriensis* showed stronger tolerance to drought stress than the tetraploid and diploid under short-term drought stress.

Conclusion

Genome doubling has improved the activities of some drought stress tolerance-related enzymes (such as SOD, POD and CAT) and the productivity of some drought stress tolerance-related metabolites (such as proline and MDA) in *P. ussuriensis* polyploid clones (T12 and F20). In our analysis, 6 physiological and biochemical indices (which are related to drought stress tolerance) of three different ploidy lines of *P. ussuriensis* were evaluated. We conclude that the triploid showed stronger drought stress tolerance than the tetraploid and diploid plants under short-term drought stress. In order to discover the drought stress tolerance mechanisms of polyploid *P. ussuriensis*, further molecular experiments on gene and protein expression are needed. Taken together, the results and conclusions of this study can guide research on drought stress tolerance mechanisms in different ploidies of *P. ussuriensis* while producing alternative germplasm resources for work on poplar stress tolerance breeding in the future.

Acknowledgements This work was supported by the National Key R&D Program of China (Grant No. 2016YFD0600404).

Author's contribution JX and KL: conceived and designed the experiments; JX, JJ and HZ: performed the experiments; JX and HZ: analyzed the data; KL: contributed reagents/materials/analysis tools; JX: wrote the paper; HZ: critically read the manuscript.

Compliance with ethical standards

Conflict of interest There is no interest conflict.

References

- Abuduwaili J, Zhang ZY, Jiang FQ, Liu DW (2015) The disastrous effects of salt dust deposition on cotton leaf photosynthesis and the cell physiological properties in the Ebinur Basin in Northwest China. *PLoS one* 10:e0124546. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4430278/>
- Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53:1331–1341
- Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. *Afr J Agric Res* 6:2026–2032
- Bajji M, Kinet JM, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul* 36:61–70
- Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann For Sci* 63:625–644
- DaMatta FM (2004) Exploring drought tolerance in coffee: a physiological approach with some insights for plant breeding. *Braz J Plant Physiol* 16:1–6
- Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot* 32:93–101
- Eifler I (1960) The individual results of crosses between *B. verrucosa* and *B. pubescens*. *Silvae Genetica* 9:159–165
- Fan HF, Du CX, Guo SR (2012) Effect of nitric oxide on proline metabolism in cucumber seedlings under salinity stress. *J Am Soc Hortic Sci* 137:127–133
- Fang GG, Deng YJ, Li P (2001) Evaluation of pulping performance of triploid *Populus tomentosa*. *For Sci Technol Manag (Suppl.)*:87–90
- Flowers TJ (2004) Improving crop salt tolerance. *J Exp Bot* 55:307–319
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci* 169:313–321
- Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int J Genomics*. Article ID 701596. https://www.researchgate.net/publication/262114029_Mechanism_of_Salinity_Tolerance_in_Plants_Physiological_Biochemical_and_Molecular_Characterization. Accessed 3 Apr 2014
- Hasheminasab H, Assad MT, Aliakbari A, Sakhafi SR (2012) Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. *J Agric Sci* 4:20
- He C, Zhang W, Gao Q, Yang A, Hu X, Zhang J (2011) Enhancement of drought resistance and biomass by increasing the amount of glycine betaine in wheat seedlings. *Euphytica* 177:151–167
- He Q, Zhao S, Ma Q, Zhang Y, Huang L, Li G, Hao L (2014) Endogenous salicylic acid levels and signaling positively regulate *Arabidopsis* response to polyethylene glycol-simulated drought stress. *J Plant Growth Regul* 33:871–880
- Hsiao TC (1973) Plant responses to water stress. *Ann Rev Plant Physiol* 24:519–570
- Johnsson H (1944) Triploidy in *Betula alba* L. *Bot Notiser Lund* 1:85–96
- Jung S (2004) Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. *Plant Sci* 166:459–466
- Li SW, Zhang ZY, Luo JM, He CZ, Pu YS, An XM (2005) Progress and strategies in cross breeding of poplars in China. *For Stud China* 7:54–60
- Li RH, Guo PG, Michael B, Stefania G, Salvatore C (2006) Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agric Sci China* 5:751–757
- Lisar SYS, Motafakkerzad R, Hossain MM, Rahman IMM (2012) Water stress in plants: causes, effects and responses. In: Rahman IMM (ed) *Water stress*, vol 175–178(117). InTech, London, pp 635–642
- Liu W, Yu K, He T, Li F, Zhang D, Liu J (2013) The low temperature induced physiological responses of *Avena nuda* L., a cold-tolerant plant species. *Sci World J*. Article ID 658793. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3693167/>. Accessed 11 June 2013
- Martin MA, Ramos S, Mateos R, Granado Serrano AB, Izquierdo-Pulido M, Bravo L, Goya L (2008) Protection of human HepG2 cells against oxidative stress by cocoa phenolic extract. *J Agric Food Chem* 56:7765–7772
- Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J Environ Stud* 15:523–530
- Morgan JM (1984) Osmoregulation and water stress in higher plants. *Ann Rev Plant Physiol* 35:299–319
- Niwa Y, Sasaki Y (2003) Plant self-defense mechanisms against oxidative injury and protection of the forest by planting trees of triploids and tetraploids. *Ecotoxicol Environ Saf* 55:70–81
- Osmond CB, Bjorkman O, Anderson DJ (1980) Physiological processes in plant ecology. *Toward a synthesis with atriplex*. *Agro-Ecosystems* 8(3):268–271
- Otto SP, Whitton J (2000) Polyploid incidence and evolution. *Ann Rev Genet* 34:401–437
- Pan Y, Wu LJ, Yu ZL (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch). *Plant Growth Regul* 49:157–165
- Panchuk II, Volkov RA, Schöffl F (2002) Heat stress-and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. *Plant Physiol* 129:838–853
- Pembrey RS, Marshall KC, Schneider RP (1999) Cell surface analysis techniques: What do cell preparation protocols do to cell surface properties? *Appl Environ Microbiol* 65:2877–2894
- Pérez-Pérez JG, Syvertsen JP, Botia P, García-Sánchez F (2007) Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedlings during drought stress and recovery. *Ann Bot* 100:335–345
- Ramsey J (2011) Polyploidy and ecological adaptation in wild yarrow. *Proc Natl Acad Sci U S A* 108:7096–7101
- Randolph LF (1941) An evaluation of induced polyploidy as a method of breeding crop plants. *Am Nat* 75:347–363

- Sairam RK, Srivastava GC (2001) Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. *J AgronCrop Sci* 186:63–70
- Sita GL, Ram NVR, Vaidyanathan CS (1980) Triploid plants from endosperm cultures of sandalwood by experimental embryogenesis. *Plant Sc Lett* 20:63–69
- Sivakumaran S, Horgan R, Heald J, Hall MA (1980) Effect of water stress on metabolism of abscisic acid in *Populus robusta* × *schnied* and *Euphorbia lathyris* L. *Plant, Cell Environ* 3:163–173
- Stebbins GL (1970) Variation and evolution in plants: progress during the past twenty years. In: Stebbins GL (ed) *Essays in evolution and genetics in honor of Theodosius Dobzhansky: a supplement to evolutionary biology*. Appleton-Century-Crofts, New York, pp 173–208
- Storchova Z, Pellman D (2004) From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 5:45–54
- Sutka J, Farshadfar E, Kőszegi B, Friebe B, Gill BS (1995) Drought tolerance of disomic chromosome additions of *Agropyron elongatum* to *Triticum aestivum*. *Cereal Res Commun* 23:351–357
- Toivonen PMA, Sweeney M (1998) Differences in chlorophyll loss at 13 C for two broccoli (*Brassica oleracea* L.) cultivars associated with antioxidant enzyme activities. *J Agric Food Chem* 46:20–24
- Tsugane K, Kobayashi K, Niwa Y, Ohba Y, Wada K, Kobayashi H (1999) A recessive Arabidopsis mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. *Plant Cell* 11:1195–1206
- Weimberg R, Lerner HR, Poljakoff-Mayber A (1984) Changes in growth and water-soluble solute concentrations in Sorghum bicolor stressed with sodium and potassium salts. *Physiol Plant* 62:472–480
- Xiong L, Zhu JK (2003) Regulation of abscisic acid biosynthesis. *Plant Physiol* 133:29–36
- Xu ZZ, Zhou GS (2005) Effects of water stress and high nocturnal temperature on photosynthesis and nitrogen level of a perennial grass *Leymus chinensis*. *Plant Soil* 269:131–139
- Yan B, Dai Q, Liu X, Huang S, Wang Z (1996) Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil* 179:261–268
- Zhang J, Kirkham MB (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytol* 132:361–373
- Zhu ZT, Kang XY (1998) Studies on selection of natural triploids of *Populus tomentosa*. *Scientia Silvae Sinicae* 34:22–31