Responses of *H. vulgare* L. Seedlings to Basic Salt and Drought under Freeze-thaw Condition

Wenyi Tang
Jilin University
Guozhang Bao (baogz@jlu.edu.cn)
Jilin University
Bairu Yan
Environmental Monitoring Center Station of Jilin Province
Yan Qu
Jilin University
Jiancai Guo
Jilin University
Saning Zhu
Jilin University
Hongwei Zhao
The Administration of Jingyu Water Conservation

Research article

Keywords: basic salt, freeze-thaw, Hordeum vulgare L., drought, soluble protein, physiological responses

Posted Date: July 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41827/v1

Abstract

Background Qinghai-Tibet Plateau is known for characteristics of high altitude, low rainfall and varying temperature, and the crops in this area are susceptible to abiotic stresses such as drought, basic salt and freeze-thaw that caused damages in different perspectives such as the permeability of biological membrane, osmotic adjustment, and antioxidant enzyme system. *Hordeum vulgare* L. is an indispensable crop in plateau and plays an important role in agricultural ecosystem as well.

Result In this experiment, *H. vulgare* L. was used as experimental material and the physiological characteristics soluble protein (SP) content, malondialdehyde (MDA) content, antioxidant enzyme activity and relative water content (RWC) of seedlings were examined under freeze-thaw condition combined with drought and alkali stress.

Conclusion Research results indicated that under the combined stresses of basic salt and drought, *H. vulgare* L. seedlings were damaged by lipid peroxidation, weakened superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities, while enhanced osmotic adjustment ability in plants cell. It was suggested that, in agricultural management, the simultaneous occurrence of two stresses, basic salt and drought, should be avoided in the early stage of *H. vulgare* L. planting to reduce the physiological stress on plants.

Background

Lying in the mid-latitude westerly zone and subtropical region, the Qinghai-Tibet Plateau, with an average altitude of more than 4000 m, was known for the complex and changeable terrain, forming a unique plateau climate characteristic [1]. The fact that, during early spring, the freeze-thaw often occurs due to the long sunshine hours and the changeable temperature between day and night has various effects on the external morphology and internal physiological metabolism of plants [2–4]. The seasonal precipitation that is uneven and the evaporation that generally exceeds precipitation on the Tibetan Plateau make drought stress prone to occur during crop growth [5], which often causes oxidative stress by the accumulation of reactive oxygen species in plants [6], affecting the structure and growth of plant [7]. Attributing to current climate, the content of sodium bicarbonate ($NaHCO_3$) and sodium chloride (NaCl) in soil has increased in Qinghai-Tibet Plateau, where salinization becomes increasingly serious. It has been confirmed that alkali stress imposed more harm to crops than salt stress does [8]. Besides, one paper has previously reported that high pH of alkali stress can short root length and seedlings height of rye, reduce the content of water and chlorophyll and decrease the relative transpiration rate [9].

Barley, a cereal crop of the genus Gramineae, can grow at an altitude of 3000 ~ 3400 m. Among them, *Hordeum vulgare* L. has good resistance to cold and drought [10, 11], is the main crop in Tibet and Qinghai. In this experiment, as materials, the *H. vulgare* L. seedlings were treated with basic salt, drought and freeze-thaw stress to artificially simulate the growing environment of plants. The relative water content (RWC), antioxidant enzyme activity, contents of malondialdehyde (MDA) and soluble protein (SP)

were examined in order to study the response characteristics of plants to drought, basic salt and freeze-thaw.

Results

Effect on relative water content of seedlings

In this experiment, the relative water content (RWC) in of 8 treatment groups decreased during freeze-thaw cycle. As shown from Fig. 1, RWC in *H. vulgare* L. seedlings had a maximum decrease under combined stresses of basic salt and drought. The RWC in seedlings of groups FB0, F0D and F00 had no significant differences compared to that in groups 0B0, 00D and 000, respectively. However, RWC in seedlings of group FBD showed significant differences compared with that of group 0BD after T3. Notably, the RWC in *H. vulgare* L. seedlings of 4 freeze-thaw groups (F**) showed a sequence as F00 > FB0 > F0D > FBD. Consistently, a similar order of RWC in seedlings can be observed among 4 non-freeze-thaw groups (0**) as well, that is, 000 > 0B0 > 00D > 0BD.

Effect on soluble protein content of seedlings

It can be observed that the soluble protein (SP) content in *H. vulgare* L. seedlings of 4 treatment groups under freeze-thaw stress (F**) was higher than that of 4 treatment groups without freeze-thaw stress (0**), respectively (Fig. 2). The SP content in seedlings of groups 0B* was significantly higher than that of groups 00* ($P < 0.05$), which indicated that under non-freeze-thaw conditions, the SP content in seedlings increased due to the occurrence of basic salt stress. Nevertheless, the SP content in seedlings of groups under either single freeze-thaw stress or single drought stress had no significant difference compared with that of control group ($P > 0.05$). In the case of freeze-thaw stress, the SP content in seedlings of groups F** increased during the period of freeze-thaw (T1-T7). Among them, group FAD reached the maximum at T5, which exhibited a further 41.2% increase than the minimum (T3). Somewhat differently, the other 3 groups FB0, F0D and F00 all reached the maximum value at T6, and were 46.4%, 86.6% and 72.7% higher than the minimum value at T5, T1 and T5, respectively.

Effect on MDA content of seedlings

Figure 3 shows that the malondialdehyde (MDA) content in *H. vulgare* L. seedlings of all experimental groups was higher than that of the control group. The non-freeze-thaw groups (0**) fluctuated little during a 14-hour freeze-thaw period, however the MDA content in *H. vulgare* L. seedlings of groups 0B* was significantly higher than that of other groups 00* ($P < 0.05$). Besides, we have noticed that MDA content in *H. vulgare* L. seedlings in response to group 00D was significantly higher than that in response to blank treatment ($P < 0.05$). Under the freeze-thaw stress, the MDA content in *H. vulgare* L. seedlings of single freeze-thaw group (F00) was significantly lower than that of groups F0D and FBD ($P < 0.05$).

Effect on SOD activity

The SOD activity in *H. vulgare* L. seedlings of groups *BD had no significant difference compared with control group (000) (Fig. 4). The SOD activity significantly enhanced owing to the occurrence of drought stress (00D) in *H. vulgare* L. seedlings ($P < 0.05$), while significantly weakened due to the basic stress (0B0) in seedlings ($P < 0.05$). Under freeze-thaw stress, the SOD activity in *H. vulgare* L. seedlings of groups F** decreased at first and then increased. During the freeze-thaw cycle, except for T4, the SOD activity in *H. vulgare* L. seedlings of group F0D was significantly higher than that of groups FBD, FB0 and F00 ($P < 0.05$).

Effect on CAT activity

It can be seen from Fig. 5 that, under non-freeze-thaw conditions, the CAT activity in *H. vulgare* L. seedlings of groups 0B0, 00D and 0BD was significantly lower than that of the control group (000) ($P < 0.05$). CAT activity in seedlings of either group F00 or group FB0 showed a trend of initially increasing and then decreasing, while that of group F0D showing a general downward trend, and that of group FBD showing an upward trend. Take if further, the CAT activity in seedlings of group FBD was significantly lower than that of group F00 ($P < 0.05$), and that of the groups FB0 and F0D was significantly lower than that of group F00 only in the latter thawing case ($P < 0.05$).

Effect on POD activity

In this experiment, during freeze-thaw cycle, the POD activity in *H. vulgare* L. of groups F00 and F0D showed a trend of initially increasing and then decreasing, while that of groups FB0 and FBD showing an increasing trend (Fig. 6). When the temperature dropped to 10 °C (T1), the POD activity in *H. vulgare* L. seedlings of groups F00, 0B0 and 00D were significantly lower than that of control group ($P < 0.05$). Under basic salt stress, the POD activity in seedlings significantly decreased within a freeze-thaw cycle ($P < 0.05$). Nevertheless, no significant difference was observed in POD activity between groups F0D and 00D. Accordingly, under freeze-thaw stress, there was a significant reducing of POD activity of groups subjected to basic salt treatment, but no effect on that of groups subjected to drought treatment.

Correlation Analysis

It can be observed from Table 1 that the content of SP and MDA in seedlings were significantly positively correlated under freeze-thaw conditions ($P < 0.01$). There was a significant negative correlation between SP content and antioxidant enzyme activity ($P < 0.01$). MDA was significantly negatively correlated with antioxidant enzyme activity and RWC, while CAT and POD were positively correlated ($P < 0.01$).

Table 1
Pearson correlation analysis between relative water content (RWC), soluble protein (SP) content, malondialdehyde (MDA) content, SOD, CAT and SOD activity in *H. vulgare* L. seedlings of freeze-thaw treatment groups (F**)

	RWC	SP	MDA	SOD	CAT	POD
RWC	1	-0.524**	-0.427*	-0.341	0.726**	0.640**
SP		1	0.750**	-0.351	-0.799**	-0.868**
MDA			1	-0.407*	-0.663**	-0.798**
SOD				1	0.052	0.213
CAT					1	0.833**
POD						1

* Significant correlation at 0.05 level (both sides). ** Significant correlation at the 0.01 level (both sides).

Discussion

Either drought or alkaline salt can lead to a large amount of water loss in seedlings by reducing the osmotic pressure of plant cells [12]. It is one important conclusion from Alexander's research that the RWC of leaves is positively related to plant's stress resistance [13]. It has been reported that under the combined effects of water loss and high temperature, the reduction of RWC in Australian durum is greater than that under single stress [14]. Consistently, in this experiment, the combined effects on RWC in *H. vulgare* L. seedling of basic salt and drought were severer than the additive of basic salt and drought alone; thus, interactions between the two stress factors were synergistic for RWC. Under freeze-thaw condition, a decrease in temperature can not only ice the water in plant cells, but also caused a dehydration, resulting in a decrease in RWC [15]. As have shown that at the freezing stage, the decreased RWC contributed to alleviate the damage caused at freezing stage to plants and maintain the osmotic balance of plant cells [16].

Most of the soluble proteins in plants are enzymes involved in metabolism [17]. An increase of soluble proteins can maintain the cell's higher osmotic potential, enhance the capacity of water absorption and holding, maintaining plant growth and improving resistance to stress [18]. Here we observed from experiments that the SP content of *H. vulgare* L. seedlings increased under either drought or freeze-thaw stress (F00 and 00D), while a higher accumulation of SP was measured under the basic-salt compound stress (*B*). These observations may attribute to the expression of resistant proteins in plant cells stimulated by alkaline stress, increasing the content of SP participating in osmotic adjustment in cells, thus making plants adapt to the external environment [19]. Under freeze-thaw stress, the SP content in seedlings of groups F** decreased with the dropping temperature, which may be due to the accelerated decomposition of soluble proteins in cells, providing plants with energy to relieve the damage caused by

stresses [20]. At thawing stage, with the temperature rising to 10 ℃, the SP content in seedlings increased. These findings are similar to the results of Lee's research, in which they examined the proteomic changes of rice roots under low temperature stress and found that the expressions of 27 proteins were up-regulated at 10 ℃ [21].

The activity changes of antioxidant enzyme in plants caused by abiotic environmental stresses may have an effect on physiological characteristics to reduce damage [22]. In a previous study, Zeng et al [23], using methods of indoor cultivating of soybean seedlings and experiments, disclosed that a large amount of CAT transcription and significant enhancement of enzyme activity were observed under high aluminum stress. Researches have shown that the antioxidant enzyme activities in leaves of *Pyracantha fortuneana* and *Rosa cymosa* are significantly enhanced under severe drought stress, indicating the strong resistance to drought stress of these species, however the antioxidant enzyme activities is greatly weakened in leaves of *Broussonetia papyrifera* and *Cinnamomum bodinieri* under same conditions, indicating the weaker resistance to drought stress [24]. Here, our experiment showed that CAT and POD activities in *H. vulgare* L. seedlings significantly weakened under non-freeze-thaw stress. The results suggested a possible reason of lipid peroxidation on the cell membrane affected by stresses duration, leading to the damage to cells and the effect on the synthesis of substances like proteins in cells, at last reducing the antioxidant enzyme activity. This is consistent with the study by Gao et al [25]. Moreover, an observed decrease in SOD activity in the leaves of *Camptotheca acuminata* seedlings accompanied low temperature stress, which was found by Feng et al. (2002). It was worth noting that CAT and POD activities increased, while SOD activity decreased with a decrease of temperature, which could be explained by the role played by SOD as the first line in defensing and eliminating reactive oxygen species (ROS). A large consumption of SOD in the process of eliminating ROS and an inefficient synthesis of enzymes in the case of low temperature were confirmed in the research results of Bao et al [17].

Environmental stress can disrupt the homeostasis of cells and the dynamic balance between production and clearance of ROS, leading to excessive accumulation of ROS in cells, causing the oxidative damage to biomolecules such as lipids [27], proteins [28] and nucleic acids [29], and the disruption of osmotic balance in plants [30], which were discussed in detail in numerous studies. MDA is the end product of lipid peroxidation and can be induced by stress in plants organ, e.g. leaves, shoots or roots [15, 31]. In this paper, MDA content in *H. vulgare* L. seedlings increased under the single or combined stress of basic salt and drought, importantly, MDA content accumulated more under single basic salt stress than that under combined stress. In addition to osmotic stress on seedlings, basic salt stress, compared with drought, is accompanied by high pH stress as well, causing damage to plant cell membranes and eventually leading to the accumulation of MDA, which has been confirmed in a study by Ali et al. [32]. The freeze-thaw manipulation treatments decreased the MDA content of *H. vulgare* L. seedlings of groups F**, during which, the activities-enhanced CAT played a key regulatory role [33].

Conclusion

In summary, as an important crop on the Qinghai-Tibet Plateau, *H. vulgare* L. has equipped with great resistance to the freeze-thaw environment within the long-term evolution. Considering current global warming, soil salinization and drought have become increasingly serious, resulting in physiological responses like the accumulation of MDA and the changes in antioxidant enzymes activity. Herein we showed that either single or compound stresses of drought, basic salts and freeze-thaw could make MDA accumulated excessively in seedlings because of the imbalance between oxygen free radical reaction and lipid peroxidation reaction, which caused oxidative stress on plants, affecting the stability of plant cells. Moreover, the contents of MDA and SP in *H. vulgare* L. seedlings increased significantly under the combined stresses of basic salt and drought, while the RWC significantly reduced. As a conclusion, to avoid simultaneous occurrence of basic salt and drought stress, the intensity of spring irrigation is supposed to increase in areas with severe basic salt stress. Though the resistance characteristics of plants under one freeze-thaw cycle were studied in this paper, in view of multiple freeze-thaw cycles in nature, one important future direction of physiological responses to freeze-thaw stress is studying the different resistance characteristics of plant between under one freeze-thaw cycle and under multiple freeze-thaw cycles.

Methods

Seeds cultivation and basic salt treatment

The full-grained *H. vulgare* L. seeds from Plateau Crop Cultivation Laboratory of Tibet College of Agriculture and Animal Husbandry were selected and soaked with 0.1% $KMnO_4$ solution for 2 h for disinfection, after which the seeds were rinsed with deionized water until the water becoming clear and then evenly placed on 8 culture dishes randomly numbered FBD, FB0, F0D, F00, 0BD, 0B0, 00D and 000 (Table 2). 1/2 of Hoagland nutrient solution was used to prepare 60 mM $NaHCO_3$ mixed solution (pH = 8.06), 500 ml of which was added to the cultivated dishes numbered like *B*, at the same time, 500 ml 1/2 Hoagland nutrient solution was added to the others numbered like *0*. 8 dishes of seeds were placed in MGC-450BP light incubator for germination, of which the cultivated conditions were set as 12 h light (25 ℃) and 12 h non-light (15 ℃). Daily watering (50 ml) was necessary during the cultivation.

Table 2
Experimental design of groups under basic salt (B), drought (D) and freeze-thaw (F) stress

	FBD	FB0	F0D	F00	0BD	0B0	00D	000
Basic salt	+	+	-	-	+	+	-	-
Drought	+	-	+	-	+	-	+	-
Freeze-thaw	+	+	+	+	-	-	-	-
+ add stress, - no stress								

Drought treatment

After seedlings were cultivated to 15 cm high with 2 or 3 leaves (around 1 week), they were treated with drought stress. $NaHCO_3$ mixed solution was used to prepare 20% PEG-6000 mixed solution for combined treatment of basic salt and drought stress, and 1/2 Hoagland nutrient solution was used to prepare 20% PEG-6000 solution. The solution in the cultivated dishes numbered like *BD was replaced with 500 ml PEG-$NaHCO_3$ mixed solution, in the cultivated dishes numbered *0D replaced with 500 ml PEG solution, in the cultivated dishes numbered *B0 replaced with 500 ml $NaHCO_3$ solution, in the cultivated dishes numbered *00 replaced with 500 ml 1/2 Hoagland nutrient solution. The drought treatment lasted for 48 h without watering.

Freezing and thawing stress treatment and sampling

After drought treatment, the cultivated dishes numbered like F** were put into BPHJ-120A high-low-temperature test chamber to carry out a freeze-thaw cycle for a period of 14 h, with the temperature curve being set as 15, 10, 5, 0, − 5, 0, 5 and 10 ℃, while other cultivated dishes numbered like 0** were maintained in light incubator under previous culture conditions. Initially, the cultivated dishes were placed in the chamber at 15 ℃ that closed to room temperature at night. Controlled precisely by program, the temperature decreased to − 5 ℃ steadily at a speed around 0.04 ℃/min, and then the temperature increased from − 5 to 10 ℃ at a speed around 0.04 ℃/min. After the freeze-thaw cycle being started, five parallel samples were taken every 2 hours from 8 cultivated dishes at random according to the required amount of the measurement, the corresponding sampling temperature was 10, 5, 0, − 5, 0, 5, 10 ℃ and recorded as T1-T7 respectively [34]. All the samples were firstly wrapped up with tin foil paper, secondly fixed in liquid nitrogen immediately for 50 s and finally put into the ultra-low-temperature freezer at − 80 ℃ for storage in order to measure the content of MDA and soluble protein, SOD, POD and CAT activity. At the same time, fresh leaves were taken to determinate RWC.

Determination

Relative water content (RWC)

The relative water content of seedlings was determined by the oven drying method [35]. For each sample (around 0.1 g), fresh weight supposed to be measured and recorded as F_W after drying the surface of leaves with filter paper. Completely being immersed in distilled water until the weight of leaves being constant, the leaves were taken out and wiped up with filter paper. The saturated fresh weight of the leaves at this time was measured and recorded as the T_W. Finally, the leaves were de-enzymed for 15 min in oven that was heated up to 105 ℃, and then dried to a constant weight in 80 ℃. The dry weight was measured and recorded as D_W. The RWC of leaves is calculated by formula (1)

$$\mathrm{RWC} = \left(\mathrm{T_w} - \mathrm{D_W}\right) / \left(\mathrm{F_W} - \mathrm{D_W}\right) \times 100\%$$

Soluble protein (SP) content

The soluble protein content in seedlings was determined by the Coomassie brilliant blue method [36]. 0.1 g leaves were selected randomly and shredded into a mortar, and then ground until homogenized with 5 ml distilled water, which next was centrifuged at a speed of 3000 r/min for 10 min. 1 ml of the supernatant was diluted in 5 times with 4 ml distilled water, of which 1 ml diluted supernatant was taken into a test tube with 5 ml of Coomassie brilliant blue solution being added. After the mixed solution being shaken and placed for 2 min, the absorbance of the solution was measured at 595 nm. The soluble protein content was calculated by standard curves.

Malondialdehyde (MDA) content

Malondialdehyde (MDA) content in seedlings was determined by the thiobarbituric acid method [36]. 0.5 g leaves were selected randomly and shredded into a mortar, and then ground into a homogenate with 5 ml 10% trichloroacetic acid (TCA) solution, which next was centrifuged at a speed of 4000 r/min for 10 min. Then 2 ml of the supernatant into was taken and fixed with 2 ml 0.6% thiobarbituric acid (TBA) solution. Mixtures was bathed in 99 °C water for 15 min, then cooled quickly in 5 min and centrifuged again at a speed of 4000 r/min for 10 min. The absorbance of supernatant was measured at 532 nm, 600 nm, and 450 nm. The MDA concentration and MDA content were calculated according to formulas (2) and (3).

$$\text{MDA concentration (μmol/L)} = 6.45 \times (\mathrm{D532} - \mathrm{D600}) - 0.56 \times \mathrm{D450} \quad (2)$$

$$\text{MDA content (μmol/g)} = \mathrm{cMDA} \times \mathrm{V_T} / \mathrm{F_W} \quad (3)$$

Where:

D450, D532, D600 are the absorbance at 450 nm, 532 nm and 600 nm, respectively.

cMDA is MDA concentration (μmol/L).

V_T is the volume of TCA solution (ml)

F_W is the fresh weight of seedlings (g);

Catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) activities

The activities of CAT, SOD and POD were determined with the CAT, SOD and POD kits provided by Nanjing Jiancheng Biological Institute [37]. A parallel sample (around 0.25 g) were randomly taken and ground to a homogenate with 5 ml phosphate buffer on ice. After centrifugations at a speed of 2500 r/min, 3500 r/min and 3500 r/min, respectively for 10 min, the supernatant was used for following measurements according to instructions of kits.

Data processing

The experiments were repeated five times, and the data were expressed as mean ± standard error (SE) (n = 5), which statistically analyzed using SPSS 19.0 software for one-way analysis of variance (ANOVA). When the variables were uniform, the significance analysis of data was analyzed using Duncan model, otherwise using Games-Howell model [38]. Pearson correlation coefficient was used to describe the correlation between variables. All results were shown in bars in figures plotted by Origin 8.0 software. Different letters presented in figures indicated significant differences between different treatment groups at the same time.

Abbreviations

MDA: malondialdehyde; TBA: thiobarbituric acid; TCA: trichloroacetic acid

Declarations

Ethics approval and consent to participate: Not applicable.

Consent to publish: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was supported by the National Natural Science Foundation of China under Grant No. 31772669 & 31270367. The funding bodies were not involved in the design of the study, collection, analysis, and interpretation of data, and in writing the manuscript.

Authors' Contributions: WY Tang, GZ Bao and BR Yan designed the experiments; WY Tang, Y Qu and JC Guo performed most of the experiments; SN Zhu performed part of the experiments; JC Guo and HW Zhao analyzed the data; and WY Tang and Y Qu wrote the manuscript. All authors agree with the manuscript contents and with its submission. All authors read and approved the final manuscript.

Author information

Affiliations

Key Laboratory of Groundwater Resources and Environment of the Ministry of Education (Jilin University); Jilin Provincial Key Laboratory of Water Resources and Environment; College of New Energy and Environment, Jilin University, Changchun, 130012 China

WY Tang, GZ Bao, Y Qu, JC Guo, SN Zhu

Contributions

WY Tang, GZ Bao and BR Yan designed the experiments; WY Tang, Y Qu and JC Guo performed most of the experiments; SN Zhu performed part of the experiments; JC Guo and HW Zhao analyzed the data; and WY Tang and Y Qu wrote the manuscript. All authors agree with the manuscript contents and with its submission. All authors read and approved the final manuscript.

Corresponding authors

Correspondence to Guozhang Bao.

References

1. Zhang Y, Xu G, Li P, Li Z, Wang Y, Wang B, et al. Vegetation Change and Its Relationship with Climate Factors and Elevation on the Tibetan Plateau. International journal of environmental research and public health. 2019;16(23).
2. Arfan M, Zhang C, Zhang D-W, Li D-X, Yan J-J, You M-H, et al CO2-EXCHANGE AND CHLOROPHYLL FLUORESCENCE RESPONSES OF FORAGE GRASSES DURING SALT STRESS AND RECOVERY IN QINGHAI-TIBETAN PLATEAU (QTP). Pakistan Journal Of Botany. 2019;51(5):1615–28.
3. Wang F, Gou X, Zhang F, Wang Y, Yu A, Zhang J, et al. Variations in leaf traits of Juniperus przewalskii from an extremely arid and cold environment. Science Of the Total Environment. 2019;689:434–43.
4. Xu HP, Zhang J, Pang XP, Wang Q, Zhang WN, Wang J, et al. Responses of plant productivity and soil nutrient concentrations to different alpine grassland degradation levels. Environmental Monitoring And Assessment. 2019;191(11).
5. Bibi S, Wang L, Li X, Zhang X, Chen D. Response of Groundwater Storage and Recharge in the Qaidam Basin (Tibetan Plateau) to Climate Variations From 2002 to 2016. Journal Of Geophysical Research-Atmospheres. 2019;124(17–18):9918–34.
6. Souza RP, Machado EC, Silva JAB, Lagoa A, Silveira JAG. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. Environmental Experimental Botany. 2004;51(1):45–56.
7. Duan B, Yang Y, Lu Y, Korpelainen H, Berninger F, Li C. Interactions between water deficit, ABA, and provenances in Picea asperata. Journal Of Experimental Botany. 2007;58(11):3025–36.
8. Alvarez-Acosta C, Marrero-Dominguez A, Gallo-Llobet L, Gonzalez-Rodriguez AM. Effects of NaCl and NaHCO3 stress on morphological growth and nutrient metabolism on selected avocados (Persea americana Mill.). Journal Of Plant Nutrition. 2019;42(2):164–77.
9. Guo H, Hu T, Fu J. Effects of saline sodic stress on growth and physiological responses of Lolium perenne. Acta Prataculturae Sinica. 2012;21(1):8.
10. Ahmed IM, Nadira UA, Bibi N, Cao F, He X, Zhang G, et al. Secondary metabolism and antioxidants are involved in the tolerance to drought and salinity, separately and combined, in Tibetan wild barley. Environmental Experimental Botany. 2015;111:1–12.

11. He X, Zeng J, Cao F, Ahmed IM, Zhang G, Vincze E, et al. HvEXPB7, a novel beta-expansin gene revealed by the root hair transcriptome of Tibetan wild barley, improves root hair growth under drought stress. Journal Of Experimental Botany. 2015;66(22):7405–19.
12. Shereen A, Khanzada MA, Baloch MAW, Asma, Shirazi MU, Khan MA, et al EFFECTS OF PEG INDUCED WATER STRESS ON GROWTH AND PHYSIOLOGICAL RESPONSES OF RICE GENOTYPES AT SEEDLING STAGE. Pakistan Journal Of Botany. 2019;51(6):2013–21.
13. Alexander RD, Wendelboe-Nelson C, Morris PC. The barley transcription factor HvMYB1 is a positive regulator of drought tolerance. Plant Physiology Biochemistry. 2019;142:246–53.
14. Liu H, Able AJ, Able JA. Genotypic performance of Australian durum under single and combined water-deficit and heat stress during reproduction. Scientific Reports. 2019;9.
15. Iseri OD, Korpe DA, Sahin FI, Haberal M. Hydrogen peroxide pretreatment of roots enhanced oxidative stress response of tomato under cold stress. Acta Physiol Plant. 2013;35(6):1905–13.
16. Hao W, Arora R, Yadav AK, Joshee N. Freezing Tolerance and Cold Acclimation in Guava (Psidium guajava L.). Hortscience. 2009;44(5):1258–66.
17. Bao G, Zhang M, Li Y, Chang Y, Tang W, Zhu S, et al PHYSIOLOGICAL RESPONSES OF ALFALFA SEEDLINGS TO FREEZE-THAW CYCLES AND ALKALINE SALT STRESS. Fresenius Environ Bull. 2019;28(5):4114–22.
18. Yin CY, Duan BL, Wang X, Li CY. Morphological and physiological responses of two contrasting Poplar species to drought stress and exogenous abscisic acid application. Plant Sci. 2004;167(5):1091–7.
19. Hazman M, Hause B, Eiche E, Riemann M, Nick P. Different forms of osmotic stress evoke qualitatively different responses in rice. Journal Of Plant Physiology. 2016;202:45–56.
20. Bae H, Kim MS, Sicher RC, Bae H-J, Bailey BA. Necrosis- and ethylene-inducing peptide from Fusarium oxysporum induces a complex cascade of transcripts associated with signal transduction and cell death in arabidopsis. Plant Physiol. 2006;141(3):1056–67.
21. Lee D-G, Ahsan N, Lee S-H, Lee JJ, Bahk JD, Kang KY, et al. Chilling stress-induced proteomic changes in rice roots. Journal Of Plant Physiology. 2009;166(1):1–11.
22. Ahsan N, Lee D-G, Lee S-H, Kang KY, Lee JJ, Kim PJ, et al. Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere. 2007;67(6):1182–93.
23. Zeng C-Q, Liu W-X, Hao J-Y, Fan D-N, Chen L-M, Xu H-N, et al. Measuring the expression and activity of the CAT enzyme to determine Al resistance in soybean. Plant Physiology Biochemistry. 2019;144:254–63.
24. Liu C, Liu Y, Guo K, Fan D, Li G, Zheng Y, et al. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. Environmental Experimental Botany. 2011;71(2):174–83.
25. Gao Y, Zhu Y, Yang Z, Du H. Effects of Drought Stress and Recovery on Antioxidant Enzyme Activities of Agropyron cristatum. Acta Agrestia Sinica. 2012;20(2):6.

26. Feng J, Zhang Y, Yang T. Effect of Low Temperature Stress on the Membrane-lipid Peroxidation and the Concentration of Free Proline in Camptotheca acuminata Seedling. Forest Research. 2002(2):6.
27. Mano Ji. Reactive carbonyl species: Their production from lipid peroxides, action in environmental stress, and the detoxification mechanism. Plant Physiology Biochemistry. 2012;59:90–7.
28. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 1997;324(Pt 1):1–18.
29. Cadet J, Douki T, Gasparutto D, Ravanat JL. Oxidative damage to DNA: formation, measurement and biochemical features. Mutation Research-Fundamental Molecular Mechanisms Of Mutagenesis. 2003;531(1–2):5–23.
30. Bian W, Bao G, Qian H, Song Z, Qi Z, Zhang M, et al. Physiological Response Characteristics in Medicago sativa Under Freeze-Thaw and Deicing Salt Stress. Water Air And Soil Pollution. 2018;229(6).
31. Karagoz H, Cakmakci R, Hosseinpour A, Kodaz S. ALLEVIATION OF WATER STRESS, AND PROMOTION OF THE GROWTH OF SUGAR BEET (BETA VULGARIS L. PLANTS BY MULTI-TRAITS RHIZOBACTERIA. Applied Ecology Environmental Research. 2018;16(5):6801–13.
32. Ali S, Bai P, Zeng F, Cai S, Shamsi IH, Qiu B, et al. The ecotoxicological and interactive effects of chromium and aluminum on growth, oxidative damage and antioxidant enzymes on two barley genotypes differing in Al tolerance. Environmental Experimental Botany. 2011;70(2–3):185–91.
33. Wu Y, Wu J, Bai T, Liu z, Zheng D. Physiological response and cold resistance of C. japonica‘Naidong'and C. japonica var. decumbens under different low temperature stress. Journal of Henan Agricultural University. 2018;52(4):9.
34. Gong Z, Chen W, Bao G, Sun J, Ding X, Fan C. Physiological response of Secale cereale L. seedlings under freezing-thawing and alkaline salt stress. Environmental science and pollution research international. 2020:8.
35. Colom MR, Vazzana C. Drought stress effects on three cultivars of Eragrostis curvula: photosynthesis and water relations. Plant Growth Regul. 2001;34(2):195–202.
36. Kong X, Yi X. Experimental techniques for plant physiology. 1st ed. Beijing: Chinese Agricultural Press; 2008.
37. Bao G, Ao Q, Li Q, Bao Y, Zheng Y, Feng X, et al. Physiological Characteristics of Medicago sativa L. in Response to Acid Deposition and Freeze-Thaw Stress. Water Air And Soil Pollution. 2017;228(9).
38. Warner R. Applied Statistics: From Bivariate Through Multivariate Techniques. 2nd ed. Thousand Oaks: SAGE Publications; 2007.

Figures

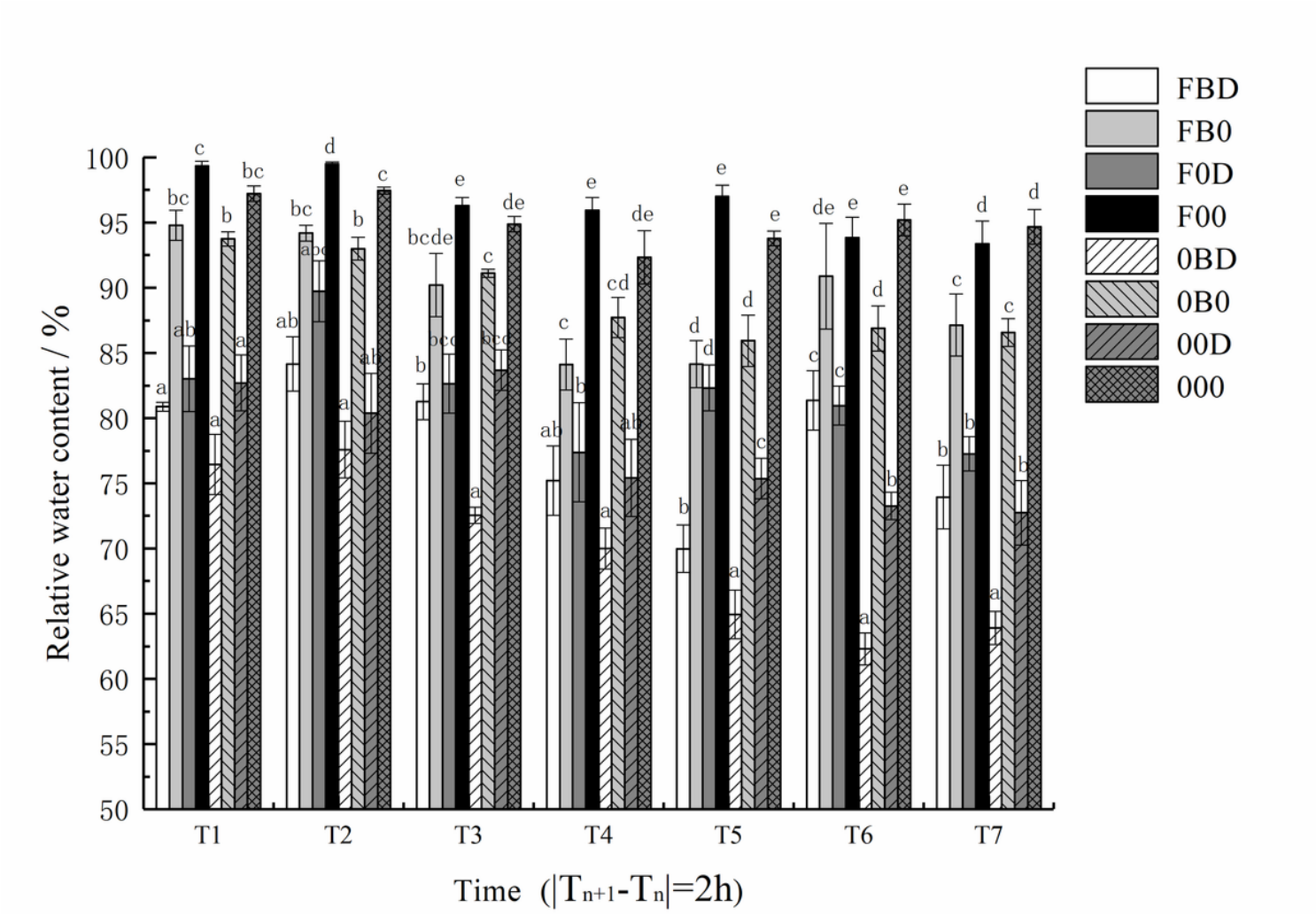

Figure 1

The relative water content in H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 ℃. The different low-case letters mean the significant difference at the same temperature ($P < 0.05$).

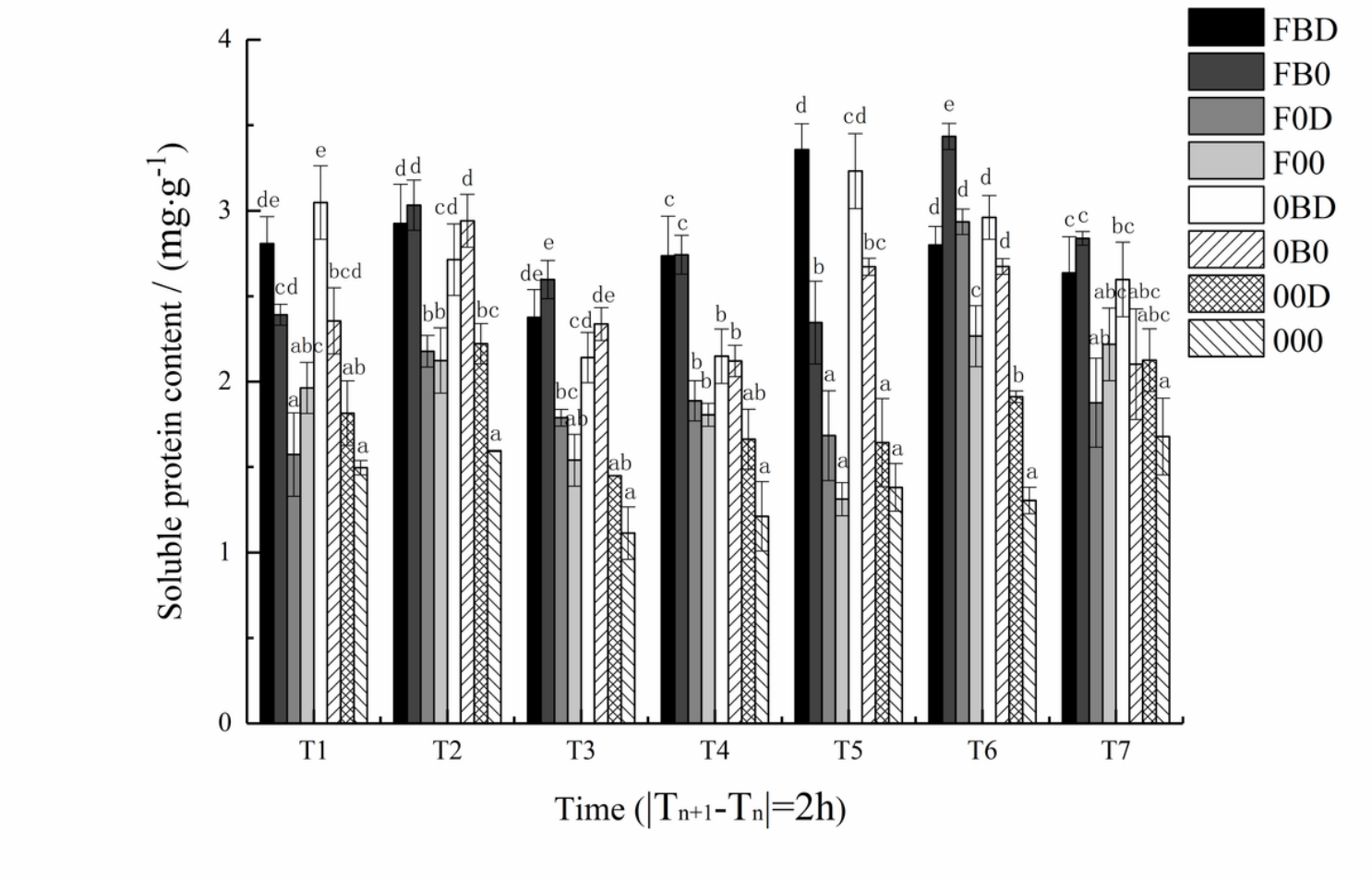

Figure 2

The soluble protein content in H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 ℃. The different low-case letters mean the significant difference at the same temperature ($P < 0.05$).

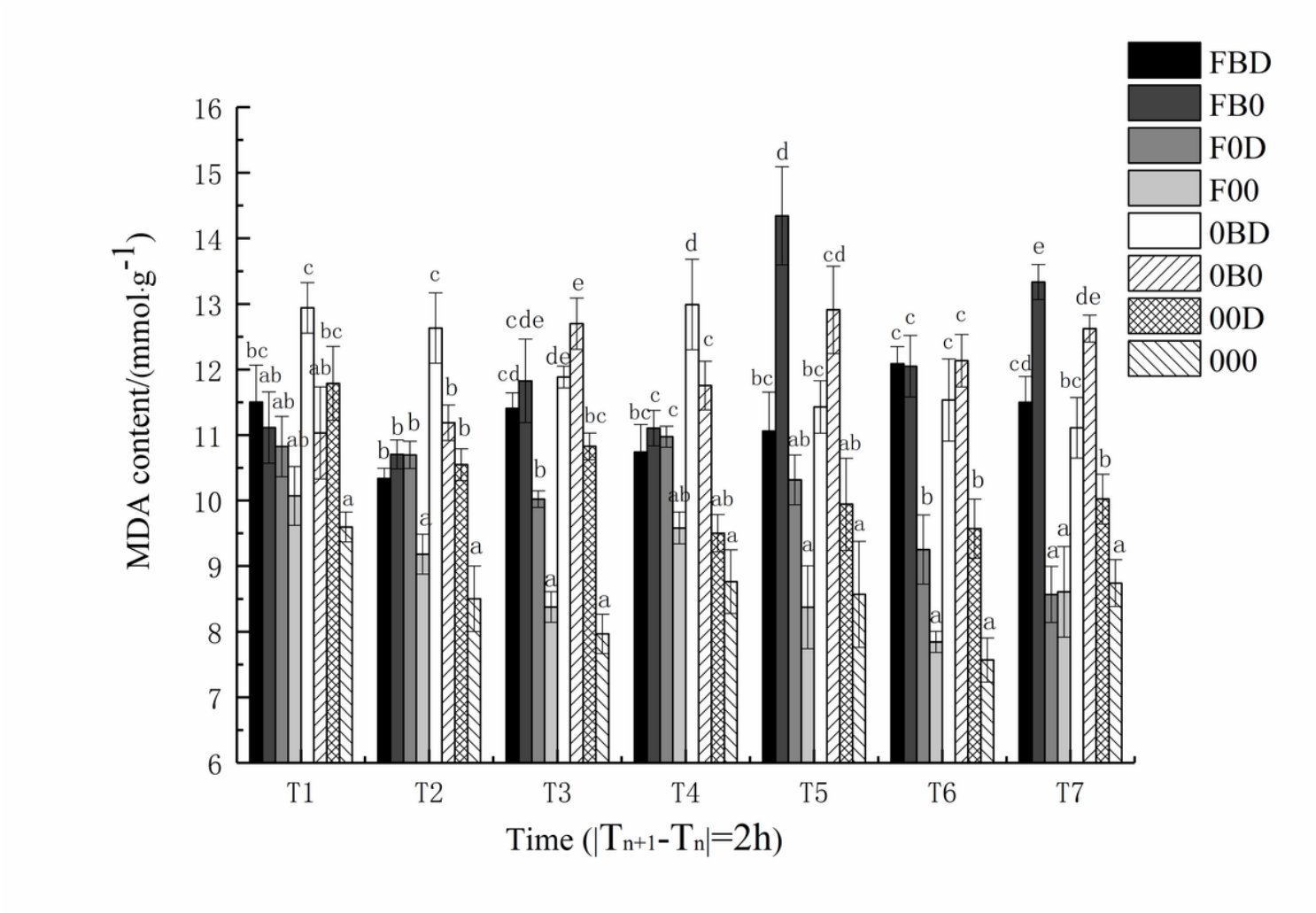

Figure 3

The malondialdehyde (MDA) content in H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 ℃. The different low-case letters mean the significant difference at the same temperature ($P < 0.05$).

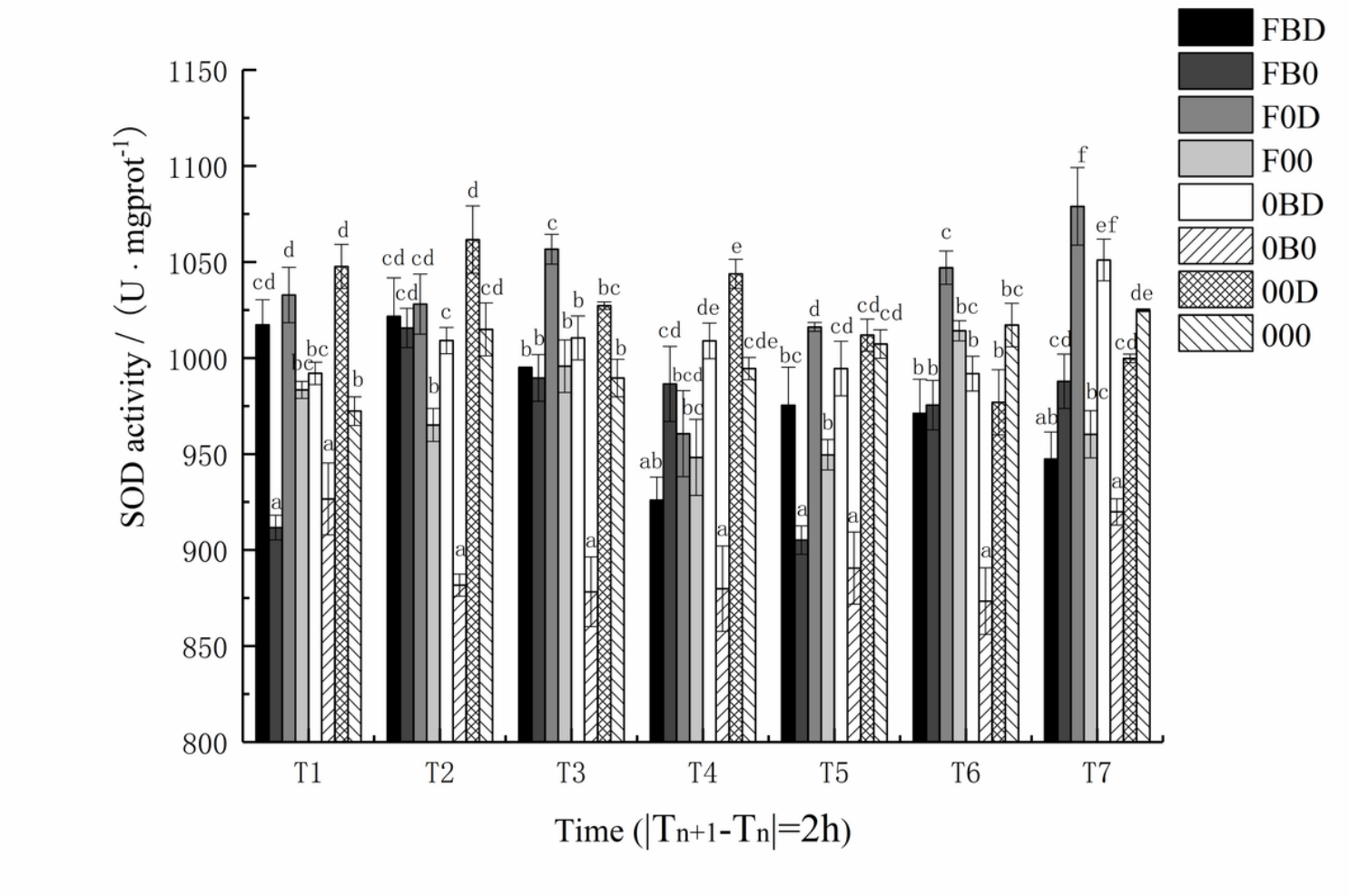

Figure 4

The SOD activity of H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 °C. The different low-case letters mean the significant difference at the same temperature (P < 0.05).

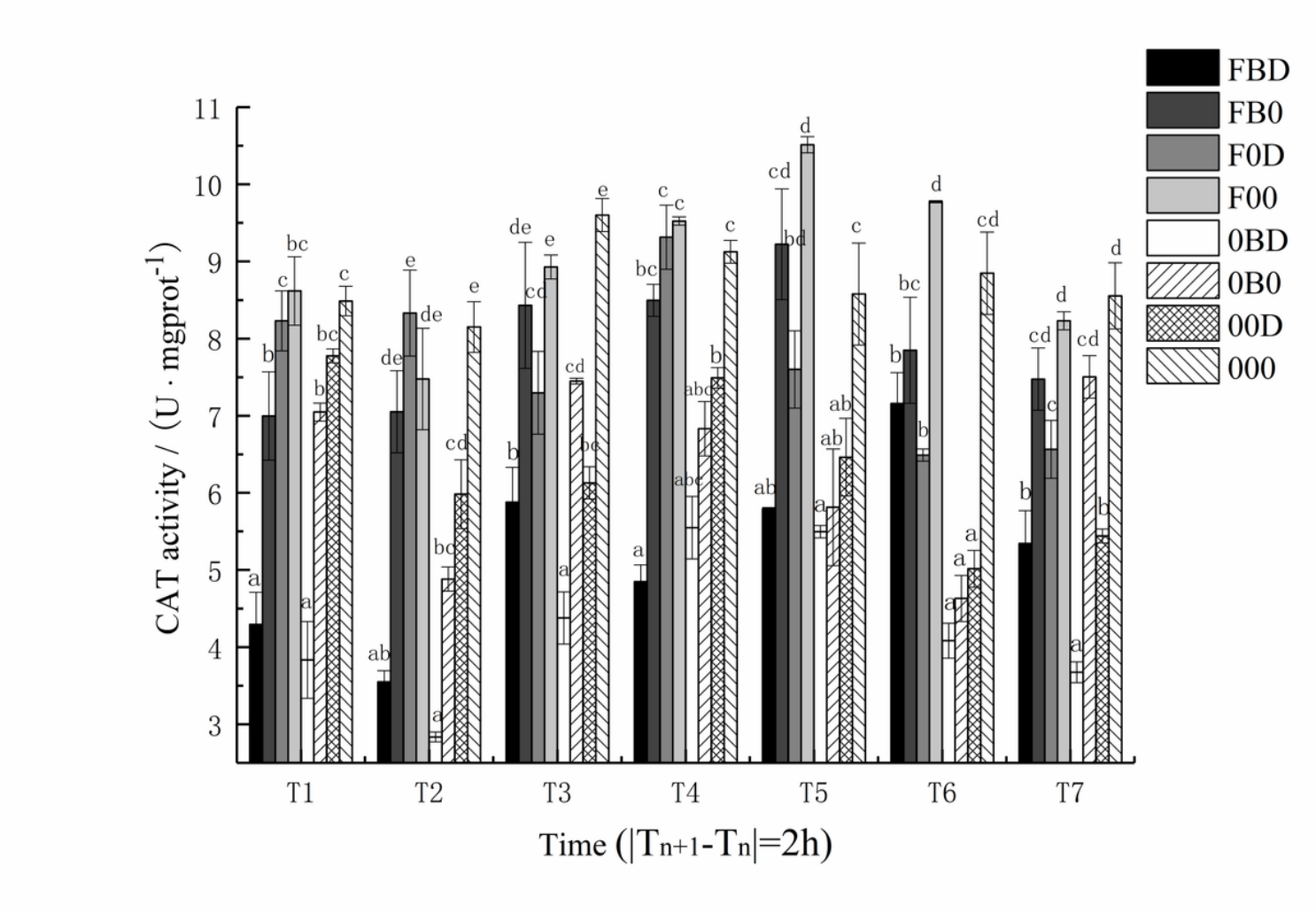

Figure 5

The CAT activity in H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 °C. The different low-case letters mean the significant difference at the same temperature ($P < 0.05$).

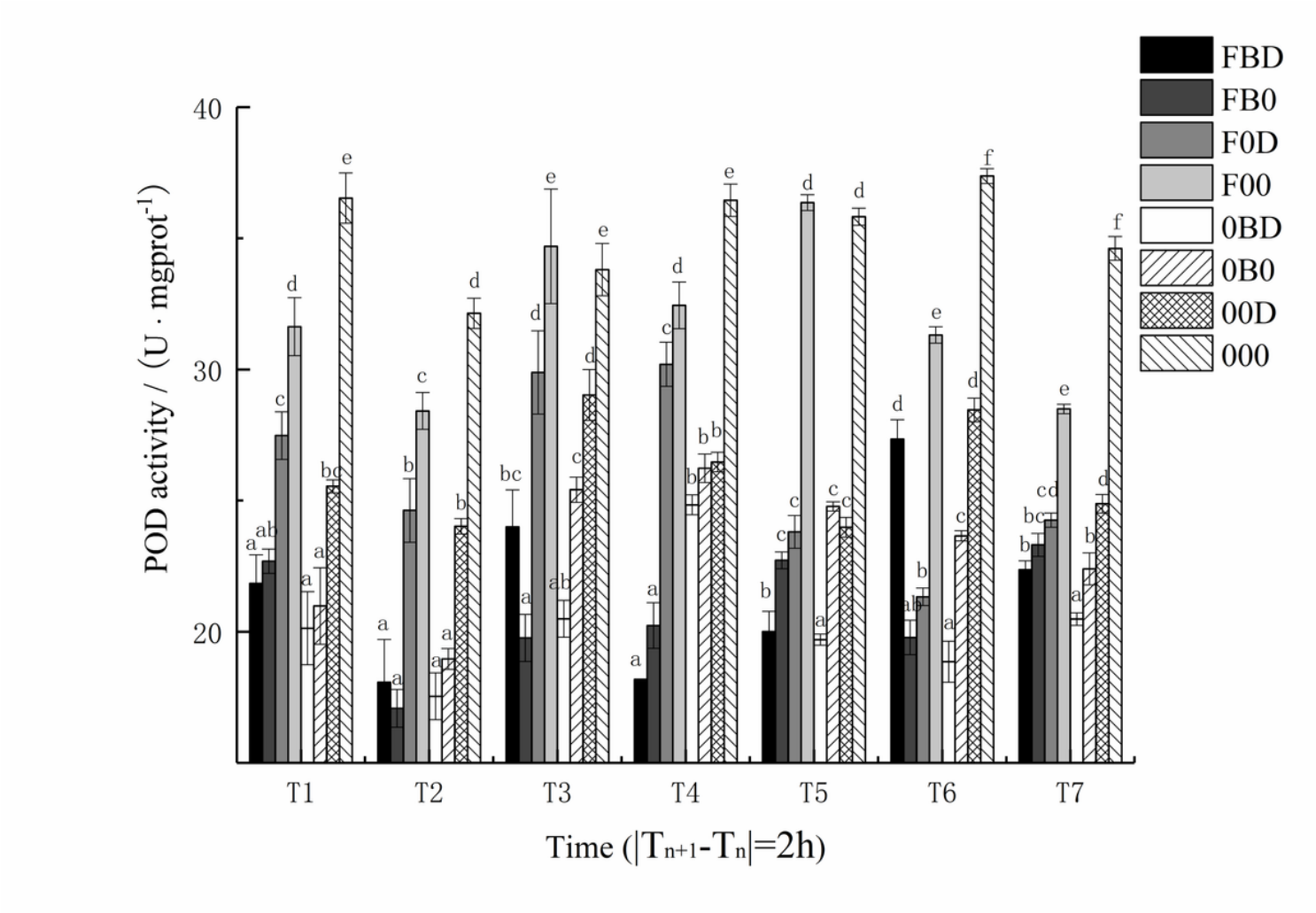

Figure 6

The POD activity in H. vulgare L. seedlings under different treatment. The initial letter F and number 0 represent whether freeze-thaw stress was added or not. The middle letter B and number 0 represent whether basic salt stress was added or not. The last letter D number 0 represent whether drought stress was added or not. T1-T7 means the sampling time intervals corresponding to 10, 5, 0, -5, 0, 5 and 10 °C. The different low-case letters mean the significant difference at the same temperature ($P < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx
- Additionalfile2.xlsx
- Additionalfile3.xlsx
- Additionalfile4.xlsx
- Additionalfile5.xlsx

- Additionalfile6.xlsx