Full Length Research Paper

Effects of waterlogging on growth, biomass and antioxidant enzymes on upper ground and roots of two peony cultivars

Xiangtao Zhu¹, Wen Ji¹, Erman Hong¹, Yufei Cheng¹, Xin Lin¹, Haojie Shi², Xueqin Li¹
and Song Heng Jin¹*

¹Jiyang College, Zhejiang A&F University, Zhuji 311800, Zhejiang, China.
²School of Agriculture and Food Science, Zhejiang A&F University, Linan, Hangzhou 311300, Zhejiang, China.

Received 29 September, 2018; Accepted 12 November, 2018

Tree peony (Paeonia suffruticosa Andr.) is a perennial deciduous shrub with ornamental and medicinal value. Waterlogging stress is an agricultural problem for peony in Jiangnan of China. This study investigated the growth, biomass, cell membrane permeability and antioxidant enzymes of two P. suffruticosa cultivars ‘Feng Dan Bai (FDB)’ and ‘MingXing (MX)’. The response of roots and upper ground to different levels of waterlogging stress was also evaluated. Results showed that mild waterlogging stress (MWS) and severe waterlogging stress (SWS) significantly decreased the increment of seedling height, diameter and biomass of leaves. The root biomass increased in FDB but no significant changes in MX. Moreover, cell membrane permeability of leaves and roots also increased, while the chlorophyll content of leaves decreased. Antioxidant enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of leaves and roots all increased, along with a gradual increase in malondialdehyde (MDA). Of this two cultivars, the root system of FDB is more susceptible to waterlogging than the upper ground, and the root system can improve the resistance to waterlogging by increasing the root system biomass, Peony adapted to the waterlogging environment by changing its external form.

Key words: Peony, waterlogging stress, growth, antioxidant systems, malondialdehyde (MDA) content.

INTRODUCTION

Tree peony (Paeonia suffruticosa Andr.) is a perennial deciduous shrub of excellent ornamental and medicinal value. It is indigenous to China where ornamental cultivation has a history of more than 2000 years (Li et al., 2009; Picerno et al., 2011). Because of its large flowers, range of colors, attractive shape and fragrance, tree peony has attracted increasing attention around the world both as a pot plant and for cut flower production.
(Han et al., 2008). There are nearly 3000 cultivars of peony in China. Four large tree peony groups have been described of the central, northwest, southwest, and Jiangnan peony groups; the variety of peony in Jiangnan group are the least (Zhang et al., 2007). Peony has large flowers of ornamental value. In recent years, oil peony has also been suggested as an important oil crop. Understanding the factors affecting peony cultivation in Jiangnan of China is important in terms of cultivation management. Changing rainfall patterns have resulted in increased flood events in many regions, so that development of flood tolerant crops is a priority (Yamauchi et al., 2017). Jiangnan of China has a subtropical to tropical monsoon climate. Rainfall is also frequent and heavy during the peony growth period. As a result, the duration of waterlogging is relatively long, often up to 6 months (from April to September), the growth of peony was affected by the waterlogging. Because of this climate, only about 20 peony cultivars remain in Jiangnan group, with some rare cultivars such as ‘Ziyunfang’ and ‘fengwei’ are on the verge of extinction. Therefore, understanding the characteristics of waterlogging tolerance in peony is important in terms of selecting cultivars suitable for growth in Jiangnan regions of China. In line with this, determining the physiological and biochemical characteristics under waterlogging stress is important from both a theoretical and practical viewpoint.

Waterlogging is a major abiotic stress to plants. Globally, it is estimated that 10% of all irrigated land is affected by waterlogging, which might reduce crop productivity as much as 20% (Ren et al., 2016). Waterlogging disturbs plants growth and development, delays growth process, leading to a significant morphological response to stress (Ghobadi et al., 2017; Sauter, 2013; Huang et al., 2015). Waterlogging results in an anaerobic environment, which inhibits aerobic respiration in the mitochondria, inducing anaerobic respiration in the root system. As a result, electron transport is blocked and ATP cannot be produced via the aerobic pathway and the cells rapidly suffer an energy crisis that can lead to cell death, resulting in the accumulation of a large amount of reactive oxygen species (ROS) (Le et al., 2017; Jin et al., 2010; Lesk et al., 2016). Overproduction ROS and subsequent oxidative stress may be the common mechanism of phytotoxicity and cause of damage to important organic constituent of plant cells (Petrov et al., 2015). To eliminate the toxic effects of ROS, plants have different enzymatic or non-enzymatic antioxidants, signaling pathways and metabolites (Ahammed et al., 2013). Rapid biochemical changes are easily induced through short-term soil waterlogging, however, anatomical and morphological changes such as the formation of adventitious roots, hypertrophied lenticels and aerenchyma are more likely to be involved in long-term acclimation (Yamauchi et al., 2017).

Plant responses to waterlogging also vary by species, genetic characteristics, age, waterlogging duration and waterlogging depth (Zhou et al., 2017; Nyman and Lindau, 2016). While extensive research has been carried out on ROS-induced injury and plant defense response systems under waterlogging stress (Wang et al., 2016; Pociecha et al., 2016). Roots and upper ground part of plant will be destroyed when plants are under waterlogging stress; little is known about the relationship between roots and upper ground parts. The physiological and biochemical changes in peony under different levels of waterlogging stress were studied (Kong, 2011), but little is known about the differences between water-tolerant species and water-sensitive species. Long-term field observations suggest that the ‘Feng Dan Bai (FDB)’ cultivar is highly tolerant of natural waterlogging conditions. In contrast, the MX is unable to survive for long time under waterlogging stress. Despite of this, little is known about how waterlogging affect growth, morphology, physiological and biochemical characteristics in Jiangnan of China and wetland areas.

The purposes of this research were the following: (1) assessment of the damage of waterlogging in tolerant and sensitive peony used in the region in different degree of waterlogging separately; (2) evaluation of quantitative traits such as roots and upper ground parts growth and other physiological characteristics; and (3) study of stress tolerance indexes in peony varieties, clarifying the mechanisms of waterlogging tolerance in peony. The results of this study will provide a theoretical basis for breeding and cultivation of waterlogging-tolerant cultivars suitable for growth in the Jiangnan of China.

MATERIALS AND METHODS

Plant and growth conditions

Experiments were conducted in Zhejiang A & F University, Zhejiang Province, China (N29°71ʹ, E120°23ʹ). Four-year-old healthy FDB and MingXing (MX) seedlings were planted in plastic containers (top diameter: 27 cm; height: 22 cm). A completely randomized design was followed with three replications per treatment and three plants per replication. Containers (18) were used in this experiment. Each container was filled with a mixed matrix consisting of garden soil, sand and perlite (v/v/v = 5: 3: 2; pH 6.4), the depth of soil was 16 cm and grown in a shaded greenhouse with natural sunlight during the day and relative humidity of 65% (±5%). The temperature of greenhouse was 20 to 25°C.

Experimental treatment

In June 2016, three waterlogging stress treatments were implemented as follows: control, standard nutrient-water management with a soil moisture content of 75% field capacity (control by weigh); mild waterlogging stress (MWS), with a flood height of 4 to 5 cm lower than the soil surface; severe waterlogging stress (SWS), with a supersaturated soil water content of 4 to 5 cm above the soil surface. A completely randomized design was followed with three replications per treatment and three plants per replication. Containers (18) were used in this experiment. All other environmental conditions were kept constant throughout the
experiment. All measurements were determined simultaneously after 15-days treatment.

**Determination of growth parameters**

Seedling height was measured with a tape and ground diameter with a vernier caliper at a height of 6 cm from the soil surface. Each plant was measured twice then the average determined. After measuring height and ground diameter, one intact plant per each treatment replicate was uprooted for roots and upper ground biomass analysis. Green tissues and roots were oven-dried at 65°C to a constant weight then weighed using an electronic scale to determine biomass. All measurements were determined simultaneously after 15-days treatment.

**Measurement of leaf chlorophyll**

The chlorophyll content was measured using the portable chlorophyll apparatus (SPAD-502Plus).

**Cell membrane permeability**

Membrane permeability was estimated by measuring the relative electrolyte conductivity in the leaves and roots according to Shi et al., 2006. Discs (0.2 g) were briefly rinsed with deionized water then immersed in a test tube with 30 ml deionized water for 12 h. Initial electrical conductivity (EC) of the solution was subsequently measured with a conductivity meter (Model DJS-1C, Leici, Shanghai). The samples were then heated at 100°C for 20 min and final EC in the bathing solution re-read. Membrane permeability was calculated as EC(%) = initial EC / final EC × 100%.

**Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities**

Leaf tissue samples (0.5 g) and roots (0.5 g) were cut into pieces then ground in 10 ml of 50 mmol phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone (PVP), respectively. The homogenate was centrifuged at 10,000 × g for 15 min at 4°C, and the supernatant used to determine SOD and POD activities. SOD activity was determined based on the inhibition of nitroblue tetrazolium reduction to blue formazan via superoxide radicals (Ries, 1977). The reaction mixture (3 ml) consisted of 50 mmol potassium phosphate buffer (pH 7.8) with 0.3 mol ethylene diaminetetraacetic acid, 39.15 mol methionine, 0.225 mol nitroblue tetrazolium, 0.006 mol riboflavin and 0.05 ml enzyme extract. POD activity was determined using the guaiacol method (Sun et al., 2011). The reaction mixture (3 ml) contained 0.05 ml enzyme extract, 2.75 ml of 50 mmol phosphate buffer (pH 7.0), 0.1 ml of 1% H$_2$O$_2$ and 0.1 mL of 4% guaiacol solution. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min then one unit of enzyme activity defined as the amount of enzyme causing a change in absorbance of 0.01 per min.

CAT activity was determined by tracking the consumption of H$_2$O$_2$ at 240 nm for 3 min (Aeby, 1984). The assay mixture (3 ml) consisted of 100 mmol potassium phosphate buffer (pH 7.0), 15 mmol H$_2$O$_2$ and 50 ul leaf extract.

**Malondialdehyde (MDA) content**

MDA content was determined according to the method of Jin et al. (2011). The degree of lipid peroxidation was determined from the content of 2-thiobarbituric acid (TBA) reactive metabolites. Fresh leaf tissue and roots were respectively homogenized and then extracted in 10 ml of 0.25% (w/v) TBA dissolved in 10% (w/v) trichloroacetic acid (TCA). The extract was heated to 95°C for 30 min then cooled quickly on ice. Absorbance of the supernatant was measured at 532 nm after centrifugation at 10,000 × g for 10 min, and correction of non-specific turbidity carried out by subtracting the absorbance at 600 nm.

**Statistical analysis**

Data were analyzed using analysis of variance (ANOVA) with SPSS version 19.0. Means were separated by calculating the least significant difference (LSD), and simple correlation analysis to determine the relationship between each physiological variable.

**RESULTS**

**The growth of roots and upper ground of peony during waterlogging stress**

The results illustrated the effects of waterlogging on peony growth (Figure 1). The increment of height, ground diameter and upper ground biomass was decreased as the increasing waterlogging stress, but the increment of roots biomass increased in FDB and has no significant difference in MX. Moreover, the increases in height, diameter, upper ground biomass under waterlogging stress were lower than the control of two cultivars, but the roots biomass were higher than control in FDB. Values of each morphological indicator were also lower in MX than FDB, suggesting that FDB was less impacted by waterlogging stress than MX. In two cultivars, the increase in ground diameter was lower under MWS than control, but higher than that under SWS, ranging from 0.16 to 0.21. Significant differences in ground diameter between the two cultivars were also observed under stress, MX showing consistently lower values than FDB. The increases in seedling height were also lower in MX than FDB; however, no significant differences in above ground biomass were observed between the MWS and SWS treatments in two cultivars. Roots biomass of FDB increased during MWS and SWS treatments than control; roots biomass of MX has no significant change between MWS and SWS treatments.

**Cell membrane permeability and chlorophyll content**

As shown in Figure 2, no significant differences in cell membrane permeability in leaves of the two cultivars can been seen under normal management and MWS condition. A gradual increase with increasing waterlogging stress was observed in two cultivars, with a larger increase in MX than FDB. However, under SWS condition, cell membrane permeability was significantly greater in MX than in FDB. The roots cell membrane permeability was also increased as the increase in degree waterlogging treatments. No significant differences
Figure 1. Changes in seedling height, ground diameter, upper ground biomass and roots biomass under waterlogging stress in two peony cultivars. Each bar represents the mean+SE calculated from three independent experiments. Bars with different letters are significantly different at P < 0.01.

Antioxidant enzyme activities and MDA content

As shown in Figures 3 and 4, antioxidant enzyme activity of leaves and roots increased gradually with increasing waterlogging stress, as did the MDA content. Enzyme activities and MDA content were both the highest under severe waterlogging stress, with significant differences as compared to the control (P<0.01).

In this study, SOD activity of leaves increased slightly with increasing waterlogging stress (Figure 3). Under control condition, SOD activity was higher in MX than FDB, under MWS the activity of SOD of two cultivars were higher than the control. Under SWS, an increase of 37.5 and 16.1% compared to the control was observed in FDB and MX, respectively. SOD activity of roots was also increased (Figure 3); under control condition, no significant differences were observed between FDB and MX. Under MWS and SWS condition, SOD activity of roots was increased both in FDB and MX. Above all, when peony is in water, the root system and the upper ground part show the same reflection between two cultivars.

Under waterlogging stress, the changes in CAT activity of roots and leaves were similar between the two cultivars (Figure 3), and the activity of CAT was higher in FDB than MX. In FDB, no significant differences in CAT...
Figure 2. Changes in cell membrane permeability of leaves and roots and chlorophyll content under waterlogging stress in two peony cultivars. Each bar represents the mean±SE calculated from three independent experiments. Bars with different letters are significantly different at $P < 0.01$. 

Zhu et al.          345
Figure 3. Changes in antioxidant enzyme SOD and CAT activities under waterlogging stress in two peony cultivars. Each bar represents the mean±SE calculated from three independent experiments. Bars with different letters are significantly different at P < 0.01.

activity of leaves were observed between MWS and SWS condition; however, significant differences were observed when compared with the control (P<0.01). But significant differences were observed in the CAT activity of roots among control, MWS and SWS (P<0.01). In MX, no significant differences were observed between control and MWS; however, a significant difference was observed under SWS (P<0.01). That is, an increase in CAT activity of 19.9 and 42.1% was observed under SWS compared to control. Under MWS and SWS condition, CAT activities of roots increased by 5% (P<0.01) and 9% (P<0.01) than control, respectively. Above all, it can be observed that when peony is in waterlogging, the root system and the upper ground part show the same reflection.

POD activity of roots and leaves showed a similar trend in two cultivars, increasing trend were observed with increasing waterlogging stress (Figure 4). Under all treatments, POD activity of leaves were lower in FDB than MX, with significant differences in both cultivars between control, MWS and SWS condition (P<0.01). Under SWS condition, an increase of 58.2 and 35.9% was observed in FDB and MX compared to the control. POD activity of roots was increased with increasing waterlogging stress in FDB and MX, with significant differences observed in two cultivars in control, MWS and SWS conditions (P<0.01). Under SWS condition, an increase of 53.2 and 51.2% was observed in FDB and MX compared to the control, respectively. Similar trend was observed between roots and leaves of two cultivars.

MDA content was found to be increased due to flooding treatment. However, the increase in MDA content of leaves under MWS were significantly lower in FDB (30.7%) followed by MX (34.2%). In SWS condition, an increase of 52.4 and 52.1% was observed in FDB and MX compared to the control. MDA content of roots also
increased gradually with increasing waterlogging stress, with significant differences between treatments in two cultivars. Under SWS, an increase of 39.1 and 39.2% was observed in FDB and MX compared to the control.

**DISCUSSION**

**Effects of waterlogging stress on morphological characteristics**

Under waterlogging stress, the external morphology of peony undergoes a series of changes, with a decrease in growth rate and gradual increase in biomass, the effect in intolerant cultivars greater than that in tolerant cultivars. In this study, no significant differences in the biomass increment were observed between FDB and MX with increasing waterlogging stress. In contrast, a greater increase in height was observed in FDB. Overall, FDB was less affected by waterlogging stress, suggesting stronger waterlogging tolerance. The decrease in seedling height, seedling diameter and biomass under waterlogging stress is mainly the result of waterlogging of the soil system, which causes soil hypoxia and a subsequent decrease in root activity (Kong, 2011). Water saturation of the roots results in root anaerobic respiration and subsequent production of harmful substances such as ethanol, thus hindering seedling height and ground diameter growth. Roots are challenged by various abiotic and biotic constraints in soils, with water status of too little or too much being a major factor resulting in plant stress. An increased number of newly-
emerged adventitious roots can compensate, at least partially, for the growth inhibition or even death of distal portions of roots present when waterlogging occurs. Many plant species produce adventitious roots (Visser and Voesenek, 2004), with some emerging into the soil, others along the soil surface and during deeper floods some even grow into the water column. The roots of MX were not increased because the adventitious roots biomass not produced in the experiment. That is to say, MX has no ability to produce new roots when emerged in water. The roots died after a long time water emerged. In the present study, the roots biomass of FDB was increased because of the adventitious roots produced in the water, this is, the reflection of tolerant peony to flooding to alleviate the injury of waterlogging. With the formation of new roots, the respiratory area of the root system gradually increased, which enhanced the waterlogging resistance of peony. The process of the formation of new adventitious roots remains to be further studied in the future.

**Effects of waterlogging stress on physiological and biochemical characteristics**

Chlorophyll is involved in the absorption, transfer and conversion of light energy during photosynthesis. With an overall decrease in chlorophyll content, light energy conversion and the overall energy supply are inhibited, thereby affecting photosynthesis. Thus, to a large extent, the chlorophyll content of a plant reflects its growth status and photosynthetic capacity. Under stress, the chlorophyll content decreases as a result of changes in cell membrane structure (Gao et al., 2015). Stress also causes an increase in ROS and MDA, thereby accelerating chlorophyll decomposition and further decreasing the overall chlorophyll content (Yi et al., 2008). In this study, chlorophyll content decreased as increasing waterlogging stress, and the MDA content and chlorophyll content showed a negative correlation. These findings confirm the relationship between chlorophyll content and ROS, consistent with a previous study in rice (Jiang et al., 1994). It has been suggested that chlorophyll content under stress may reflect the degree of tolerance (Yi et al., 2008). In this study, the chlorophyll content of FDB was significantly higher than that of MX, suggesting stronger waterlogging tolerance in FDB.

When faced with external environmental stresses, cell membrane permeability increases due to increased leakage of electrolytes (Burgess et al., 2014; Tang et al., 2014). The reason for this was that the cell membrane was damaged under adverse stress and the membrane permeability increased, so that the electrolyte infiltration inside the cell increased the conductivity. Cell membrane damage can therefore be determined by calculating the electrical conductivity of fluid. The higher the electrical conductivity, the greater the leakage of fluid and the more electrolytes are present, thus, the more serious the cell membrane damage. In this study, electrical conductivity increased with waterlogging stress in two cultivars and was greater in MX than FDB. This results further suggests that MX is more greatly affected, and therefore, less tolerant to waterlogging stress.

Under normal circumstances, there is a dynamic balance between generation and elimination of ROS. However, stress breaks this balance, causing a substantial accumulation in ROS, increasing the generation of MDA (Jin et al., 2010). In turn, this causes further damage to the membrane structure, inducing a series of physiological and biochemical changes (Jin et al., 2010). ROS can be eliminated via antioxidant enzyme activity, alleviating damage to the plant. In antioxidative systems of plants, SOD can remove O$_2^-$ as SOD may control other activated species (H$_2$O$_2$ and OH$^-$), it is defined as a key antioxidative enzyme in the system. POD is an important enzyme involved in morphogenesis and auxin oxidation. It is the enzyme which is very sensitive to environmental fluctuations being considered as a measure of plant resistance to stress. The main enzymes involved in this process are SOD, POD and CAT (Jin et al., 2011). As shown in this study, SOD, POD and CAT activity increased with increasing waterlogging stress along with MDA content. Thus, under waterlogging stress, peony plants activate an automatic adjustment mechanism, however, at a certain level of stress, the effect on growth and development is unavoidable. SOD initiates membrane lipid peroxidation both directly and indirectly, increasing the content of MDA, the accumulation in MDA in turn inhibits SOD, reducing the protective effects of the enzyme system and further promoting damage.

In this study, the relationship between roots and upper ground were discussed; at the later period of waterlogging, adventitious roots were produced in FDB. Due to the new roots, the tolerant of waterlogging will be strengthened. This study also suggests that this is the main physiological response and damaging effect of soil waterlogging stress in peony. A great difference was observed in waterlogging tolerance between two peony cultivars. Such differences were caused by the different level of chlorophyll and different antioxidant enzyme activities, so that various indices characterizing growth activity, as well as the cell membrane permeability and MDA content, changed to a different degree. The accumulation of MDA in FDB was lower than in MX; it was mainly due to the sharp increase in antioxidant enzymes.

**ACKNOWLEDGEMENTS**

The work was partly supported by the Zhejiang Provincial Natural Science Foundation of China (LY16C160011), the National Natural Science Foundation of China (Nos.
31170584 and 31200525) and National College students’ Innovation and Entrepreneurship Training Program of China (201813283008).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES