Drought resistance of ten ground cover seedling species during roof greening

Pengqian Zhang¹,²*, Jiade Bai¹, Yanju Liu³*, Yuping Meng¹, Zheng Yang¹, Tian Liu¹

¹ Beijing Biodiversity Conservation Research Center, Beijing, China, ² Beijing Gardening and Greening Bureau, Beijing, China, ³ Beijing Center for Physical and Chemical Analysis, Beijing, China

☯ These authors contributed equally to this work.
* liuyanju@hotmail.com

Abstract

Roof greening is an important national policy for maintaining the hydrological balance in China; however, plant growth is limited by drought stress. This study aims to identify strong drought resistant plant species for roof greening from ten common species: Paeonia lactiflora, Hemerocallis dumortieri, Meehania urticifolia, Iris lactea var. chinensis, Hylotelephium erythrostictum, Sedum lineare, Iris germanica, Cosmos bipinnata, Hosta plantaginea, and Dianthus barbatus. By controlling the soil relative water content (RWC), we designed three treatments: moderate drought stress (40±2% < RWC < 45±2%), severe drought stress (RWC < 30±2%) and well-watered control (RWC > 75±2%). After the seedlings were provided different levels of water, their membrane permeability (MP), chlorophyll concentration (Chl), and superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) activity were measured. Finally, the membership function method was used to assess the drought resistance of these species. The results showed that C. bipinnata and M. urticifolia were not suitable for moderate or severe drought stress and did not survive. The other species presented variations in physiological and biochemical parameters. The MP of H. dumortieri, I. lactea and Ho. plantaginea showed minor changes between the well-watered control and drought stress. Most of the species showed reduced SOD activity under moderate drought stress but increased activity under severe stress. All of the plant species showed decreases in the protective enzymes POD and APX with increasing drought stress. The membership function method was applied to calculate the plant species’ drought resistance, and the following order of priority of the roof-greening plant species was suggested: H. dumortieri > I. germanica > I. lactea > D. barbatus > Hy. erythrostictum > S. lineare > Ho. plantaginea > P. lactiflora.

1 Introduction

Roof greening, which is regarded as the “fifth surface greening”, is one of the fundamental measures for sponge cities and represents an important national policy for improving the relationship between city development and nature protection to maintain the hydrological balance in China [1,2]. As an important supplement of urban landscaping, roof greening can help
mitigate the urban heat island effect [3], improve air quality [4] and enrich the biodiversity of cities [5]; hence, this landscaping style has expanded throughout all of China. Green roofs can be categorized roughly into two types: those that consist of diverse types plants (shrubs, trees, grasses, and flowers), namely, intensive green roofs (IGRs), and those that consist of simple herbaceous plant species, namely, extensive green roofs (EGRs) [6]. To grow on roofs, plants face many challenges. Taking Beijing as an example, plants that compose green roofs suffer from restricted rainfall in winter, spring, and autumn and evaporation always increases with high summer temperatures. Drought is considered as one of the most common environmental stresses that currently affects plant growth [7,8]. When plants experience drought stress, reactive oxygen species (ROS) are produced [9] including singlet oxygen (\(^{1}\text{O}_2\)), superoxide radical (\(\text{O}_2^-\)), hydroxyl free radical (\(\cdot\text{OH}\)), and hydrogen peroxide (\(\text{H}_2\text{O}_2\)) [10]. ROS can reduce crop productivity and plant viability because they can cause oxidative damage to proteins, DNA, and lipids [11]. Accordingly, drought stress can not only disrupt leaf membrane permeability (MP) [12] but also reduce the chlorophyll concentration (Chl) [13] and the activity of superoxide dismutase (SOD) [14], peroxidase (POD) [15] and ascorbate peroxidase (APX) [16]. These indicators are used for measuring the degree of plant drought stress, and they are usually analyzed as a whole due to their close associations, such as the ability of antioxidative enzymes SOD, POD and APX [17] to quench ROS and protect the cell from damage.

Biological membranes are crucial aspects of living systems that control the organization and distribution of different chemical components [18], and maintain sufficient water in plant tissue to protect the organism from dehydration and carboxylation and prevent enzymes from inactivating [19]. Liposomes are colloid vesicles composed of a lipid bilayer membrane and a watery internal compartment [20], and they serve as transport carriers for the efflux of secreted proteins. Low temperature [21], drought [22], salt [23] and heavy metal [24] stress break the stability of the plant cell membrane system and proteins, thereby increasing biofilm fluidity, altering the conformation of proteins, and then leading to physiological, biochemical and metabolic imbalance and abnormalities [25]. MP is determined by electrolyte leakage [26] and could be estimated by measuring electrolytes seeping from the plant cells under environmental stress. Generally, the greater the value of MP, the more cell damage there is. Under the same water condition, a lower MP value implies a stronger adaptation of the plant species to the environment.

Chlorophyll, a green pigment, is widely distributed in plant leaves and stems [27]. It helps convert absorbed solar radiation into stored chemical energy [28] and binds to proteins within chloroplasts and affects the light-harvesting capability and photosynthesis of plants [29, 30]. Upon drought stress, plant Chl is mainly affected by the physical destruction of chloroplasts and the inhibition of Chl a and Chl b functionality. Drought stress also causes the chloroplast matrix lamella to bend and swell [31], thereby impeding Chl synthesis and reducing its production [32]. In addition, reactive oxygen species (ROS, \(^{1}\text{O}_2\), \(\text{O}_2^-\) and \(\cdot\text{OH}\)) can directly or indirectly lead to lipid peroxidation and thus Chl damage [33].

The antioxidant system in plants consists mainly of nonenzymatic antioxidants and antioxidative enzymes. The most important antioxidant system in plants is composed of the antioxidative enzymes in chloroplasts and the cytoplasm [34]. SOD is an important enzyme that is ubiquitously expressed in aerobic organisms and catalyzes the dismutation of superoxide anions to hydrogen and molecular oxygen, which constitutes the first line of defense against ROS at the cellular level [35, 36]. Based on the prosthetic metal at the active site, SODs are classified into three groups, namely, CuZn-SODs, Mn-SODs, or Fe-SODs [37], of which Mn-SODs are closely related to mitochondria [38] and CuZn-SODs are mainly located in the cytoplasm and chloroplasts of plant cells [39]. McCord and Fridovich [40] described the principle chemical reaction under the elimination of ROS by SODs as \(\text{O}_2^-+\text{O}_2^-+2\text{H}^+\rightarrow\text{O}_2+\text{H}_2\text{O}_2\).
Existing in peroxisomes, glyoxysomes, vacuoles, the nucleus, and the extracellular matrix, SODs play a critical role in drought tolerance [41]. The SOD activity reflects the ability of plant species to adapt to environmental stress. Higher SOD activity values represent a stronger adaptation ability [42].

The antioxidant enzyme POD can scavenge and breakdown ROS [43] via the reaction \( \text{RH}_2^+ + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{R} \), in which \( \text{H}_2\text{O}_2 \) is thoroughly converted into \( \text{H}_2\text{O} \) [44, 45]. Chen et al [46] and Wu et al [47] found that increased POD activity helped cucumber and *Dendrobium moniliforme* alleviate oxidative damage under drought stress. POD can further scavenge peroxides induced by SOD, and the synergistic action between these enzymes constitutes the protective enzyme system of the organism. Changes in the activity of these enzymes under stress may reflect the plant resistance ability in adverse environments.

APX is a member of the class I heme peroxidases and an important enzyme in plant antioxidant defense systems, and APX with several isoenzymes has a strong ability to scavenge ROS [48–51]. APX has been found in most eukaryotes, including higher plants [52], where it plays a key role in the metabolism of \( \text{H}_2\text{O}_2 \). Stronger APX activity would more quickly remove \( \text{H}_2\text{O}_2 \), thus preventing oxidative damage [53]. Different kinds of POD isoenzymes have obvious tissue and organ specialization. Similarly, APX is distributed in chloroplasts and the cytoplasm. POD and APX differ in their composition, structure, substrate specialization affinity, and stability during the purification process [54].

The membership function method is widely used to assess plant stress resistance. For example, it has been used to assess the drought tolerance of *Malus* [55], maize [56] and potato [57] and the salt tolerance of *Sorghum bicolor* [58], *Lactuca sativa* [59], sugar beet [60], etc. The membership function weighted average method (\( D \) value) not only eliminates the one-sidedness associated with individual indexes but is also a relatively reliable evaluation method because the \( D \) value is the pure number within the closed interval of [0,1], which makes the difference in drought resistance of each test material comparable [61].

According to "Beijing local standards, roof greening specification (DB11/T 281–2005)" [62], more than 20 species can be used for ground cover and roof greening. Studies have focused on most of these species for their drought resistance, although these studies were limited to one species or one family. Because of the lack of studies comparing the drought resistance between these species, this study aims to screen plant species with strong resistance under drought stress to provide government policymakers with scientific plant species choices to improve plant survival rates and save maintenance costs during roof greening.

### 2 Study area overview

Milu Park is located 2 km far away from the South 5th Ring Road in Beijing and surrounded by Nan-Haizi Suburb Park. The drought stress experiment was conducted under a rain shed in the core-protection area for David's Deer in Milu Park (39.78˚N, 116.47˚E). During the test period, the daily average temperature was approximately 18.7˚C, the daily average humidity was approximately 55.2%, and the daily average illumination intensity (at 12:00) was approximately 2000 lx. The experiment was performed in the middle of April to the end of May 2015.

### 3 Materials and methods

This research has been held in the Beijing Milu Ecological Research Center (also known as Milu Park), located in Daxing district, Beijing, China. Milu Park is a place dedicated to ecological science research, as well as offered popular science education for the public for free. The authors, as staffs of Milu Park, in charge of conducting scientific research including biological science and environmental science. No additional permission is required for the authors to
carry out the experiments here. Also, the 10 plant species used for experiments were all market-purchased, common ground cover plants. These plants are not endangered rare plants, not be protected.

3.1 Seedlings
One-year-old seedlings of ten species, i.e., *Paeonia lactiflora, Hemerocallis dumortieri, Meehania urticifolia, Iris lactea var. chinensis, Hylotelephium erythrostictum, Sedum lineare, Iris germanica, Cosmos bipinnata, Hosta plantaginea, and Dianthus barbatus*, were provided by the Yu-Quanying flower market, a large and popular wholesale market in Fengtai District, Beijing that supplies most ornamental plants for Beijing City. The plant species present various propagation modes and other characteristics (Table 1).

3.2 Field soil collection and preparation
The field soil was collected from a wild wetland area in Milu Park, and it was then air-dried and ground to powder for the transplantation experiment. The soil was moderately saline (pH = 7.89) and presented available nitrogen, available phosphorus, and available potassium contents of 24.7 mg kg⁻¹, 18.9 mg kg⁻¹, and 322 mg kg⁻¹, respectively [63].

3.3 Plant transplanting
The transplantation program was as follows: first, approximately 400 g powdered soil was placed in a plastic pot that was 20 cm tall and 13 cm in diameter and had 3 small holes at the bottom for drainage. Second, after removing the plastic wrap surrounding the roots, the seedlings were carefully planted at the pot’s center. Third, another approximately 300 g of powdered soil was placed into the pot to cover the roots and then compressed tightly by hand. The seedlings were watered every 10 min, three times in total, to ensure that enough water was available to support plant growth. The transplantation was a success if new leaves and fresh stems were developed. The seedling survival rate reached 99% one week after replanting.

3.4 Drought stress treatment design
Three treatments were designed for each of the ten species: two drought stress level treatments, which included moderate drought stress (MDS or moderate; the water content in the soil

| Latin name                  | Propagation mode | Life cycle | Family      | Species characteristics                                                                 |
|-----------------------------|------------------|------------|-------------|------------------------------------------------------------------------------------------|
| *Paeonia lactiflora*        | division of suckers | perennial | Ranunculaceae | Popular in gardening, and roots used as traditional Chinese medicine                       |
| *Hemerocallis dumortieri*   | sowing of seeds  | perennial | Liliaceae    | Native to Northeast China, North Korea, Japan and Russia                                  |
| *Meehania urticifolia*      | sowing of seeds  | annual or perennial | Lamiaceae | Adapted to dark and moist environments                                                    |
| *Iris lactea var. chinensis*| sowing of seeds  | perennial | Iridaceae    | Tolerant to saline-alkaline conditions and presents a well-developed root system          |
| *Hylotelephium erythrostictum* | cuttings of seeds | perennial | Crassulaceae | Traditional Chinese medicine                                                              |
| *Sedum lineare*             | sowing of seeds  | perennial | Crassulaceae | Traditional Chinese medicine                                                              |
| *Iris germanica*            | rhizome cuttings | perennial | Iridaceae    | Native to Europe                                                                         |
| *Cosmos bipinnata*          | sowing seedling  | annual or perennial | Asteraceae | Native to Mexico                                                                        |
| *Hosta plantaginea*         | division of suckers | perennial | Liliaceae    | Traditional Chinese medicine                                                              |
| *Dianthus barbatus*         | sowing of seeds  | perennial | Caryophyllaceae | Native to Europe                                                                         |

https://doi.org/10.1371/journal.pone.0220598.t001
varied from 40 ± 2% ~ 45 ± 2%) and severe drought stress (SDS or severe; the water content in the soil was less than 30 ± 2%), and one control group (CG or well-watered), which was under sufficient soil water conditions (the water content in the soil was over 75 ± 2%) [64, 65]. For each treatment, three replicates were performed. Drought stress was dependent on natural evaporation. During the drought stress period, a WET-2™ sensor made by Delta-T Devices, Ltd., Cambridge, UK, was applied to measure the water content. Once the relative water content (RWC) of the soil met the requirements of the experiment, the plant seedlings were maintained under those conditions for approximately two days to ensure that changes in plant physiology and biochemistry had occurred. For the well-watered treatment, the seedlings were watered every four days.

3.5 Leaf sampling
All plants grew new leaves ten days after transplanting, which indicated the plants’ roots had developed by the time leaves could react to the plant’s physiological status. Referring to the sampling method in VDI-Guideline 3975 Part 11 [66], at least 15 g of healthy leaves was collected for each replicate. The leaf samples were placed into sealed plastic bags under a portable ice-box at 0~4˚C before being transferred to the lab for further physiological and chemical analysis.

3.6 Determining the MP, Chl, SOD activity, POD activity, and APX activity
The MP (%) of the leaves was calculated as $MP = \frac{L_t}{L_{Cg}} \times 100$, where $L_t$ is the relative electrical conductivity of the plant material in the drought stress treatments and $L_{Cg}$ is the relative electrical conductivity of the material in the control group. The relative electrical conductivity $L = \frac{S_1 - S_2}{S_0}$, where $S_1$ is the original conductivity of the deionized water with fractured fresh leaves, $S_2$ is the conductivity of the boiled deionized water with fractured leaves, and $S_0$ is the conductivity of deionized water [67]. The leaf MP was determined using a Thermo Scientific™ Orion 3-star inductivity- measuring device. Before the test, all sample leaves were flushed with deionized water 3 times and residual water on the leaf surface was removed by absorbent paper.

Chl was estimated according to the method described by Arnon [68] and Zhang et al [69] in detail. Three grams of fresh leaf material was crushed with a mortar and extracted with 10 mL of 80% acetone for 15 min. The extracted solution was then centrifuged at 2500 rpm (F = 34.9 g) for 3 min and measured at wavelengths of 643 nm, 645 nm, and 663 nm via a spectrophotometer (Metash™ UV-6100A). Calculations were performed via the formulas below.

Chlorophyll a (Chl a, mg · L⁻¹) = 12.7A₆₄₃ - 2.69A₆₄₅

Chlorophyll b (Chl b, mg · L⁻¹) = 22.9A₆₄₅ - 4.68A₆₆₃

The total chlorophyll of the solution (C_T, mg · L⁻¹) = Chl a + Chl b

Chlorophyll concentration (Chl, mg · g⁻¹) = C_T * V/W/1000

where Chl a and Chl b refers to the concentration of chlorophyll a and chlorophyll b of the extracted solution; $A_{643}$, $A_{645}$ and $A_{663}$ refer to the absorbance of the measured solution at wavelengths of 643 nm, 645 nm and 663 nm, respectively; $C_T$ (mg/L) is the total chlorophyll of the solution; V represents the total volume of the extracted solution (mL); and W is the weight
of the extracted leaf (g). In the final result, Chl (mg g\(^{-1}\)) refers to the chlorophyll content contained within each gram of leaf sample.

Crude enzyme extracts from the leaves were used to measure the SOD, POD, and APX activity. Approximately 0.5 g fresh leaves was added with a slight amount of CaCO\(_3\), high-purity quartz sand and 5 mL of phosphate buffer (0.05 mol L\(^{-1}\)) and then crushed into a powder in a mortar under freezing conditions. The mixture was subsequently transferred to a 10 mL centrifuge tube and then diluted with deionized water to 10 mL. The samples were then centrifuged at high speed (F = 13000 g) for 20 min at 0~4˚C.[67]

The SOD and POD reaction systems were established as described by Zhang et al.[67], and the APX reaction system was described by Tang et al.[70] as shown below:

Two copies of the reaction system solution for each leaf sample were configured following Table 2-SOD mentioned above, one of which was put into a test tube and illuminated with 4000 lx for approximately 20~30 min at room temperature, the other one was put into the check tube wrapped in aluminum foil to avoid illumination. Once the color of the solution transition started, the reaction was immediately stopped. The final solution absorbance value was determined with a Metash™ UV-6100A spectrophotometer at a wavelength of 560 nm.

\[
\text{SOD activity (U \cdot mg^{-1})} = \frac{(A_0 - A_s) \times V_T}{A_0 \times 0.5 \times W \times V_1} \times \text{dilution ratio}
\]

where \(A_0\) is the absorbance of the check tube solution; \(A_s\) is the absorbance of the test tube solution; \(V_T\) (mL) is the total volume of the samples; \(V_1\) (mL) is the volume of the reaction system; and \(W\) (g) is the weight of the fresh leaves.

All the components were put into a test tube, and then the components of the POD reaction system were added (Table 2-POD). The solution’s light absorption value was recorded for each tube (the wavelength was maintained at 470 nm). The solution was read every 1 min, and each solution was recorded 5 times in 5 min.

\[
\text{POD activity (U \cdot g^{-1} \cdot min^{-1})} = \frac{\Delta A_{470} \times V_T}{W \times V_1 \times 0.01 \times t}
\]
where $\Delta A_{470}$ is the change in absorbance during the reaction period, $W$ (g) is the weight of the sample, $t$ (min) is the reaction time, $V_s$ (mL) is the volume of the reaction system, and $V_T$ (mL) is the total volume of the sample.

With respect to the APX reaction system (Table 2-APX), the mixture was put into a test tube, after which the light absorption values were recorded at 290 nm every minute; this step was repeated 5 times. The formula to calculate the APX activity was as follows:

$$\text{APX activity} \, (\text{U} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \cdot \text{FW}) = \frac{\Delta A_{290} \times V_1}{0.01 \times V_2 \times t \times W}$$

where $\Delta A_{290}$ is the change in absorbance during 5 min, $V_1$ (mL) is the volume of the crude enzyme, $V_2$ (mL) is the volume of the crude enzyme involved in the reaction (0.1 mL in this test), $t$ (min) is the reaction time (5 min in this test) and $W$ (g) is the weight of the fresh leaves. FW is short for fresh weight.

### 3.7 Data analysis

SPSS 17.0 and Excel 2010 for Windows were used to calculate the mean, SD, etc. Multiple comparisons of the means by the least significant difference (Tukey’s honestly significant difference [HSD]) test were performed on the 5 parameters (MP, Chl, SOD activity, POD activity, and APX activity) under the two drought stress treatments and the control group. ANOVA was used to determine significant differences between 10 species and between three treatments ($P<0.05$).

Following a drought resistance assessment method [71] for plant species based on the membership function value in fuzzy mathematics was used, the MP, Chl, SOD activity, POD activity, and APX activity results can be integrated into a single value for each species. The membership function value was calculated as follows:

$$\bar{X}_{ij} = \frac{X_{ij} - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}}$$

(1)

$$\bar{X}_{ij} = 1 - \frac{X_{ij} - X_{\text{min}}}{X_{\text{max}} - X_{\text{min}}}$$

(2)

where the lowercase “i” and “j” represent the plant species and the parameter type, respectively; “$\bar{X}_{ij}$” is the mean value of the parameter “j” of the species “i”; “$X_{\text{max}}$” and “$X_{\text{min}}$” represent the maximum and minimum of the parameter “j” of the “i” species; and “$\bar{X}_{ij}$” is the membership function value and represents the drought resistance of the seedlings. The average of the membership function value was then applied to estimate the adaptive capability of the plants under drought stress. After calculation according to formula (1) or (2), only positive “$\bar{X}_{ij}$” value was chosen as the result. The formula for the average was as follows (where “n” represents the number of parameters, and “$\bar{X}_i$” represents the average “$\bar{X}_{ij}$”):

$$\bar{X}_i = \frac{\sum \bar{X}_{ij}}{n}$$

### 4 Results and discussion

#### 4.1 Soil relative water content

The soil relative water content between the three drought stress levels are significantly different, with the average of 74.8~85.3% for the well-watered control group, 38.3~45.2% for the
moderate drought level, and 15.4~24.3% for the severe drought level (Table 3). These findings were consistent with the designed levels.

4.2 Membrane permeability

Plant cells dehydrate when they suffer drought stress, which leads to mechanical damage to the membranes [72]. Greater MP values led to more cytosolic exosmosis and further damage to the plant cellular structure. However, it was hard to distinguish which species had stronger or weaker drought resistance when they were under the well-watered control because they had not been affected by drought yet. In this study, *C. bipinnata* and *D. barbatus* presented significantly higher MP values than the other species (Fig 1), followed by the MP value of *M. urticifolia* and *Hy. Erythrodictum*, for which the MP value was significantly different relative to the remaining species. On the contrary, Liliaceae and Iridaceae family species presented low MP values under the well-watered control. The change of MP values implies the different physiological characteristics of various plants.

With the drought stress treatment, the above four plant species with higher MP values expressed different tolerance features. *C. bipinnata* did not survive under severe drought stress, and *M. urticifolia* did not survive under moderate or severe drought stress. Both species are annual herbs, and their root growth is strongly inhibited by the lack of water [73,74]. Although leaf sampling occurred only ten days after transplanting, the plant roots were transplanted with the original moist rooting medium and the seedlings were shaded and fully watered, which was beneficial for root development. Previous studies identified a strong relationship between new leaf germination and plant survival rate [75]. In addition, leaf biomass was positively correlated with root biomass, implying that root length developed when the plants grew new leaves [76]. In this study, plants under the well-watered control have all grown new leaves and even buds at sampling, thus demonstrating that the root has developed. Leaf sampling could be conducted in 3 days, 5 days, 10 days, etc. once the soil RWC matched the designed drought levels [77–79]. Therefore, the withering of *C. bipinnata* and *M. urticifolia* was induced by drought stress instead of a short growth period. The MP values of *D. barbatus* did not change significantly.

### Table 3. Soil relative water content.

| Plant species | Well-watered group (%) | Moderate stress group (%) | Severe stress group (%) |
|---------------|------------------------|--------------------------|------------------------|
|               | 27th, April 2015*      | 29th, April 2015**       | 2ed, May 2015        |
| *P. lactiflora*| 80.1±2%                | 75.8±1%                  | 45.2±4%              |
| *He. dumortieri*| 82.3±4%               | 76.8±2%                  | 41.6±2%              |
| *M. urticifolia*| 78.8±5%               | 75.2±1%                  | 41.9±3%              |
| *I. lactea*    | 85.3±1%                | 80.2±3%                  | 42.5±3%              |
| *He. dumortieri*| 82.9±2%               | 79.3±2%                  | 40.8±3%              |
| *S. lineare*   | 78.4±1%                | 76.1±2%                  | 43.4±1%              |
| *I. germanica* | 85.3±2%                | 78.5±2%                  | 44.7±2%              |
| *C. bipinnata* | 77.1±2%                | 74.8±3%                  | 40.1±3%              |
| *Ho. plantaginea*| 83.8±3%              | 78.6±2%                  | 42.8±2%              |
| *D. barbatus*  | 82.3±4%                | 79.0±4%                  | 40.3±1%              |

The values represent the mean ± SD (n = 30).

* is the day when the water content of the soil achieved the designated level.

** is the sampling day.

[https://doi.org/10.1371/journal.pone.0220598.t003](https://doi.org/10.1371/journal.pone.0220598.t003)
Under drought stress, most species showed significantly increased MP values, including *P. lactiflora*, *He. dumortieri*, *I. lactea*, *S. lineare*, *I. germanica*, and *Ho. plantaginea*, among which *P. lactiflora* showed significantly increased MP values only under severe drought stress (Fig 1). The MP value of *I. germanica* increased the most by 36% and 56% under moderate and severe conditions, respectively, and that of *S. lineare* increased by 31% and 55%, respectively. These findings indicated that these species’ membranes were damaged under drought stress.

Among all the plant species, only two, *Hy. erythrostictum* and *D. barbatus*, did not show significantly changed MP values under the drought stress treatment. *Hy. erythrostictum* was considered a kind of xerophilous plant with fleshy leaves [80], and the MP values were reduced at the early stage of drought stress [81]. Although fewer investigations have been performed on the effects of drought in *D. barbatus*, especially on its MP, the permeation regulation synchronized with damage to the protoplast membrane in *Dianthus plumarius*, another Caryophyllaceae plant [82]. Although both *S. lineare* and *Hy. erythrostictum* belong to the Crassulaceae family, their MP value variation trend was the opposite. The MP values of *S. lineare* were also significantly greater than that of *Hy. erythrostictum* under drought stress [83].

Plant drought resistance is closely related to its cell membrane system stability [31]. Usually, the cell membrane is first affected by drought stress [84], and then the cell structure is damaged and MP increases, which leads to the extravasation of extracellular electrolytes, which is why MP values increase when plants are subjected to drought stress. The stability of the MP values of these two species indicated that their cell membrane was undamaged. Therefore, the osmotic adjustment ability of multicolored carnation leaves is strong enough to avoid damage to the protoplast membrane under drought stress treatment.

### 4.3 Chlorophyll concentration

In the well-watered control, the studied plant species presented Chl concentrations from 6.06 to 47.69 mg g\(^{-1}\) FW, and the fleshy Crassulaceae species *H. erythrostictum* and *S. lineare* presented the lowest Chl concentrations (Fig 2). The plants’ Chl concentrations were affected by light intensity and environmental temperature, which affect the opening and closing of
stomata and photosynthetic rates of plant leaves and then affect the accumulation of carbohydrates, which is consistent with the Chl concentration of plants [85]. In the study, all plants grew in a stable light intensity environment because the experimental area had a roof, which can prevent the effects of strong sunshine or rain. As for the ambient temperature, no extreme temperatures were encountered during the experiment. The leaves of these plants were collected at the same time after drought stress under the same environmental temperature. Thus, the changes in Chl concentration should be caused by drought stress instead of temperature or sunlight.

Upon drought stress, change trends of plant Chl concentration were various. Four species showed significantly ($P < 0.05$) increased Chl concentrations under the different extents of drought stress, including P. lactiflora, Hy. erythrostictum, S. lineare and Ho. plantaginea (Fig 2). The increased Chl concentration under moderate or severe drought stress might be due to the increase of the stem cell mass and cell number of the leaves, thus forming a Chl condensation phenomenon, as in P. lactiflora [86]. Additionally, Liu et al [87] reported that the Chl a and b of Hy. erythrostictum would be increased during the day but decreased during the night under drought stress, which probably indicates more photosynthetic pigments were produced to promote photosynthesis of Hy. erythrostictum under drought stress. However, the photosynthetic pigment content was decreased at night for maintaining its normal physiological activities.

The species I. germanica and I. lacteal showed increased Chl concentrations under moderate stress, and then these values decreased under severe drought stress (Fig 2). Most Iridaceae plants are shade plants [88], some of which feature colorless leaves [89] and possess lower Chl concentrations than sun plants leaves [90]. Zhou [91] researched seven Iris species and also found that I. germanica had a higher Chl concentration in the early stage of drought stress than the control group.

D. barbatus did not change the Chl concentration under drought stress treatment (Fig 2), which indicated that the species had a strong self-repair and regulate ability during drought.

![Fig 2. Variance of the total chlorophyll concentration in the ten species during the drought stress tests.](https://doi.org/10.1371/journal.pone.0220598.g002)
stress, and its leaves had a relatively good physiological and biochemical state, which could maintain normal photosynthesis and strong resistance during drought.

Only one species, *He. Dumortieri*, significantly decreased the Chl concentration with the increase of drought stress (Fig 2), which indicated that chlorophyll synthesis was interrupted and the chlorophyll decomposed under drought.

### 4.4 Superoxide dismutase activity

SOD activity is very sensitive to drought [92]. In this study, the SOD activity of six species, *He. dumortieri, I. lactea, Hy. erythrodictum, I. germanica, Ho. plantaginea, and D. barbatus*, initially significantly (*P* < 0.05) decreased under moderate drought stress but increased under severe drought stress (Fig 3). The reduction of SOD activity under the moderate condition implied that a considerable amount of ROS was produced to damage plant cells and tissues, thus leading the plant cells to undergo oxidative damage. The activity of the enzyme SOD is influenced by the concentration of the O$_2^-$ substrate. Stress raises the production of O$_2^-$, thus increasing the SOD activity [93]. A previous investigation indicated that the increased SOD activity under severe drought was caused by the drought exercise under moderate drought stress [87]. The drought exercise was applied to enhance the resistance of rice to high temperatures [94] and the resistance of wheat to drought stress [95].

The change trends of SOD activity can be adopted to judge the species’ drought resistance. Those species with higher SOD activity under drought can be considered to have strong drought resistance [96]. The SOD activity of *P. lactiflora* and *S. lineare* increased under either the moderate or severe drought stress. The difference of SOD activity might be caused by the expression of various isozymes, which induced the accumulation of the antioxidant substance in plants leaves that started up the antioxidant protection system when plants were under moderate and severe drought stress [97]. The degrees of SOD increase of *S. lineare* were higher than that of *P. lactiflora*, which is consistent with a previous study [98] in which *S. lineare* had stronger SOD activity.

![Fig 3. Variance of superoxide dismutase activity in the ten species during the drought stress tests.](https://doi.org/10.1371/journal.pone.0220598.g003)
Overall, in this study, the SOD activity in most species increased after severe drought stress, which suggested that drought stress-induced SOD activity increases in these plant species to help them eliminate ROS. These plant species presenting increased SOD activity showed advantages in terms of their drought stress response, and proper drought exposure could significantly improve plant resistance to sustained drought stress [95].

4.5 Peroxidase activity

In the study, all plant species showed decreased POD activity under the moderate or severe drought stress, with most showing declining POD activity as the drought stress increased (Fig 4). Compared with the well-watered control, the POD activity of He. dumortieri, Hy. erythrostictum, S. lineare, I. germanica, Ho. plantaginea and D. barbatus was reduced significantly ($P<0.05$) during the moderate drought stress. The POD activity of these species was significantly ($P<0.05$) reduced under the severe drought stress (Fig 4). Such declining trends of POD activity with drought stress were contrary to the increased SOD activity trends.

Under drought stress, stronger POD activity might be attributed to the plant defense mechanisms against free radical formation resulting from water deficit [99]. According to Fig 4, the POD activity of I. lacteal was reduced under both the moderate and severe drought stress and was significantly ($P<0.05$) higher than that of the species that survived under severe drought stress except for He. dumortieri and Ho. plantaginea. This finding may indicate the species that have stronger resistance to drought.

Under severe drought stress, P. lactiflora, S. lineare, I. germanica and D. barbatus showed significantly lower POD activity values compared with the other species and other water conditions (Fig 4), which may be related to the POD enzyme reaching its tolerance limit and decreasing rapidly.

In general, the POD activity of all the species tended to decrease when the seedlings experienced drought stress. As a special enzyme to eliminate $H_2O_2$, the reduced POD activity in this study might have been caused by ROS elimination because different protective enzymes work together as a whole, with the elimination of $O_2^-$ SOD increasing $H_2O_2$ production. However, a very high concentration of $H_2O_2$ was beyond the reach of POD activity [100],

![Fig 4. Variance of peroxidase activity of the ten species under the drought stress tests.](https://doi.org/10.1371/journal.pone.0220598.g004)
which indicates that although SOD and POD are both antioxidases and can cooperate in scavenging ROS, drought stress might lead to a different enzyme system to resist adverse drought environments.

### 4.6 Ascorbate peroxidase activity

Under well-watered conditions, *P. lactiflora* presented the highest APX activity value at 0.98 U·min⁻¹·g⁻¹ FW, although the other species had APX activity from 0.09 to 0.26 U·min⁻¹·g⁻¹ FW. Seven species showed increased APX activity, with *P. lactiflora, He. dumortieri, Hy. erythroictum, I. germanica, Ho. plantaginea,* and *D. barbatus* presenting significantly higher APX values under moderate drought than under well-watered and severe drought stress conditions (*P*<0.05). However, the APX activity of *S. lineare* decreased significantly (*P*<0.05) under moderate and severe drought stress. At severe stress, most plant species reduced their APX value no more than 0.50 U·min⁻¹·g⁻¹ FW, with the lowest at 0.07 U·min⁻¹·g⁻¹ FW for *Ho. plantaginea* (Fig 5).

An interesting phenomenon in this study was that the SOD and APX activity of some plants seemed to complement each other. The SOD activity of *He. dumortieri, I. lactea, Hy. erythroictum, I. germanica, Ho. plantaginea,* and *D. barbatus* decreased under moderate drought stress and increased under severe stress (Fig 3), whereas the APX activity displayed the opposite pattern. Moreover, the highest values of APX activity in these species were recorded in the moderate drought stress treatment, which may suggest that APX activity was first activated by the early or moderate drought stress to scavenge ROS. When the SOD activity increased under severe drought stress, the APX activity decreased at the same time, which suggests that SOD plays a dominant role in ROS scavenging during severe drought stress. The weakening of APX activity under severe drought stress indicates that its antioxidant capability is temporary and limited [101].

![Fig 5. Variance of ascorbate peroxidase activity of the ten species under the drought stress tests.](https://doi.org/10.1371/journal.pone.0220598.g005)

The histogram shows the mean values. Above the histogram, the lowercase letters before the commas indicate statistical significance among the different plant species, and the lowercase letters after the commas indicate statistical significance among the well-watered, moderate and severe drought stress treatments. The different lowercase letters indicate a significant difference at *P*<0.05.
4.7 Assessment of plant drought resistance

We determined the drought resistance of ten ground cover species through six physiological indicators: MP, Chl, SOD activity, POD activity, and APX activity. However, it is difficult to judge which plant species has better drought resistance only based on individual parameters. Therefore, it is reasonable to use the membership function method that applies fuzzy mathematics to weigh these indicators and ultimately assess the drought resistance of the ten species. The calculated results following the formula of the membership function are shown in Table 4. The findings indicate that *He. dumortieri* was the most drought resistant species while *C. bipinnata* and *M. urticifolia* were not suitable for moderate or severe drought stress due to withering. In addition, *P. lactiflora* survived the weakest drought resistance. The order of plant resistance to drought stress was as follows: *He. dumortieri > I. germanica > I. lactea > D. barbatus > Hy. erythrostictum > S. lineare > Ho. plantaginea > P. lactiflora > M. urticifolia > C. bipinnata* (Table 4).

5 Conclusions

This study investigated how ten common plant species were tolerant to levels of drought stress and showed that drought stress disrupted plant growth because the same conditions were not observed under the well-watered treatment. Five parameters (MP, Chl, SOD, POD, and APX activity) changed under moderate and severe drought stress. The main results are as follows.

First, *C. bipinnata* and *M. urticifolia* failed to survive the drought stress and were not suitable for both moderate and severe drought stress.

Second, each plant species had quite different physiological and biochemical parameters. *He. dumortieri, I. lactea, and Ho. plantaginea* maintained a stable MP value after experiencing drought stress. Most species (except *P. lactiflora* and *S. lineare*) showed reduced SOD activity under moderate drought stress but increased activity under severe drought stress. However, the plant species showed decreased POD activity and APX activity when the drought stresses increased.

Third, complementary relationships might occur among SOD, POD and APX activity, and SOD may play a dominant role in scavenging ROS under severe drought stress while APX and POD are responsible under moderate drought stress.
Finally, *C. bipinnata* and *M. urticifolia* were very sensitive to drought stress and thus are unfit for roof greening, especially in arid regions. However, *He. dumortieri, I. germanica, I. lactea, D. barbatus, Hy. erythrostictum, S. lineare, Ho. plantaginea*, and *P. lactiflora* could be applied as roof greening in Beijing and other northern Chinese cities.

**Supporting information**

S1 Data.
(XLS)

**Acknowledgments**

The authors thank the anonymous reviewers and editor Saddam Hussain for valuable comments, and Fen Qin (a business development manager for the China office from Power Systems Research) for helping with the manuscript preparation.

**Author Contributions**

**Conceptualization:** Pengqian Zhang, Jiade Bai, Yanju Liu.

**Data curation:** Pengqian Zhang.

**Formal analysis:** Pengqian Zhang, Jiade Bai.

**Funding acquisition:** Pengqian Zhang, Yanju Liu.

**Investigation:** Pengqian Zhang, Zheng Yang, Tian Liu.

**Methodology:** Pengqian Zhang, Yanju Liu, Zheng Yang, Tian Liu.

**Project administration:** Pengqian Zhang, Jiade Bai, Yuping Meng.

**Validation:** Yanju Liu.

**Writing – original draft:** Pengqian Zhang.

**Writing – review & editing:** Yanju Liu.

**References**

1. Wang PY, Zhang Y. Discussion on the design of water storage and drainage sheaf on the green roof plate. Chinese Landscape Architecture. 2015, 31(11): 13–17. (In Chinese with English abstract)
2. Yu KJ, Li DH, Yuan H, Fu W, Qiao Q, Wang SS. “Sponge city”: theory and practice. Planning Studies. 2015, 39(6): 26–36. (In Chinese with English abstract)
3. Ondimu SN, Murase H. Combining Galerkin methods and neural network analysis to inversely determine thermal conductivity of living green roof materials. Biosystems Engineering. 2007, 96(4): 541–550.
4. Baik JJ, Kwak KH, Park SB, Ryu YH. Effects of building roof greening on air quality in street canyons. Atmospheric Environment. 2012, 61(12): 48–55.
5. Köhler M, Clements AM. Green Roofs, Ecological Functions. Springer New York. 2013.
6. Peng LLH, Jim CY. Economic evaluation of green-roof environmental benefits in the context of climate change: The case of Hong Kong. Urban Forestry & Urban Greening. 2015, 14: 556–561.
7. Nahar K, Hasanuzzaman M, Alam MM, Fujita M. Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. AoB Plants. 2015, Jul 1, 7:plv069. https://doi.org/10.1093/aobpla/plv069 PMID: 26134121
8. Romain M, Erwin D, Marc V, Francis MD, Didier D, Jean-Michel P, et al. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. New Phytologist. 2006, 169(4): 765–777. https://doi.org/10.1111/j.1469-8137.2005.01630.x PMID: 16441757
9. Sharma P, Dubey RS. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regulation. 2005, 46(3): 209–221.

10. Smirnoff N. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist. 1993, 125: 27–58.

11. Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, 55(1): 373–399.

12. Bai LP, Sui FG, Ge TD, Sun ZH, Lu YY, Zhou GS. Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of Maizet. Pedosphere. 2006, 16(3): 326–332.

13. Rulcová J, Pospíšilová J. Effect of Benzylaminopurine on rehydration of bean plants after water stress. Biologia Plantarum. 2001, 44(1): 75–81.

14. Bedard K, Krause K. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiological Reviews. 2007, 87(1):245–313. https://doi.org/10.1152/physrev.00044.2005 PMID: 17237347

15. Bahari AA, Soltanaei R, Chaghazari HR, Masoudi F, Nazari H. Effect of water deficit stress and foliar application of salicylic acid on antioxidant enzymes activity in leaves of Thymus daenensis subsp. Lancifolius. Cercetari Agronomice in Moldova. 2015, 48(1): 57–67.

16. Ghoulene I, Mohammed-Reda D, Rachid R, Houria B. ROS and antioxidant system of triticum durum after water stress. Annual Research & Review in Biology. 2014, 4(8): 1241–1249.

17. Chang EM, Shi SQ, Liu JF, Yang WJ, Jiang ZP. ROS production and its elimination in old platycladus orientalis' leaves. Journal of Northeast Forestry University. 2011, 39(11): 8–11. (In Chinese with English abstract)

18. Russell JP, Veronica V. Phenylalanine increases membrane permeability. Journal of the American Chemical Society. 2017, https://doi.org/10.1021/jacs.7b09219 PMID: 28965406

19. Sanchez-Rodriguez E, Rubio WM, Cervilla LM, Blasco B, Rios JJ, Rosales M, et al. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci. 2010, 178: 30–40.

20. Jovanović A, Balanč B, Đorđević V, Šavkina K, Nedović V, Bugarski B, et al. Fluorescence analysis of liposomal membranes permeability. Tehnika. 2019, 74: 493–498.

21. Shen M. Preliminary study on the relations between membrane permeability, endogenous hormones and cold resistance of Ivy. Acta Horticulturae Sinica. 2005, 32(1): 141–144. (In Chinese with English abstract)

22. Dai GX, Peng QK, Xiao LT, Deng GF. Effect of Drought Stress Simulated by PEG on Malonaldehyde, Proline Contents and Superoxide Dismutase Activity in Low Potassium Tolerant Rice Seedlings. Chinese Journal of Rice Science. 2006, 20: 557–559. (In Chinese with English abstract)

23. Du RF, Hao WF, Wang LF. Dynamic response on anti-oxidative defense system and lipid peroxidation of Lespedeza davurica to drought stress and re-watering. Acta Prataculturae Sinica. 2012, 21(2): 51–56. (In Chinese with English abstract)

24. De Vos CHRD, Schat H, Voojis R, Ernst Wilfried HO. Copper-induced Damage to the Permeability Barrier in Roots of Silene cucubalus. Journal of Plant Physiology. 1989, 135(2): 164–169.

25. Zhang GL, Chen LY, Zhang ST, Xiao YH, He ZZ, Lei DY. Effect of high temperature stress on protective enzyme activities and membrane permeability of flag leaf in rice. Acta Agronomica Sinica. 2006, 32(9): 1306–1310. (In Chinese with English abstract)

26. Ruchika P, Brendan RC, Richard E. Growth and Physiological Responses of Temperate Pasture Species to Consecutive Heat and Drought Stresses. Plants. 2019, https://doi.org/10.3390/plants8070227 PMID: 31315284

27. Yao LN, Zhou QY, Yang HX. Discussion on the molecular structure of chlorin. Education In Chemistry. 2005, 5: 58–59, 41. (In Chinese)

28. Croft H, Chen JM, Wang R, Mo G, Luo S, Luo X, et al. The global distribution of leaf chlorophyll content. Remote Sensing of Environment. 2020, https://doi.org/10.1016/j.rse.2019.111479

29. Paulsen H, Schmid VHR. Analysis and Reconstitution of Chlorophyll-Proteins. Heme, Chlorophyll, and Bilins. 2008, https://doi.org/10.1385/1-59259-243-0:235

30. Mork-Jansson AE, Eichacker LA. A strategy to characterize chlorophyll protein interaction in LIL3. Plant Methods. 2019, https://doi.org/10.1186/s13007-018-0385-5 PMID: 3062623

31. Jian LC, Wang H. Plant stress biology, Science Press, Beijing, China. 2009, pp 123, 174. (In Chinese)

32. Zhang MS, Xie B, Tan F, Zhang QT. Relationship among Soluble Protein, Chlorophyll and ATP in Sweet potato under water stress with drought resistance. Scientia Agricultura Sinica. 2003, 36(1): 13–16. (In Chinese with English abstract)
33. Zhang MS, Tan F. Relationship between ratio of Chlorophyll a and b under water stress and drought resistance of different Sweet Potato varieties. Seed. 2001, 4: 23–25. (In Chinese with English abstract)

34. Qin P, Liu FH, Liang XN. Superoxide Dismutase and Plant Resistance to the Environmental Stress. Heilongjiang Agricultural Sciences. 2002, (1): 31–34. (In Chinese with English abstract)

35. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Biology, Annual Review of Plant Biology. 1998, 49: 249–279.

36. Alsheer RG, Erturk N, Heath LS. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of experimental botany. 2002, 53: 1331–1341. PMID: 11997379

37. Fridovich I. Superoxide dismutases. In: Meister A, ed. Advances in Enzymology and Related Areas of Molecular Biology. New York: John Wiley & Sons. 1986, 58: 61–97. https://doi.org/10.1002/9780470123041.ch2 PMID: 3521218

38. White JA, Scandalias JG. In vitro synthesis, importation and processing of Mn-superoxide dismutase (SOD-3) into maize mitochondria. Biochimica et Biophysica Acta. 1987, 926: 16–25. https://doi.org/10.1016/0304-4165(87)90178-4 PMID: 2443181

39. Baum JA, Scandalias JG. Developmental expression and intracellular localization of superoxide dismutases in maize. Differentiation. 1979, 13: 133–140.

40. Mccord JM, Fridovich I. Superoxide Dismutase an enzymic function for erythrocuprein (Hemocuprein). Journal of Biological Chemistry. 1969, 244(22): 6049–6055. PMID: 5389100

41. Molina-Rueda JJ, Tsai CJ, Kirby EG. The Populus Superoxide Dismutase Gene Family and Its Responses to Drought Stress in Transgenic Poplar Overexpressing a Pine Cytosolic Glutamine Synthetase (GS1a). PloS One. 2013, 8(2): e56421. https://doi.org/10.1371/journal.pone.0056421 PMID: 23451045

42. Xu XY, Zhang FY, Zeng QW. Effect of NaCl and Na2SO4 stress on seed germination of Cosmos bipinnatus. Journal of Northeast Agricultural University. 2014, 45(4): 55–59. (In Chinese with English abstract)

43. Mohammadi MHS, Etemadi N, Arab MM, et al. Molecular and physiological responses of Iranian Perennial ryegrass as affected by Trinexapac ethyl, Paclobutrazol and Abscisic acid under drought stress. Plant Physiology and Biochemistry. 2016, 111: 129–143. https://doi.org/10.1016/j.plaphy.2016.11.014 PMID: 27915174

44. Asada K. Ascorbate peroxidase- a hydrogen peroxide- scavenging enzyme in plants. Physiologia Plantarum. 1992, 85(2): 235–241.

45. Qin P, Liu YJ, Liu FH. Effects of drought stress on SOD and POD activities in tobacco leaves. Chinese tobacco science. 2005, (2):28–30. (In Chinese with English abstract)

46. Chen Y, Wang M, Hu LL, Liao WB, Dawuda MM, Li CL. Carbon Monoxide Is Involved in Hydrogen Gas-Induced Adventitious Root Development in Cucumber under Simulated Drought Stress. Frontiers in Plant Science. 2017, 8(230). https://doi.org/10.3389/fpls.2017.00128

47. Wu XL, Yuan J, Luo AX, Chen Y, Fan YJ. Drought stress and re-watering increase secondary metabolites and enzyme activity in dendrobium moniliforme. Industrial Crops & Products. 2016, 94: 385–393.

48. Jiang MY, Guo SC. Oxidative stress and antioxidation induced by water deficiency in plants. Plant Physiology Community. 1996, 32(2): I44–I50. (In Chinese with English abstract)

49. Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, et al. Regulation and function of ascorbate peroxidase isoenzymes. Journal of Experimental Botany. 2002, 53(372):1305–1319. PMID: 11997377

50. Li ZQ, Li JX, Zhang GF. Expression regulation of plant ascorbate peroxidase and its tolerance to abiotic stresses. HEREDITAS (Beijing). 2013, 39(1): 45–54. (In Chinese with English abstract)

51. Sukweenadith J, Kim YJ, Rahimi S, Silva J, Myagmarjav D, Kwon WS, et al. Overexpression of a cytosolic ascorbate peroxidase from Panax ginseng enhanced salt tolerance in Arabidopsis thaliana. Plant Cell Tissue and Organ Culture. 2017, 1–14.

52. Anjum NA, Sharma P, Gill SS, Hasanuzzaman M, Khan EA, Kachhapa K, et al. Catalase and ascorbate peroxidase-representative H2O2-detoxifying haeme enzymes in plants. Environmental Science and Pollution Research. 2016, https://doi.org/10.1007/s11356-016-7309-6 PMID: 27549233

53. Wu QS, Xia RX. Effects of arbucular mycorrhizal fungi on leaf solutes and root absorption areas of trifoliate orange seedlings under water stress conditions. Frontiers of Forestry in China. 2006, 1: 312–317.

54. Tian GZ, Li HF, Qiu WF. Advances on research of plant peroxidases. Journal of Wuhan Botanical Research. 2001, 19(4): 332–344. (In Chinese with English abstract)
77. Niu W. Studies on photosynthetic characteristics and drought Resistance of four Caragana species. Lanzhou, Gansu Province, China: Gansu Agriculture University. 2018: 32. (In Chinese with English abstract)

78. Yang B. Response of Robinia pseudoacacia L. Seedlings to Soil Water Stress: Based on Growth, Physiology and the Allocation and Dynamics of Non-structural Carbohydrates. Yangling, Shanxi Province, China: Northwest A & F University. 2019: 19. (In Chinese with English abstract)

79. Wu JC, Tian SQ, Xue RR, Xu Z, Zhang XX. Physiological and biochemical response of three species of Lina seedlings to natural drought stress. Guizhou Agriculture Science. 2017, 45(12): 25–29. (In Chinese with English abstract)

80. Liu ZJ, Yuan R, Wang N, Li BY, Jia SC, Zong YZ, et al. Effects of drought Stress on leaf physiology of Hylotelephium erythrostictum. Journal of Shanxi Agricultural Sciences. 2018, 46(04), 548–553. (In Chinese with English abstract)

81. Fu BC, Bo W, Qin GJ, Wang S. Physiological response of drought stress in Sedum species and evaluation of drought resistance. Molecular Plant Breeding. 2017, 15(03): 1096–1103. (In Chinese with English abstract)

82. Sun X. Preliminary study on drought-resistance characteristics of Dianthus plumarius. Haerbin, Heilongjiang province, China: Northeast Forestry University. 2006: 20. (In Chinese with English abstract)

83. He YC, Zu WH, Wang Q, Li QB, Ma FF, Hu HR. Comparison of drought tolerance in six succulents. Acta Agriculturae Boreali-occidentalis Sinica. 2010, 19(3): 127–130. (In Chinese with English abstract)

84. Yang PH, Li GQ, Guo L, Wu SJ. Effect of drought stress on plasma membrane permeability of soybean varieties during flowering-poding stage. Agricultural research in the arid areas. 2003, 21(3): 127–130. (In Chinese with English abstract)

85. Chen JY, Feng LY, Gao J, Shi JW, Zhou YC, Tu FT, et al. Influence of light intensity on stoma and photosynthetic characteristics of Soybean leaves. Scientia Agricultura Sinica. 2019, 52(21): 3773–3781. (In Chinese with English abstract)

86. Chang QS, Zhang LX, Wang JZ, Wang Z, Xu SJ, Kang L, et al. Effects of drought stress and rewatering on physiological indexes of four Paonia lactiflora cultivars and evaluation of their drought resistance. Journal of Nanjing Forestry University(Natural Sciences Edition). 2018, 42(06): 44–50. (In Chinese with English abstract)

87. Liu YL, Guo SX, Ma MS. Effect of drought stress and rewatering at seedlings stage on light energy utilization and antioxidant enzymes activities of spring maize leaves. Journal of Soil and Water Conservation. 2018, 32(1): 339–343. (In Chinese with English abstract)

88. Tian X. The studies on the introduction and adaptability of Crassulaceae plants and their propagation techniques. Hefei City, Anhui Province, China: Anhui Agriculture University. 2009, 1. (In Chinese with English abstract)

89. Cui J. Effect of the stress on the three kinds of Crassulaceae Echeveria succulent leaves coloring. Shenyang City, Liaoning Province, China: Shenyang Agriculture University. 2016: 1. (In Chinese with English abstract)

90. Huang QC, Wei YH. Comparative Analysis of Chlorophyll Content on Several Sun Plants and Shade Plants. Hubei Agricultural Sciences. 2009, 48(8): 1923–1924, 1929. (In Chinese with English abstract)

91. Zhou Y. Studies on the drought resistance of 7 Iris species. Urumqi, Xinjiang Uygur Autonomous Region, China: Xinjiang Agriculture University. 2006: 14. (In Chinese with English abstract)

92. Ding YF, Sun JP, Shang XY, Li XJ, Sun H, Zhu JW, et al. Effects of drought on cell membrane damage and activities of protective enzyme of different flue-cured tobacco leaves. Southwest China Journal of Agricultural Sciences. 2015, 28(6): 2746–2749. (In Chinese with English abstract)

93. Yin YQ, Hu JB, Deng MJ. Latest development of antioxidant system and responses to stress in plant leaves. Chinese Agricultural Science Bulletin. 2007, 23(1): 105–110. (In Chinese with English abstract)

94. Shang RX, Yu X, You CC, He HB, Li J, Wu LQ. Adaptation physiological mechanism of rice under dual stress of drought stress and high temperature in booting stage. Journal of Gansu Agricultural University. 2019, 12(54): 39–46. (In Chinese with English abstract)

95. Li TH, Wang X, Cai J, Zhou Q, Dai TB, Jiang D. Comprehensive evaluation of drought priming on plant tolerance in different wheat cultivars. Journal of Triticaceae Crops. 2018, 38(1): 65–73. (In Chinese with English abstract)

96. Zuo YM, Yang WZ, Yang TM, Yang MQ, Xu ZL, Yang SB, et al. Comparison of resistant physiological index among four species in the Genus Panax under water stress. Crop. 2016, (03): 84–88. (In Chinese with English abstract)
97. Dong QQ, Ai X, Zhang YZ, Zhang KC, Zhou DY, Wang XG, et al. Effect of drought stress on physiological characteristics and yield in different tolerant peanut. Journal of Shenyang Agricultural University. 2020, 51(01): 18–26. (In Chinese with English abstract)

98. Chen L, Shen XR, Zhao HY, Liu K. Optimization of treatment conditions for extracting SOD in Sedum lineare. Grassland and Turf. 2009, 1(132): 1–6. (In Chinese with English abstract)

99. Ruppenthal V, Zoz T, Steiner F, Lana MDC, Castagnara DD. Silicon does not alleviate the adverse effects of drought stress in soybean plants. Semina: Ciências Agrárias, Londrina. 2016, 37(6): 3941–3954.

100. Sun GR, Peng YZ, Yan XF, Zhang R, Jiang LF. Effect of drought stress on activity of cell defense enzymes and lipid peroxidation in leaves of Betula Platypylla seedlings. Scientia silvae sinicae. 2003, 39(1): 165–167.

101. Li Y, Deng XP, Sang-Soo K, Kiyoshi T. Drought tolerance of transgenic sweet potato expressing both Cu/Zn Superoxide Dismutase and Ascorbate Peroxidase. Journal of Plant Physiology and Molecular Biology. 2006, 32(4): 451–457. (In Chinese with English abstract) PMID: 16957397