Physiological and Biochemical Responses of Two Herbaceous Peony Cultivars to Drought Stress

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Abstract. Herbaceous peony is a perennial flowering plant with strong environmental adaptability and may be a good candidate for culture in arid areas. In this study, the physiological and biochemical responses of two herbaceous peony cultivars to different soil moisture gradients in pots were assessed by analyzing changes in 13 stress-related indices. The drought damage index (DDI) and the contents of malondialdehyde (MDA), soluble sugar, proline, and abscisic acid (ABA) generally increased as drought stress intensified, whereas leaf relative water content (LRWC) decreased, and the contents of soluble protein, indole-3-acetic acid (IAA), the ratio of IAA and ABA, and the activities of four antioxidant enzymes fluctuated. For the leaves, a positive correlation was found between DDI and superoxide dismutase (SOD), MDA, soluble sugar, proline, ascorbate peroxidase (APX), and ABA, but it was negatively correlated with LRWC, peroxidase (POD), and catalase (CAT). In fibrous roots, DDI was positively correlated with MDA, soluble sugar, proline, soluble protein, and ABA but was negatively correlated with SOD, CAT, APX, and IAA/ABA. Principal component analysis and subordinate functions were used to evaluate drought resistance of the two cultivars, with ‘Karl Rosenfield’ showing greater resistance to drought than ‘Da Fu Gui’.

The shortage of water resources is a serious problem facing the world’s agriculture and horticulture. Increasingly severe drought calls for saving water in green spaces and also for more drought-resistant landscape plants. However, the effects of water deficit on plants are manifested in various stages, affecting specific physiological metabolic processes such as absorption and transportation of water and nutrients (Aranda et al., 2015; Smith et al., 2009). These physiological and biochemical processes can reflect a plant’s response to environmental stresses

(Lambers et al., 2008). *Paeonia lactiflora*, a traditional ornamental plant, has a cultivation history of more than 3900 years and is currently a sought-after cut flower in international markets (Li, 1999). With a perennial crown (metamorphosed underground shoot), herbaceous peony has developed special growth patterns to escape stress periods such as considerable changes in temperature, light, and humidity during their annual growth cycle and to resume growth in the usual season, which differentiates it from other perennial plants (Kamenetsky et al., 2003), but the resistance of herbaceous peony varieties cultivated worldwide to these stresses also varies widely.

Only a few studies on the physiological and biochemical responses of herbaceous peonies to drought stress have been published. Guo (2009) studied the phenotypic traits and leaf physiological and biochemical characteristics of four herbaceous peony

cultivars treated with different soil moisture content (SMC) ($75 \pm 5\%$, $55 \pm 5\%$, $35 \pm 5\%$, and $15 \pm 5\%$ of saturated SMC). Using principal component analysis to comprehensively analyze the indices of the test materials, Guo found that four cultivars could be ranked from strong to weak drought resistance: ‘Da Fu Gui’, ‘Da Hong Pao’, ‘Zhao Yuan Fen’, and ‘Mo Zi Lou’. Our team studied the effects of different levels of soil water content on phenotypic traits and water physiological indices of the leaves of some herbaceous peony cultivars such as ‘Duchesse de Nemours’ that had been introduced to Beijing (Wang et al., 2014a). In many cases, the drought stress response is similar to saline-alkali stress. It indicates that the influence of salt concentration on phenotypic traits is greater than pH (Wang et al., 2013), although extreme pH in irrigation water will reduce crown size, leaf area, and stem thickness of herbaceous peonies, which eventually affects the quality of flowers (Zhao et al., 2013).

In contrast, tree peony has received more attention and thus achieved more results in this area of research. Using potted *P. suffruticosa* ‘Luo Yang Hong’ and ‘Wu Long Peng Sheng’, Li (2014) assessed the dynamic changes in activity of cell defense enzymes and content of substances related to osmotic adjustment of leaves in naturally induced drought stress and rewatering, finding that after rewatering, most indices of both tree peony cultivars dropped compared with the control. A et al. (2015) used potted plants of three tree peony cultivars with five levels of SMC ($35 \pm 5\%$, $55 \pm 5\%$, $75 \pm 5\%$, $95 \pm 5\%$, and half flooded) to study the changes in soluble protein and soluble sugar content, as well as cell membrane permeability under water stress. On the basis of the changes in these indices, a comprehensive assessment of drought resistance of the cultivars was achieved, with cultivars ranked as ‘Fen Rong Su Zhuang’ > ‘Bing Shan Cang Yu’ > ‘Jin Cheng Nv Lang’, whereas their resistance to waterlogging was different. Furthermore, some researchers have conducted detailed studies on the physiology and biochemistry of cultivars of *P. rockii* and *P. suffruticosa* under drought stress (Guo et al., 2014; Kong et al., 2011; Peng et al., 2014).

The objective of this study was to determine how drought stress affects the physiology and biochemistry of two herbaceous peony cultivars. Using relative methods and indices of leaves and fibrous roots, which are more sensitive tissues to environmental changes than leaves, we established a theoretical basis for future screening of drought-resistant varieties for the application of herbaceous peony to arid areas.

Materials and Methods

Plant material and experimental treatments.

The two herbaceous peony cultivars, ‘Karl Rosenfield’ and ‘Da Fu Gui’, were collected from nurseries in Xiaotangshan, Beijing. On 28 Sept. 2016, dormant herbaceous peonies

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were pruned to the ground, the roots were lifted out of the ground and washed, and then cut into divisions containing five to eight strong buds and a fleshy root. Roots were shortened to 15 to 20 cm. One hundred plants of each cultivar that had been divided were planted with the same mass of substrate (peat: perlite: vermiculite = 3:1:1, v/v) in the same size pot. In mid-April of 2017, the buds of all plants were cut off, and all plants were moved into a greenhouse under the following conditions: light intensity: 30 to 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; photoperiod: 9 h·d⁻¹; relative humidity: 50% to 75%; and day/night temperatures: 26 to 30 °C/14 to 18 °C. On 21 April, a trial experiment was performed with 15% of the plants, and soil-saturated water content was measured with FieldScout (TDR 100; Spectrum Technologies Inc., Aurora, IL). The division of soil moisture gradients was based on the methods of Guo (2009) and Wang et al. (2014a), and appropriate improvements relied on previous trials. Since phenotypic traits changed rapidly as SMC decreased, a more intensive gradient in the severe drought stress treatment was set: control (CK), mild drought stress (T1), moderate drought stress (T2), moderately severe drought stress (T3), severe drought stress (T4), and very severe drought stress (T5). The soil water content of each treatment was 60 ± 2%, 45 ± 2%, 30 ± 2%, 15 ± 2%, 10 ± 2%, and 5 ± 2%, respectively, of saturated SMC. The experiment began on 10 May 2017. All plants were irrigated thoroughly. Watering was then stopped to allow soil to dry naturally. SMC was measured with FieldScout at 8:00 to 9:00 AM every morning. Twelve pots were selected randomly from each cultivar that reached the corresponding gradient on the same day, and those already used were no longer used as follow-up experimental materials. In addition to direct determination of phenotypic traits, LRWC, the fibrous roots and leaves of plants were collected randomly and mixed, then wrapped separately in aluminum foil, placed immediately into liquid nitrogen, and stored before the determination of other indices. All the aforementioned experiments were repeated three times.

Measurement methods of indices. Referring to the evaluation system of soil water stress on the physiological and biochemical reactions of herbaceous peony cultivars used by Guo (2009) and Wang et al. (2014a), five levels of scores were assigned based on the degree of drought stress on phenotypic traits (Table 1). The DDI of each cultivar under each treatment was obtained with the following Eq. [1], and the average score of every four plants was calculated as one replicate for further data analysis.

$$\text{DDI} = \frac{\sum \text{Score of each plant}}{\text{Number of plants in each treatment}} \quad [1]$$

The LRWC was measured using the method of Barrs and Weatherley (1962). MDA content was determined by the thiobarbituric acid reaction according to Heath and Packer

(1968). Soluble sugar content was measured based on the anthrone method (Morris, 1948) whereas free proline content was quantified using the method of Bates et al. (1973). Soluble protein content was determined using the Coomassie Brilliant Blue G-250 staining method (Bradford, 1976). The measurement and calculation of SOD (EC 1.15.1.1) activity were based on the photochemical method described by Li (2000). Using the corresponding kit for each assay (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), the activities of POD (EC 1.11.1.7), CAT (EC 1.11.1.6), and APX (EC 1.11.1.11) were measured according to the instructions of each kit. ABA and IAA were tested with enzyme-linked immunosorbent assay by the College of Agronomy and Biotechnology, China Agricultural University. All indices of each treatment were measured three times.

Statistics. Data of different treatments and indices were subjected to analysis of variance and significant differences ($P \leq 0.05$) assessed by Duncan's multiple range test (Duncan, 1955) with IBM SPSS Statistics (version 20.0, IBM Corp., Armonk, NY). In this study, correlation analysis among physiological and biochemical indices of leaves and fibrous roots was performed separately. As phenotype intuitively reflects the plant's response to drought stress, the indices whose correlation between it and DDI, which represents the phenotypic trait, is significant at the 0.05 level and 0.01 level were extracted to

calculate the eigenvectors and contribution rates (C_i) with principal components analysis. These aforementioned processes also were performed using IBM SPSS Statistics. The membership function method was used to comprehensively evaluate the two herbaceous peony cultivars using a slightly modified method of Wang et al. (2014b) by replacing the original meaning of the reference with drought resistance-related significance. At first, the specific membership function values were calculated with Eq. [2] (if the correlation result between the index and DDI was positive) or with Eq. [3] (if the correlation result between the index and DDI was negative):

$$X_{ij} = (X - X_{\min}) / (X_{\max} - X_{\min}) \quad [2]$$

$$X_{ij} = (X_{\max} - X) / (X_{\max} - X_{\min}) \quad [3]$$

where X is the value of character j for cultivar i , $X_{\min}$ is the minimum value of character j among all measurements for cultivar i , $X_{\max}$ is the maximum value of character j among all measurements for cultivar i , and X_{ij} is the drought-responsive membership value for cultivar i and character j .

Thereafter, the integrated index (I) of each cultivar was calculated with Eq. [4] (multiplication and addition):

$$I = \sum (C_i \times X_{ij}) \quad [4]$$

Finally, the values of ' I ' indicate the level of drought resistance: the lower the value, the greater the drought resistance.

Table 1. Evaluation of phenotypic traits in herbaceous peony under drought stress.

Description of the external form of the plant	Score
No obvious symptoms of drought stress	1
About 10% to 30% of leaves wilt and droop, and their tip and margin turn yellow	2
About 50% of the tip and margin of leaves turn yellow, curl and dry	3
About 70% to 90% of leaves curl and dry	4
Leaves and branches wither until the entire plant dies	5

Fig. 1. The appearance of two cultivars. Left is 'Karl Rosenfield' and right is 'Da Fu Gui'. (A) Control (unstressed). (B) T5 (strongly drought stressed).

Results and Discussion

Under drought stress, a series of changes take place in plant cells, and the damage is ultimately reflected in the external appearance of plants (Farooq et al., 2009). The degree of drought damage is judged directly by observing the phenotypic traits of plants. To relate phenotypic traits to physiological and biochemical indicators, it is necessary to quantify them. Our experiment showed that as SMC decreased, the phenotypic traits worsened, which was consistent with the results of DDI (Figs. 1 and 2).

LRWC can indicate whether the plant leaf cells are in a state of expansion, which is a commonly used indicator to measure the drought resistance of plants (Raviv and Blom, 2001). As shown in Fig. 3, LRWC of both cultivars declined as SMC decreased. Although there was no significant difference in LRWC between the two cultivars for each treatment, treatments were significantly different for each cultivar, except between T4 and T5.

MDA content is considered to be an essential indicator of plant resistance to abiotic stress. In *Paeonia* spp., MDA shows an increase in response to drought stress (A et al., 2015; Guo, 2009; Li, 2014). In our study, MDA accumulated both in leaves and fibrous roots as the severity of drought stress increased (Fig. 4). Water-stress tolerance involves subtle changes in cellular biochemistry and may result from the accumulation of compatible solutes and specific proteins that can be rapidly induced by osmotic stress (Shao et al., 2007). As shown in Fig. 4, the increasing trends of soluble sugar and proline contents observed in both herbaceous peony cultivars under drought stress mirror previous findings of some tuberous root plants such as *Dahlia pinnata* (Fan et al., 2011), *Ipomoea batatas* (Yooyongwech et al., 2017), and *P. suffruticosa* 'Luo Yang Hong' and 'Hu Hong' (Kong et al., 2011), the latter using potted plants with four levels of SMC (100%, 75% as CK, 50%, and 25%). However, the variation in soluble protein content of this study was not unanimous. Guo (2009) found that the soluble protein content of two herbaceous peony cultivars, 'Zhao Yuan Fen' and 'Mo Zi Lou', declined as SMC decreased, whereas 'Da Hong Pao' showed a down-up-down trend. In *Medicago sativa*, Zhang and Shi (2018) found that soluble protein content first increased then decreased as drought stress increased.

Plants have a protective mechanism against abiotic stresses by synthesizing antioxidants and increasing the activity of antioxidative enzymes (Egert and Tevini, 2002). Guo (2009) studied four herbaceous peony cultivars under drought stress and concluded that their SOD activity first increased then declined with decreasing SMC. In this study, the SOD activity of leaves and fibrous roots of both peony cultivars first increased, then decreased, and finally rose again (Fig. 5). Although the lowest SMC in Guo's experiment was almost the same as T3 in our study, Guo

did not test plants growing in soil with lower SMC. Curiously, SOD activity increased slightly from T4 in our study. CAT, which is present only in peroxisomes, transmutes H_2O_2 into water and molecular oxygen, whereas POD decomposes H_2O_2 through oxidation of cosubstrates such as phenolic compounds and/or antioxidants (Bowler et al., 1994). In this study, the changes in POD and CAT activities in leaves and fibrous roots of the two cultivars fluctuated but were coordinated and synergistic overall, whereas CAT activity decreased continuously throughout severe drought. In general, APX scavenges excess reactive oxygen species and its activity increases under stress, catalyzing the conversion of H_2O_2 to water using ascorbate as substrate (Hossain et al., 2012). In a study on the evaluation of heat resistance in

P. lactiflora by Zhang et al. (2016), APX was considered a reliable physiological evaluation index of heat damage in leaves, so we adopted it. In our experiment, the changes in APX activity in leaves was more regular than in fibrous roots, which may be because APX mainly exists in the chloroplast and cytoplasm and is a key enzyme for scavenging H_2O_2 in chloroplasts (Caverzan et al., 2016). On the basis of the changes observed in the activity of these four enzymes, we believe that although the antioxidant enzyme system might play a role in assisting the plant in response to severe drought stress, it was damaged at early growth stages.

Plants have developed many adaptive strategies to enhance drought stress tolerance, including changes in phytohormones (Davies, 2010). ABA regulates stomatal

Fig. 2. Drought damage index (DDI) of two herbaceous peony cultivars under drought stress. Values are means of three replicates and error bars represent sd. Different letters on the columns indicate significant differences among treatments across both cultivars according to Duncan's multiple range test at $P \leq 0.05$. CK = control; T1 = mild drought stress; T2 = moderate drought stress; T3 = moderately severe drought stress; T4 = severe drought stress; T5 = very severe drought stress.

Fig. 3. Leaf relative water contents (LRWC) of two herbaceous peony cultivars under drought stress. Values and different letters on the columns indicate the same meanings as Fig. 2. CK = control; T1 = mild drought stress; T2 = moderate drought stress; T3 = moderately severe drought stress; T4 = severe drought stress; T5 = very severe drought stress.

Fig. 4. Contents of malondialdehyde (MDA), soluble sugar, proline, and soluble protein of two herbaceous peony cultivars under drought stress. Values are means of three biological replicates and error bars represent s.d. Different letters on the columns indicate significant differences among treatments across both cultivars of each index according to Duncan's multiple range test at $P \leq 0.05$. CK = control; T1 = mild drought stress; T2 = moderate drought stress; T3 = moderately severe drought stress; T4 = severe drought stress; T5 = very severe drought stress.

Fig. 5. Activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) of two herbaceous peony cultivars under drought stress. Values and different letters on the columns indicate the same meanings as Fig. 4. CK = control; T1 = mild drought stress; T2 = moderate drought stress; T3 = moderately severe drought stress; T4 = severe drought stress; T5 = very severe drought stress.

Fig. 6. Contents of abscisic acid (ABA), indole-3-acetic acid (IAA), and IAA/ABA of two herbaceous peony cultivars under drought stress. Values and different letters on the columns indicate the same meanings as Fig. 4. CK = control; T1 = mild drought stress; T2 = moderate drought stress; T3 = moderately severe drought stress; T4 = severe drought stress; T5 = very severe drought stress.

Table 2. Correlation analysis between DDI and other drought-responsive physiological and biochemical indices of leaves and fibrous roots (pooled for both herbaceous peony cultivars).

Indices	LRWC	MDA	Soluble sugar	Proline	Soluble protein	SOD	POD	CAT	APX	ABA	IAA	IAA/ABA
DDI Leaves	-0.934**	0.930**	0.859**	0.919**	-0.152	0.410*	-0.465**	-0.590**	0.625**	0.697**	0.286	-0.239
Fibrous roots	—	0.885**	0.934**	0.904**	0.874**	-0.778**	-0.134	-0.881**	-0.675**	0.970**	-0.141	-0.790**

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

DDI = drought damage index; LRWC = relative water content; MDA = malondialdehyde; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase; ABA = abscisic acid; IAA = indole-3-acetic acid.

Table 3. Eigenvectors and contribution rates of principal components of drought-responsive indices of leaves and fibrous roots.

Indices	Leaves		Fibrous roots
	Component 1	Component 2	Component
LRWC	-0.945	0.147	—
MDA	0.973	0.026	0.890
Soluble sugar	0.939	-0.128	0.965
Proline	0.914	0.232	0.904
Soluble protein	—	—	0.946
SOD	0.487	0.726	-0.871
POD	-0.643	0.535	—
CAT	-0.515	-0.756	-0.861
APX	0.660	-0.342	-0.795
ABA	0.828	-0.181	0.957
IAA	—	—	—
IAA/ABA	—	—	-0.742
Contribution rate/%	62.165	18.070	78.182
Cumulative contribution rate/%	62.165	80.235	78.182

LRWC = relative water content; MDA = malondialdehyde; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase; ABA = abscisic acid; IAA = indole-3-acetic acid.

conductance by reducing transpiration water loss and eventually affects the regulation of water balance and osmotic stress tolerance in plants (Morkunas et al., 2014). In our experiment, an increasing trend in ABA content with more acute drought stress (Fig. 6) is consistent with previous studies in *Agrostis* spp. (Dacosta and Huang, 2007) and *Vaccinium darrowii* seedlings (Chen et al., 2017). Under drought stress, IAA also is involved in the physiological responses of plants (Mahouachi et al., 2007). The change in IAA content in

our research was irregular, but contradictory results were found regarding changes in IAA content in different plants under different drought stress conditions. With increasing drought stress, IAA content first increased then declined in *Agrostis stolonifera* (Li et al., 2015) and decreased in leaves of *Manihot esculenta*, but the content in roots varied, depending on the variety (Zhou et al., 2013). In this experiment, IAA/ABA fluctuated in leaves and decreased in fibrous roots, which had the same association in the roots

of cassava varieties (Zhou et al., 2013). In contrast, Li et al. (2010) found a low IAA/ABA ratio in the main stem leaves of cotton (*Gossypium* spp.) at an early stage of drought stress, which increased in intermediate and later stages.

The correlation analysis is shown in Table 2. For the leaves, DDI was positively correlated with SOD ($P \leq 0.05$), as well as MDA, soluble sugar, proline, APX, and ABA ($P \leq 0.01$), and was negatively correlated with LRWC, POD, and CAT ($P \leq 0.01$). In the fibrous roots, DDI was positively correlated with MDA, soluble sugar, proline, soluble protein, and ABA ($P \leq 0.01$) and was negatively correlated with SOD, CAT, APX, and IAA/ABA ($P \leq 0.01$). The former nine indices and the latter nine indices were extracted to calculate the eigenvectors and levels of contribution of principal components of drought-responsive indices of leaves and fibrous roots (Table 3). On the basis of membership function values (Table 4), integrated indices were calculated with Eq. [4], and the drought resistance of 'Karl Rosenfield' was ranked as being greater than 'Da Fu Gui' (Table 5).

In this study, drought stress triggered a series of reactions in two herbaceous peony cultivars. We have measured the physiological and biochemical indicators of both leaves and fibrous roots of herbaceous peony under drought stress, which would provide a theoretical basis for related mechanisms and

Table 4. Membership function values of drought-responsive indices of leaves and fibrous roots.

Indices	Leaves		Fibrous roots	
	'KR'	'DFG'	'KR'	'DFG'
LRWC	0.000	1.000	—	—
MDA	0.000	1.000	0.000	1.000
Soluble sugar	0.000	1.000	1.000	0.000
Proline	0.000	1.000	1.000	0.000
Soluble protein	—	—	1.000	0.000
SOD	0.000	1.000	1.000	0.000
POD	0.000	1.000	—	—
CAT	0.000	1.000	0.000	1.000
APX	1.000	0.000	1.000	0.000
ABA	1.000	0.000	0.000	1.000
IAA	—	—	—	—
IAA/ABA	—	—	0.000	1.000

'KR' = Karl Rosenfield; 'DFG' = Da Fu Gui; LRWC = relative water content; MDA = malondialdehyde; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase; ABA = abscisic acid; IAA = indole-3-acetic acid.

Table 5. Integrated index and relative drought resistance precedence of two herbaceous peony cultivars.

Indices	'KR'		'DFG'	
	Leaves	Fibrous roots	Leaves	Fibrous roots
LRWC	0.000	—	0.127	—
MDA	0.000	0.000	0.126	0.112
Soluble sugar	0.000	0.122	0.125	0.000
Proline	0.000	0.114	0.126	0.000
Soluble protein	—	0.119	—	0.000
SOD	0.000	0.110	0.090	0.000
POD	0.000	—	0.102	—
CAT	0.000	0.000	0.094	0.109
APX	0.097	0.100	0.000	0.000
ABA	0.113	0.000	0.000	0.121
IAA	—	—	—	—
IAA/ABA	—	0.000	—	0.094
Integrated index	0.775		1.226	
Precedence	1		2	

'KR' = Karl Rosenfield; 'DFG' = Da Fu Gui; LRWC = relative water content; MDA = malondialdehyde; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase; ABA = abscisic acid; IAA = indole-3-acetic acid.

allow for the improvement of herbaceous peony introduction to drought stressed zones. A comprehensive evaluation of drought resistance between the two cultivars is necessary because the trends in some indicators of different plants are not necessarily the same, whereas a simple comparison of some indicators shows a very scattered or difficult-to-interpret pattern, so that we established a correlation between DDI and other indices of leaves and fibrous roots and made use of principal component analysis and subordinate functions to obtain the final result. We also need to mention that although a greenhouse provides more controlled conditions in stress treatments, the relationship between the findings of this greenhouse experiment and a field experiment needs further study and validation.

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