

PHYSIOLOGICAL RESPONSES OF THE INVASIVE GREAT YELLOWCRESS (*RORIPPA AMPHIBIA* (L.) BESSERD) UNDER DIFFERENT WATER CONDITIONS

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Abstract. The invasion plant *Rorippa amphibia* (L.) Besser native to Europe was first reported in China in 2009 and then it spread extensively in Liaoning Province. It natively grows in flooded or shallow habitats, but it actually appears in more broadly water-related habitats in Shenyang, Liaoning Province. Individuals of *Rorippa amphibia* were treated for 1 month under 4 water gradients with waterlogging, wet, moderate (CK) and drought in Shenyang Normal University, and their physiological indexes were measured. The results showed water stresses had some adverse effects on its physiological processes including the significantly decreasing of the transpiration rate, net photosynthetic rate and stomatal conductance, the increasing of malondialdehyde and electrolyte leakage. But the change of other physiological indexes as follows may alleviate the disadvantageous effects of water stresses. Compared with the CK, the chlorophyll content increased significantly, especially under the drought treatment. The activities of SOD, POD and CAT increased. SOD and POD activities were highest under the drought treatment and CAT activity was highest under the waterlogging treatment. The osmotic adjustment substance contents including proline, soluble protein and soluble sugar were significantly increased under water stresses, especially under the drought treatment and the waterlogging treatment. The root activities increased as soil water content declined and the activity under the drought treatment was significantly higher than the other 3 treatments. The result that all the individuals of *Rorippa amphibia* could survive under extreme soil water conditions meant that *Rorippa amphibia* had a strong invasive line because it could adapt to very different soil water conditions from waterlogging to extremely drought condition.

Keywords: *invasive mechanism, water stress, photosynthetic indexes, antioxidase, proline, soluble protein, soluble sugar, malondialdehyde (MDA), electrolyte leakage*

Introduction

With the development of globalization, many plants are brought to other parts of the world with the activities of people, so that plant invasion has become a common phenomenon. Water is an important ecological factor to influence the survival state of plants. To research the capability adapting to different water condition of invasive plants is very important to evaluate their expanding ability because water stress is a major limiting factor of plant growth and physiological process.

In general, water stress can inhibit the growth of plant. Water stress can be divided into waterlogging stress and drought stress. Chlorophyll is the predominant pigment of plants, and the quantity of chlorophyll determines plant growth capacity and is an indicator of stress level. Drought stress can change the quantity of chlorophyll a and chlorophyll b in plants leading to changes in photosynthesis capacity (Shukla et al., 2015; Yang et al., 2015). The study of Jennifer and Hulme (2021) on the invasive plant *Rumex obtusifolius* L. found that its chlorophyll content increased under drought stress. But the study of Pintó-Marijuan et al. (2016) on the invasive plant *Aptenia cordifolia*

(L.f.) Schwantes found that its chlorophyll content decreased under drought stress. Net photosynthetic rate has long been recognized as one of the most sensitive processes. Water stress can affect the indicators of photosynthesis. For example, stomatal closure limits air exchange and reduces photosynthetic CO₂ assimilation, whereas water deficiency decreases RuBP contents, thereby suppressing photosynthetic CO₂ assimilation, which becomes the dominant limitation at severe drought (Lewis et al., 1994). A study on invasive *Eriobotrya japonica* (Thunb.) Lindl. found a decrease in stomatal conductance (Cond) (Williams-Linera et al., 2021). Malondialdehyde (MDA) and Relative water content (RWC) can indicate the degree of stress. MDA, a product of lipid peroxidation, is an indicator of oxidative damage (Blokhina, 2003). RWC is also a key indicator to describe the water related physiological function in plants (Wang et al., 2019; Feizabadi et al., 2020). Under drought stress, plasma membrane permeability changes and electrolyte leakage had been found (Bajji et al., 2002).

Waterlogging stress is an important natural disturbance (Mack et al., 2000). Waterlogging can cause stunted growth or even death of plants. Because the decreasing of air exchange between soil and the atmosphere O₂ in the soil declines rapidly, and the soil may become hypoxic or anoxic within a few hours during waterlogging (Malik et al., 2002). One of the initial responses of plant to waterlogging stress appears to involve the closing of stomata, with a subsequent down-regulation of the photosynthetic machinery (García-Sánchez et al., 2007). The study of Jennifer and Hulme (2021) on the invasive plant *Rumex obtusifolius* L. found that its chlorophyll content increased under waterlogging stress. The study on invasive *Ligustrum sinense* Lour. found a decrease in stomatal conductance (Cond) and the net photosynthetic rate (Brown and Pezeshki, 2000). Similarly, Smaoui et al. (2011) found in their study on the invasive plant *Cotula coronopifolia* L. that chlorophyll content increased under waterlogging stress, while net photosynthetic rate and transpiration rate decreased. Many researchers found that waterlogging can facilitate invasion with exotic plants (Kercher and Zedler, 2004; Diez et al., 2012), mainly because of their tolerance to flooding, which is another highly relevant factor for the success of invasive plant species (Dalmagro et al., 2013).

Some plants may have developed complex mechanisms to cope with water stress, such as adaptive morphology and structural changes (Moore et al., 2008; Lawlor, 2012; Komatsu and Yanagawa, 2013), enhanced biochemical and physiological responses such as solute accumulation, redox homeostasis, and changes in cellular turgor, membrane fluidity and composition (Reddy et al., 2004; Valliyodan and Nguyen, 2006). Plant tissues can reduce their cellular osmotic potential by synthesizing osmotic adjustment substances such as soluble protein (SP), proline (PRO) and soluble sugars to cope with water stress (Gao et al., 2008; Blum, 2016). Similarly, the antioxidant enzyme system is switched on. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) are key enzymatic antioxidants. Some studies have indicated that enzymatic antioxidants increased in response to mild and/or moderate water deficit, but progressive severe drought stress impaired the function of these enzymes (Fan et al., 2009; Liu et al., 2011; Ge et al., 2014).

Rorippa amphibia (L.) Besser (yellowcress) is a perennial herb of the genus *Rorippa* Scop in the Brassicaceae family. Native to Europe, its invasion to the Caucasus, Central Asia, North Africa, North America and even New Zealand had been reported. Some studies have shown that it is most likely to invade with the introduced turf grass species (Elton, 1977). *Rorippa amphibia* have a strong ability of asexual reproduction and sexual reproduction, which is very helpful for them to become the dominant species in

lawns and other habitats. In addition, they have strong adaptability and expansibility, and the destructive power to some ecosystems is great (Ruprecht et al., 2013). *Rorippa amphibia* was first reported in China in 2009 and then it appeared in most areas of Liaoning Province, China, mainly distributed on the roadside and lawns (Zhang et al., 2009). In China, there are a few reports on the study of *Rorippa amphibia*. *Rorippa amphibia* grows in habitats are frequently flooded or where the groundwater level is relatively stable and shallow (Akman et al., 2012, 2014). But we found it appeared from moist and shade habitat to sunny and dry habitat in Shenyang, China. In this study, *Rorippa amphibia* was subjected to different gradient of soil water contents, and its physiological indexes under different water conditions were determined. We expect to reveal the adaptability of *Rorippa amphibia* to cope with water stress and to provide some theoretical basis for evaluating its potential invasive ability by this study.

Materials and methods

Plant material

This study was conducted from September to November in 2020 at Shenyang Normal University (Shenyang, Liaoning Province, China; 41°54'22"N 123°24'36"E). We put 15.0 kg mixed soil (loam soil: peat soil: perlite: vermiculite=15:4:3.5:1 v/v) in each pot (54*30*18 cm) and there were 8 pots to be used in this experiment. Well-grown and uniform in size individuals of *Rorippa amphibia* which were dominant species in a lawn in Shenyang Normal University were dug out and 40 individuals were transplanted in each pot. The pots were put in a greenhouse near windows for 30 days and plenty watered to guarantee the plants growing normally. Then 30 individuals which were similar in height and size were kept in each pot and they were nearly equidistant. The environmental indicators during the experiment were measured and the average temperature, humidity and illuminance were 20.17°C, 22.01%, 28853.67 lux respectively.

Experimental design and water treatments

Four different soil water contents were 5-10%, 15-20%, 25-30%, >100% (kept water surface higher than 2 cm over soil surface), which were equivalent to drought treatment, moderate treatment (CK), wet treatment and waterlogging treatment, respectively. There were two pots in each treatment (i.e., n=2 pots per treatment). The total 8 pots with 4 soil water gradients were random sorting (*Figs. 1,2*) in order to reduce the influence of other environmental factors on the experiment. When the water content was close to the high line of a specific designed treatment, the experiment began. The water content of soil samples in each pot was measured every day by drying method (Wang and Gao, 2006) and the overall weight of the whole pot was weighed to monitor the soil water condition. When the soil water content of each pot was close to the low line of each design water gradient, supplement water was given to meet the high line of the designed treatment. The *Rorippa amphibia* was treated under the designed water gradient for 1 month.

The superoxide dismutase, peroxidase, and catalase activities, soluble protein, soluble sugars, proline, malondialdehyde (MDA) content, electrolyte leakage, relative water content (RWC), chlorophyll content, photosynthetic indicators of the fresh fourth leaf and root activity were measured at the 35th days after the beginning of treatment.

There were 3 repetitions for each indicator in every water treatment and samples were taken from different pots (*Fig. 1*).

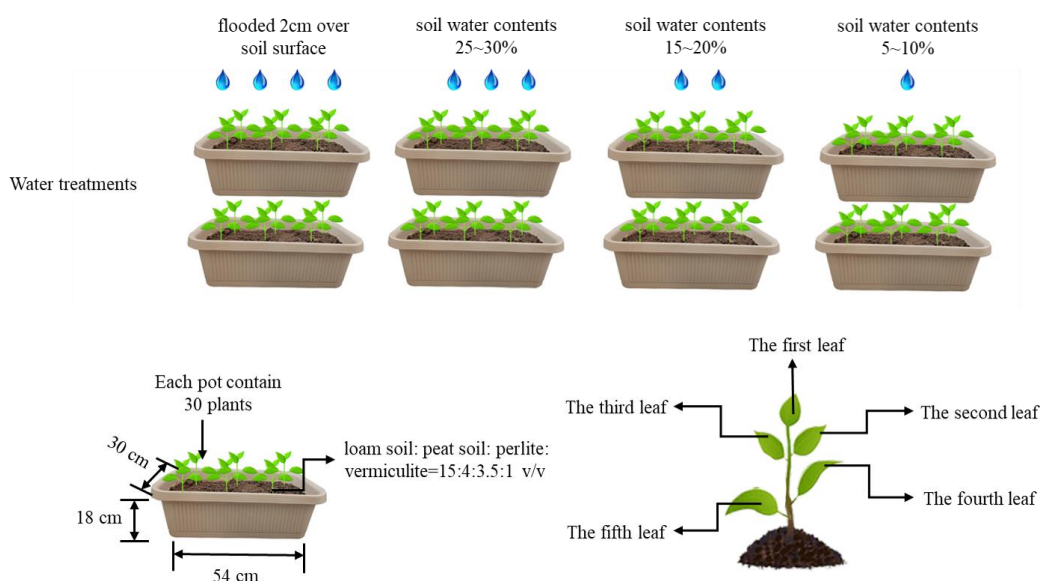


Figure 1. Different water treatments

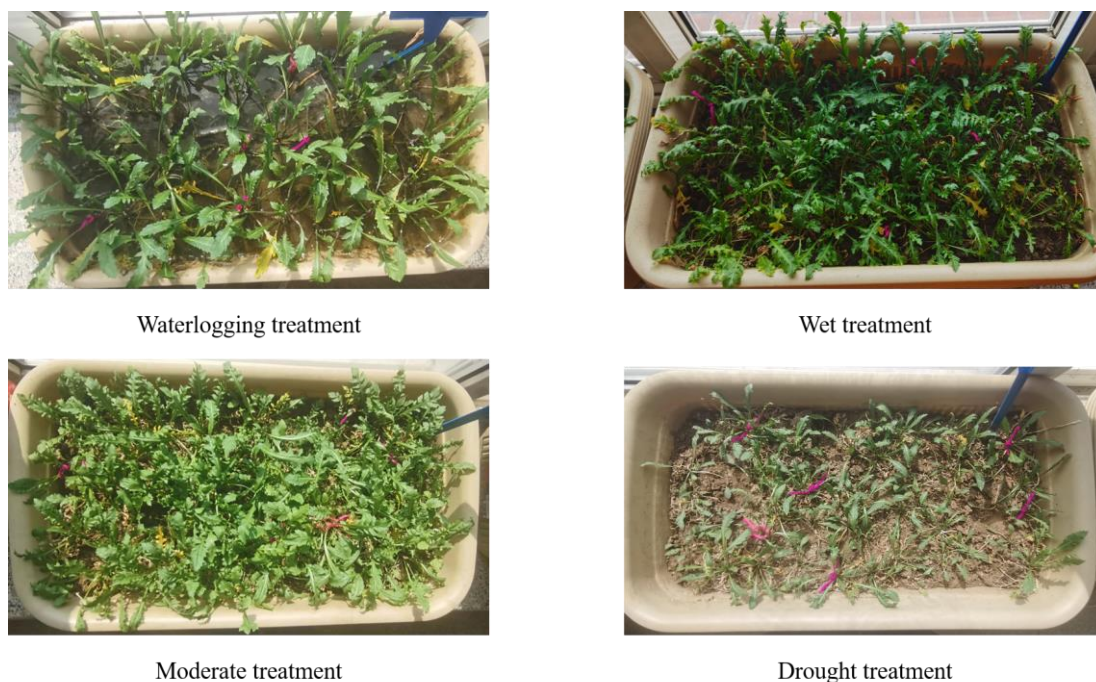


Figure 2. Plants under different water treatments of the experiment

Determination of activities of superoxide dismutase, peroxidase, and catalase

The leaves (0.2 g) were ground into homogenate in ice-bath using a mortar and pestle. 0.1 mol/L phosphate-buffered saline (pH 7.8) was added during grinding. After grinding,

the homogenate was centrifuged at 10,000 r/min for 20 min at 4°C by centrifuge (D-16C, Sartorius Lab Instruments GmbH & Co. KG 37070 Goettingen, Germany). The supernatant was collected to determine. Superoxide dismutase (SOD; EC 1.15.1.1) activity, assayed using the photochemical nitroblue tetrazolium (NBT) method (Beyer and Fridovich, 1987) measured estimate at 560 nm by spectrophotometer (UV-5900, Shanghai Metash Instruments Co., Ltd).

Fresh leaves (0.2 g) were ground into homogenate in ice-bath using a mortar and pestle. 20 mmol/L KH_2PO_4 was added during grinding. After grinding, the homogenate was centrifuged at 10,000 r/min for 20 min at 4°C. The supernatant was collected to determine. The peroxidase (POD; EC 1.11.1.7) activity use the Guaiacol method measured estimate at 470 nm (Chancea and Maehly, 1955).

The leaves (0.2 g) were ground into homogenate in ice-bath using a mortar and pestle. 0.1 mol/L phosphate-buffered saline (pH 7.0) was added during grinding. After grinding, the homogenate was centrifuged at 4,000 r/min for 15 min at 4°C. The catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decomposition of H_2O_2 directly at 240 nm for 3 min as described by Aebi (1984).

Estimation of soluble protein content

For the contents of soluble proteins, 0.2 g fresh leaves were extracted in 2 mL buffer phosphate (0.1 M and pH = 7.8). The extract was then centrifuged at 3,000g for 10 min at 4°C and supernatant was collected. Soluble protein contents were determined according to the method of Bradford (1976), using the reagent Coomassie Brilliant Blue G-250, followed by absorbance readings at 595 nm using bovine serum albumin as standard.

Estimation of soluble sugars content

Fresh leaves (0.2 g) were homogenized in deionized water, heated to 100°C for 30 min and then cooled to room temperature, and this process was repeated twice. The extract was moved into 25 mL volumetric flasks and volume completed to scale. The anthrone-sulfuric acid method was used to quantify the total soluble sugars. The absorbance at 630 nm was measured using a spectrophotometer using sucrose as standard (Chen et al., 2007).

Estimation of proline content

Leaves (50 mg) were blended in 3% sulfosalicylic acid (10 mL) followed by filtration to determine the leaf proline contents. Taking 2 mL supernatant and acid ninhydrin reagent (2 mL) along with CH_3COOH (2 mL) were reacted in glass vials, subsequently cooled in ice and the resulting amalgam was extracted with toluene (4 mL) using a vortex shaker for 15-20 s. The change in color was measured at 520 nm using a spectrophotometer at room temperature with toluene as blank (Bates et al., 1973). A calibration curve based on proline standard was developed to assess the proline concentrations.

Estimation of malondialdehyde (MDA) content

Leaves (0.2 g) were ground with 5 mL 10% trichloroacetic acid and centrifuged at 10,000 g under 4 °C for 15 min. The reaction mixture containing 2 mL supernatant and 2 mL 0.6% thiobarbituric acid was incubated at 100 °C in water bath for 20 min and

then cooled quickly in an ice bath. The reaction mixture was centrifuged at 4,000 g and 4 °C for 10 min. The absorbance of supernatant was measured at 532, 600, and 450 nm. The content of MDA was calculated as the *Eq.1* showed (Li et al., 2012).

$$MDA\ content = 6.45 \times (A532 - A600) - 0.56 \times A450 \quad (Eq.1)$$

Estimation of root activity

A 0.25 mL of 0.4% TTC solution was prepared and put in a 10 mL container with a small amount of hyposulphuric acid sodium (Na₂S₂O₄) powder and shaken gently to homogenize the chemicals completely. Red color triphenylformazan (TPF) was produced immediately. Ethyle acetate was added to the container to the 10 mL level. The solution was stored in the dark. Six TPF solutions, respectively of 0, 0.25, 0.50, 1.00, 1.50, 2.00 mL concentration were put in 10 mL capacity containers. Ethyle acetate was added to each container to the 10 mL level. A series of TPF concentrations were prepared. The TPF measurements were recorded with a spectrophotometer at 485 nm. Plot of standard curves was established.

Using a spatula, carefully dug 3 individuals out randomly from each treatment and then soaked them repeatedly in water and rinsed gently until no soil was left on the root surface. Using absorbent paper removed superfluous water from the surface of roots. Fresh root materials (0.5 g) were immersed in a mixture of 5 mL TTC and 5 mL 0.1 mol/L Phosphate-buffered saline (pH 7.8), incubated in the dark at 37 °C for 2 hours prior to addition of 2 mL 1 mol/L sulfuric acid to stop the reaction. Then roots were ground into homogenate using a mortar and pestle. Ethyl acetate and quartz sand (small amount) were added during grinding. After grinding, the homogenate was then measured at 485 nm with spectrophotometer (Wang et al., 2006). We determined the root activity using TTC as the standard.

Estimation of relative water content (RWC)

For relative water content (RWC) measurement, leaves were blotted dry on filter papers weighed (Fresh weight, FW); immersed in ice-cold water for 10 h to rehydrate, blotted on filter papers and reweighted (hydrated weight, HW). The plants were subsequently dried at 80°C for 48 h prior to determination of dry weight (DW) by drying oven (DHG-9240A, Shanghai Yiheng Technical Co., Ltd.; Shanghai Bluepard). RWC was calculated as the *Eq.2* showed (Taheri-Garavand et al., 2021):

$$RWC = \left\{ \frac{(FW - DW)}{(HW - DW)} \right\} \times 100\% \quad (Eq.2)$$

Estimate electrolyte leakage

Leaves were washed with deionized water, each leaf was put into a marked vial filled with 20 mL deionized water respectively, and incubated at room temperature in the dark for 6 h. The electrolytic conductivity (EC₁) of the solution was measured using a conductivity meter (SA29-DDB11A, Midwest Group, Beijing, China). The solution was then heated to 100°C, cooled to room temperature, and the electrolytic conductivity (EC₂) was measured once again. The percentage electrolyte leakage (EL) of the leaf discs was calculated as the *Eq.2* showed (Dionisio-Sese and Tobita, 1998).

$$EL = \left(EC_1 / EC_2 \right) \times 100\% \quad (\text{Eq.3})$$

Determination of chlorophyll content (SPAD units)

Chlorophyll content was determined using a SPAD-calibrated portable chlorophyll meter (SPAD-502Plus, Konica Minolta (China) Investment LTD.) which can read chlorophyll content of leaf directly. 3 the fourth leaves of different *Rorippa amphibia* individuals per treatment were measured and the average value was used to represent the chlorophyll content of the whole leaf (Costa et al., 2015).

Determination of photosynthetic indicators

Photosynthetic indicators of the fourth leaf (3 repetitions per treatment) were measured using a portable photosynthetic system (LI-6400, Li-Cor Inc., Lincoln NE, USA) at ambient climatic conditions. During the measurement (10:00-12:00) the irradiance was $1,200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and temperature was around 20°C (He et al., 2014). The net photosynthetic rate, transpiration rate (Tr), stomatal conductance (Cond) and intercellular carbon dioxide concentration (Ci) were measured.

Statistical methods

We performed our statistical analysis using version 26.0 of the SPSS statistics software. One-way ANOVA followed by LSD's multiple-range test for multiple comparisons was used to detect differences among treatments. We defined significance at $P < 0.05$. We used version 9.0 of the Origin Pro software (<https://www.originlab.com/>) to prepare the graphs.

Result

Variations in the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)

SOD and POD activities of *Rorippa amphibia* reached maximum value under drought treatment (Fig. 3A,B), while CAT activity was maximum under waterlogging treatment (Fig. 3C). SOD activities under the drought and waterlogging treatments were significantly higher than those of the moderate and wet treatments ($P < 0.01$). SOD under the drought treatment increased by 8.85%, 41.82%, 52.44% over the corresponding activity under the waterlogging, wet and moderate treatments, respectively. The corresponding increments of POD activity were 8.93%, 15.15%, 103.69%. CAT activity under the waterlogging treatment, on the other hand, displayed 38.10%, 141.67% and 7.41% increments over the wet, moderate and drought treatment, respectively. Under the moderate treatment, activity of POD was significantly lower than those displayed by the other 3 water stress treatments ($P < 0.01$) and activity of CAT was very significantly lower than the drought and waterlogging treatments ($P < 0.01$), and significantly lower than the wet treatment ($P < 0.05$).

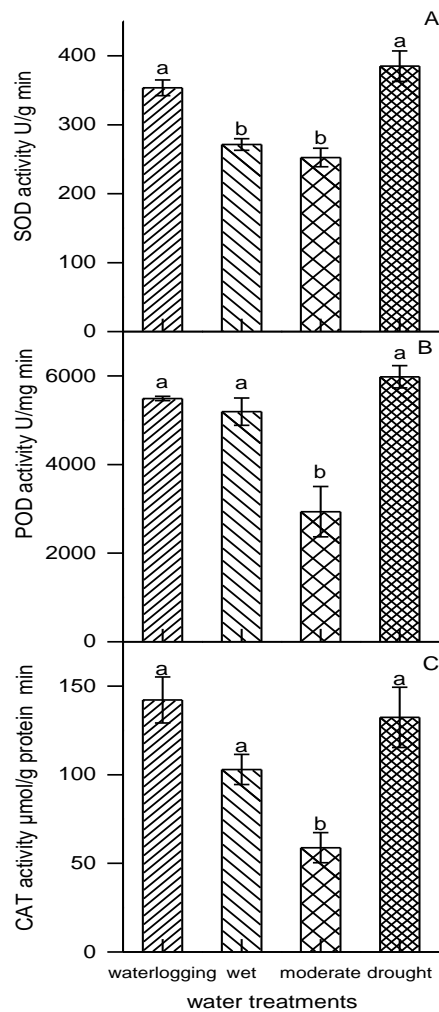


Figure 3. Variations of the SOD, POD, and CAT activities of different water treatments. Bars indicate standard deviations ($n = 3$). Different lowercase letters in columns within the different treatments indicate significant difference. Note: the meaning of the bars and lowercase letters were the same as figure 3 for Figures 4~12

Variations of proline content

Water stresses increased proline content in *Rorippa amphibia* (Fig. 4). The proline content was the highest under the drought treatment ($2.875 \cdot 10^{-4} \mu\text{g/g}$), followed in descending order by the waterlogging treatment ($1.245 \cdot 10^{-4} \mu\text{g/g}$), wet treatment ($6.267 \cdot 10^{-5} \mu\text{g/g}$), and moderate treatment ($4.85 \cdot 10^{-5} \mu\text{g/g}$). The drought treatment was significantly higher than the other 3 treatments ($P < 0.01$). The waterlogging treatment were significantly higher than the wet and moderate treatments ($P < 0.01$).

Variations of soluble sugars content

The leaf soluble sugar contents were the lowest ($0.0067 \mu\text{g/g}$) and highest ($0.0111 \mu\text{g/g}$) for the moderate treatment and drought treatment, respectively (Fig. 5). The differences were significant among the treatments except between the waterlogging treatment and drought treatment. The drought treatment and waterlogging treatment

were significantly higher than moderate treatment ($P < 0.01$), and the wet treatment was significantly higher than moderate treatment ($P < 0.05$).

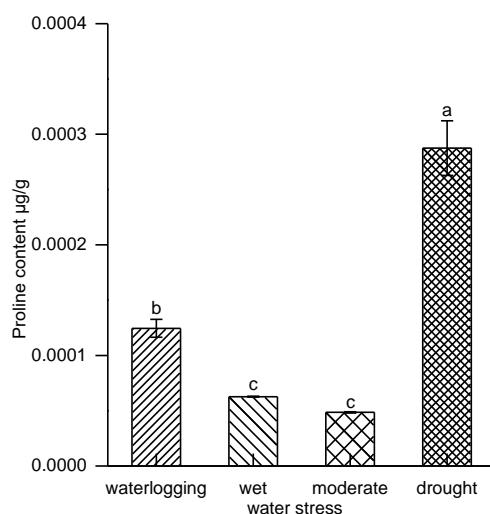


Figure 4. Variations of proline content of different water treatments

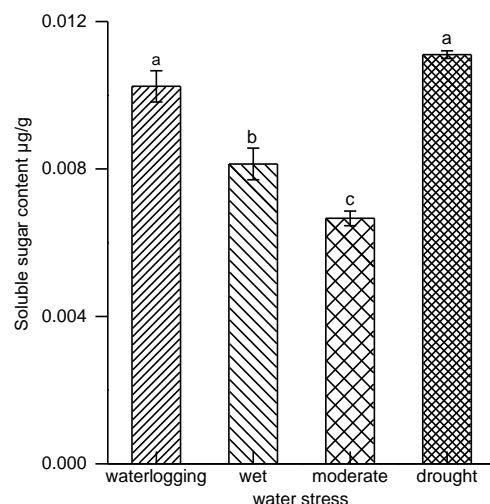


Figure 5. Variations of soluble sugars content of different water treatments

Variations of soluble protein content

The lowest (8.1675 mg/g) and highest (9.1895 mg/g) protein contents were displayed by the moderate treatment and waterlogging treatment, respectively (Fig. 6). The values for the waterlogging treatment and drought treatment were significantly higher than the moderate treatment ($P < 0.01$, $P < 0.05$, respectively) (Fig. 6).

Variations of malondialdehyde (MDA) content

Water stresses increased the MDA content of *Rorippa amphibia*. The MDA content was the highest under waterlogging treatment (12.2216 µmol/g) followed in descending order by the wet treatment (9.2333 µmol/g), drought treatment (7.4469 µmol/g),

moderate treatment (5.1248 $\mu\text{mol/g}$). The differences among the 4 treatments were significant, which was embodied in that the waterlogging treatment was much significantly higher than the other 3 treatments ($P<0.01$), and the moderate treatment was much significantly lower than the wet and drought treatments ($P<0.01$), while the difference between the wet and drought treatments was significant ($P<0.05$) (Fig. 7). Compared with the moderate treatment, MDA contents of the other 3 treatments were higher ($P<0.01$) which may generate higher lipid peroxidation and the damage of membrane.

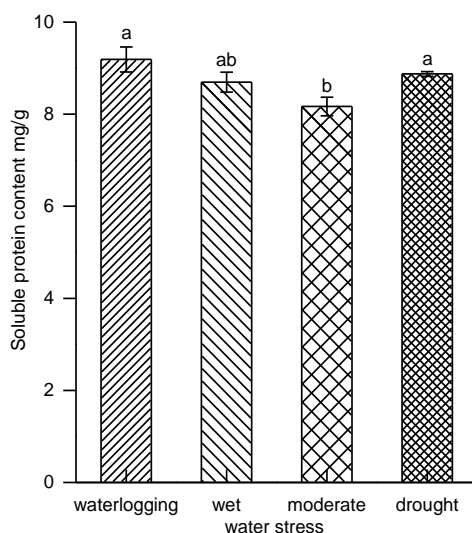


Figure 6. Variations of soluble protein content of different water treatments

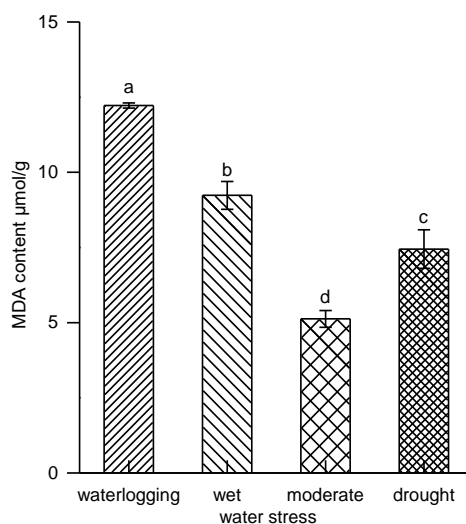


Figure 7. Variations of MDA content of different water treatments

Variations of root activity

The root activities of *Rorippa amphibia* increased as soil water content declined (Fig. 8). Root activity under the drought treatment was about 2.1 times as large as the waterlogging treatment. The differences of root activities were significant among

different treatments except between the wet treatment and moderate treatment, the wet treatment and waterlogging treatment. The drought treatment was significantly higher ($P<0.01$).

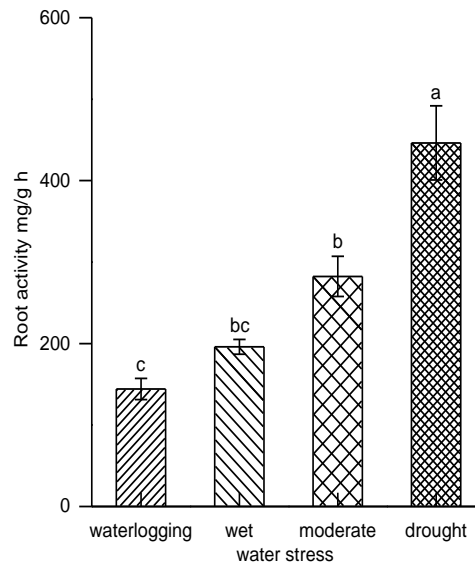


Figure 8. Variations of root activity of different water treatments

Variations of relative water content (RWC)

The RWC of leaves decreased with the decline of soil water contents. The RWC under the drought treatment was significantly lower than the other 3 treatments ($P<0.01$). The value of RWC was the highest under the waterlogging treatment and increased by 36.81% compared with the drought treatment (*Fig. 9*).

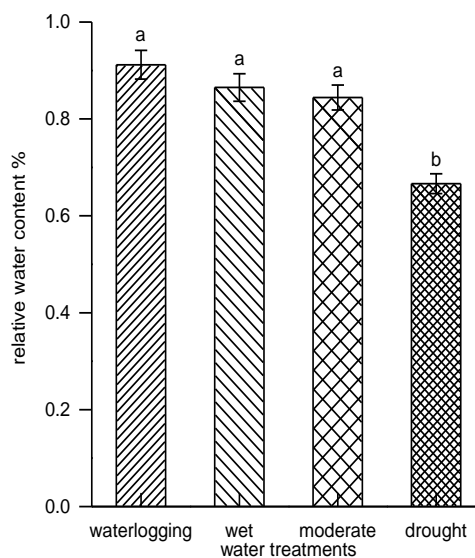


Figure 9. Variations of relative water content of different water treatments

Variations of electrolyte leakage

The waterlogging treatment evoked the highest electrolyte leakage and the value which was about 1.5 times as large as the lowest level of moderate treatment was significantly higher than other 3 treatments ($P < 0.01$) (Fig. 10). The differences of electrical conductivity among the drought, wet and moderate treatments were not significant. The trend of electrolyte leakage was similar to MDA, which reflected the damage degree of membrane of *Rorippa amphibia* under different water treatments.

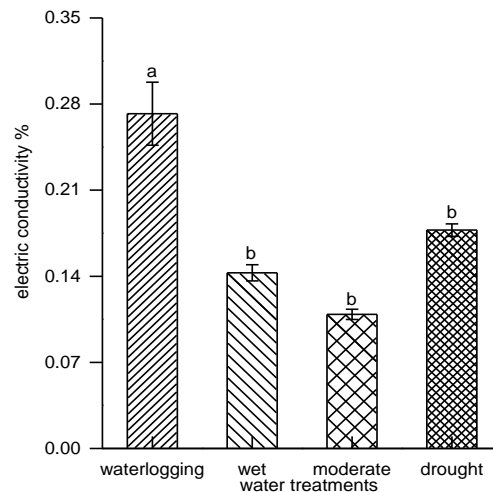


Figure 10. Variations of electrolyte leakage of different water treatments

Variations of chlorophyll content (SPAD units)

As shown in Figure 11, chlorophyll content was the highest for the drought treatment (44.00) followed in descending order by the wet treatment (39.20), waterlogging treatment (38.61), moderate treatment (36.28). Chlorophyll content of the drought treatment was significantly higher than those of the other 3 treatments ($P < 0.01$), while chlorophyll content of the moderate treatment was significantly lower than those of the other 3 treatments ($P < 0.01$).

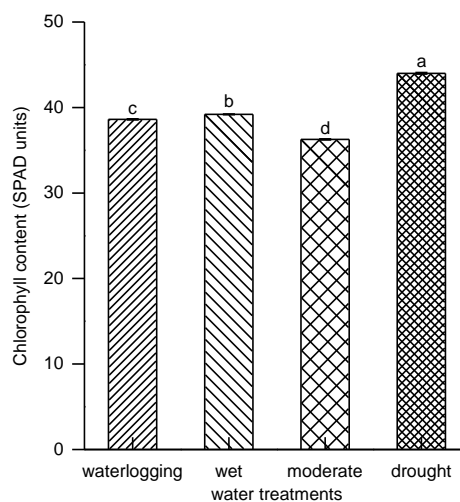


Figure 11. Variations of chlorophyll content (SPAD units) of different water treatments

Variations of photosynthetic indicators

Water stress had an obvious impact on the photosynthetic indicators of *Rorippa amphibia* (Fig. 12). Net photosynthetic rate (photo) was the highest under the moderate treatment ($5.5960 \mu\text{mol}/\text{m}^2\cdot\text{s}$) followed in descending order by the wet treatment ($5.2129 \mu\text{mol}/\text{m}^2\cdot\text{s}$), drought treatment ($3.0701 \mu\text{mol}/\text{m}^2\cdot\text{s}$), waterlogging treatment ($1.9714 \mu\text{mol}/\text{m}^2\cdot\text{s}$). The transpiration rate (Tr) and stomatal conductance (Cond) had the same changing trend as photo rate, while the intercellular carbon dioxide concentration (Ci) had just an opposite trend. All the photosynthetic indicators were significantly different among the 4 treatments ($P < 0.01$).

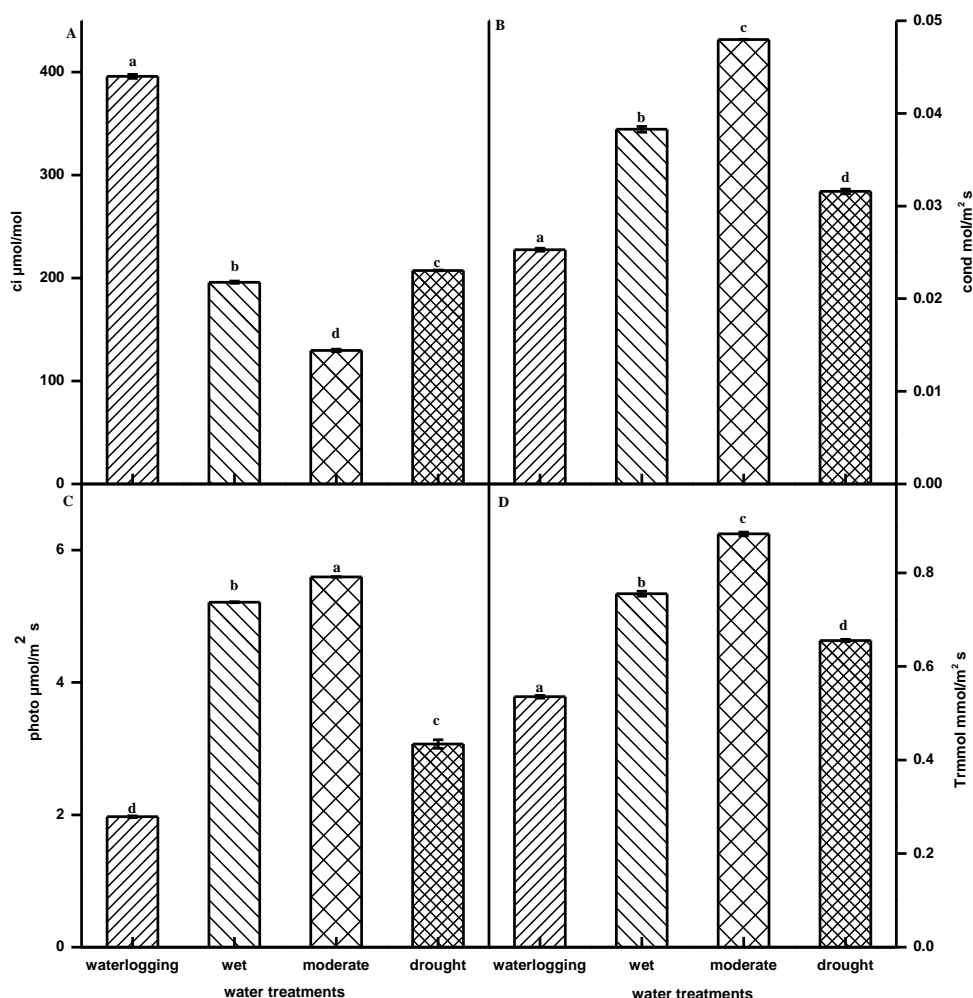


Figure 12. Variations of photosynthetic indicators of different water treatments

Discussion

Water stress is an important environmental factor that could influence the physiological and biochemical characteristics of plants. Water stress retards growth and metabolic activity (Pasala, 2016).

An increase in antioxidant enzyme activity is a common adaptive response of plants to water stress (Gill and Tuteja, 2010). The main function of SOD is to transform $O_2^{\cdot-}$ into O_2 and H_2O_2 , the latter is toxic, but can be eliminated by POD and CAT (Vince et al., 2004). The POD and CAT activities increased as a result of drought treatment in this study, which may protect plants from oxidation. POD also plays an important role in eliminating MDA, thus protecting permeability of cell membranes (Hojati et al., 2010). In this study, POD and CAT activity increased significantly under the waterlogging, wet and drought treatments, which may limit the increases of MDA. The increases of POD activity under the waterlogging, wet and drought treatments were in line with those reported by Hojati et al. (2010). Further, the increase of SOD activity was congruent with the report of Dehury et al. (2012).

Plants can reduce their cellular osmotic potential by synthesizing osmotic adjustment substances such as soluble protein (SP), proline (PRO) and soluble sugars to reduce effects of water stresses. The notable increase in proline content observed in this study in consistency with several reports (Pei et al., 2009; Liu et al., 2016; Li et al., 2020). The result that proline content increased significantly under waterlogging and drought treatments suggest that *Rorippa amphibia* may have a strong adaptability to extreme water conditions. Water stress induces soluble sugar accumulation, particularly sucrose, glucose, and fructose, which helps to improve osmoregulation (Praxedes et al., 2006). In this paper, the soluble sugar increased significantly under the drought treatment and waterlogging treatment compared with moderate treatment, which was similar to previous reported of Wu et al. (2016), Balsamo et al. (2015), and Pintó-Marijuan and Munné-Bosch (2013), but contradicted those reports for Li et al. (2020). Soluble protein contains some antioxidant enzymes. The significant increase in soluble proteins under the waterlogging and drought treatments showed by this study was consistent with the notable increase in some antioxidant enzymes. However, it was at variance with a previous report by Jalil et al. (2018). The observed discrepancy may be due to excessive stresses and/or to subtle difference between plants taxa and/or environments.

Malondialdehyde (MDA), which is a byproduct of enzymatic and oxygen radical-induced lipid peroxidation, is widely used as a biomarker of oxidative stress in plants (Davey, 2005). The increase of malondialdehyde content leads to the increase of plasma membrane peroxidation, which leads to electrolyte leakage. In this paper, MDA content increased significantly under the drought, wet and waterlogging treatments compared with the moderate treatment. This result was similar to Quinet's et al. (2015). Electrolyte leakage level had the same trend with MDA level. The results of this paper were similar to those of Zhang et al. (2020) and Faria et al. (2012). In addition, previous studies showed RWC as an eminent trait to measure stress tolerance (Dapanage and Bhat, 2017; Hemmati, 2018). RWC of *Rorippa amphibia* decreased significantly with the decrease of soil water content. In this paper, we found root activity reached the highest under the drought treatment, and this indicated that *Rorippa amphibia* continuously strengthened the water absorption capacity of root system in order to adapt to drought condition, which further indicated that *Rorippa amphibia* had strong adaptability.

Chlorophyll is another indicator of water stress level. A result showed that drought stress enhanced chlorophyll content in wheat leaves (Xu et al., 2017). In contrast, Li's study of *Lilium brownii* var. *viridulum* Baker found that reduced water content led to reduced chlorophyll content (Li et al., 2020). In this paper, the chlorophyll content of

Rorippa amphibia increased significantly under water stress conditions especially under drought treatment.

Photosynthesis is the most sensitive physiological process of plants to water stress (Centritto et al., 2011; He et al., 2017). Soil moisture and photosynthetically active radiation intensity affect plants by affecting plant photosynthesis, transpiration and respiration (Mitton et al., 1998; Deans et al., 2018). In this paper, the photosynthesis physiological parameters of *Rorippa amphibia* changed under different water conditions. Photosynthetic rate, stomatal conductance and transpiration rate decreased and intercellular carbon dioxide concentration increased under the drought and waterlogging treatments compared with the moderate treatment. The reason for the changes in these indicators under the drought treatment may be when water was scarce, root water intake reduced, and then stomatal conductance, photosynthetic rate, transpiration rate decreased, and intercellular carbon dioxide concentration increased. Some studies suggest *Rorippa amphibia* could even withstand submergence to some degree (Sasidharan et al., 2013; Akman, 2014). However, Prolonged flooding could have deleterious effects on nutrient uptake, carbohydrates translocation, hormonal balance, respiration of *Rorippa amphibia*. Then the root activity decreased and the water absorption was reduced, which led to the stomatal closure and the decreasing of stomatal conductance, photosynthetic rate as well as transpiration rate. So intercellular carbon dioxide concentration increased. The study of *Bupleurum chinense* DC also showed the same results (Yang et al., 2019).

Conclusion

The wet treatment had slight impact on the physiological indexes of *Rorippa amphibia*. The differences of most measured physiological indexes were not significant between the moderate treatment (CK) and wet treatment except the MDA content, soluble sugar content, activities of POD and CAT.

The drought and waterlogging treatments had some severe adverse effects on physiological process of *Rorippa amphibia*. The photosynthetic capacity and root activity were the lowest and the MDA content and electrical conductivity were the highest under the waterlogging treatment. The RWC of leaf was the lowest and the MDA content and electrical conductivity significantly increased under the drought treatment compared with CK.

But some changes of physiological indexes may alleviate the adverse effects of water stresses. *Rorippa amphibia* significantly increased the enzyme activities of antioxidant defense system, the contents of osmotic adjustment substance including proline, soluble sugar and soluble protein under the waterlogging and drought treatments. The activities of SOD and POD, chlorophyll content and root activity under drought treatment were the highest among all the treatments.

All the results indicated that *Rorippa amphibia* could tolerate extremely adverse soil water conditions. Its invasive capability to some ecosystems under different water conditions could not be ignored and further research should be carried out on *Rorippa amphibia*.

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REFERENCES

- [1] Aebi, H. (1984): Catalase in vitro. – *Oxygen Radicals in Biological Systems* 105: 121-126.
- [2] Akman, M., Bhikharie, A. V., McLean, E. H., Boonman, A., Visser, E. J. W., Schranz, M. E., Tienderen, P. H. (2012): Wait or escape? Contrasting submergence tolerance strategies of *Rorippa amphibia*, *Rorippa sylvestris* and their hybrid. – *Annals of Botany* 109: 1263-1276.
- [3] Akman, M., Bhikharie, A. V., Mustroph, A., Sasidharan, R. (2014): Extreme flooding tolerance in *Rorippa*. – *Plant Signaling & Behavior* 9: e27847.
- [4] Bajji, M., Kinet, J. M., Lutts, S. (2002): The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. – *Plant Growth Regulation* 36: 61-70.
- [5] Balsamo, R., Boak, M., Nagle, K., Peethambaran, B., Layton, B. (2015): Leaf biomechanical properties in *Arabidopsis thaliana* polysaccharide mutants affect drought survival. – *Journal of Biomechanics* 48: 4124-4129.
- [6] Bates, L. S., Waldren, R. P., Teare, I. D. (1973): Rapid determination of free proline for water-stress studies. – *Plant and Soil* 39: 205-207.
- [7] Beyer, W. F., Fridovich, I. (1987): Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. – *Analytical Biochemistry* 161: 559-566.
- [8] Blokhina, O. (2003): Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: A Review. – *Annals of Botany* 91: 179-194.
- [9] Blum, A. (2016): Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. – *Plant, Cell & Environment* 40: 4-10.
- [10] Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – *Analytical Biochemistry* 72: 248-254.
- [11] Brown, C. E., Pezeshki, S. R. (2000): A study on waterlogging as a potential tool to control *ligustrum sinense* populations in western Tennessee. – *Wetlands* 20: 429-437.
- [12] Bufford, J. L., Hulme, P. E. (2021): Increased adaptive phenotypic plasticity in the introduced range in alien weeds under drought and flooding. – *Biological Invasions* 23: 1-14.
- [13] Centritto, M., Brillì, F., Fodale, R., Loreto, F. (2011): Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (*Populus nigra*) saplings. – *Tree Physiology* 31: 275-286.
- [14] Chance, B., Maehly, A. C. (1955): Assay of catalases and peroxidases. – *Methods in Enzymology* 2: 764-775.
- [15] Chen, P., Chen, X., Wang, H., Qian, L. N., Zhang, C. C. (2007): Determination of polysaccharide from *Panax japonicus* of Hubei by anthrone-sulfuric acid Method. – *Chinese Journal of Hospital Pharmacy* 27: 1654-1656.
- [16] Costa, J. P. R., Caputti, G. P., Galzerano, L., Silva, W. L., Ruggieri, A. C., Malheiros, E. B. (2015): Relative chlorophyll contents in the evaluation of the nutritional status of nitrogen from *xaraes palisade* grass and determination of critical nitrogen sufficiency index. – *Acta Scientiarum Animal Sciences* 37: 109-114.
- [17] Dalmagro, H. J., Lobo, F. A., Vourlitis, G. L., Dalmolin, Â. C., Antunes, M. Z., Ortíz, C. E. R., Nogueira, J. S. (2013): Photosynthetic parameters of two invasive tree species of the Brazilian Pantanal in response to seasonal flooding. – *Photosynthetica* 51: 281-294.
- [18] Dapanage, M., Bhat, S. (2017): Physiological responses of commercial sugarcane (*Saccharum* spp. hybrids) varieties to moisture deficit stress tolerance. – *Indian Journal of Plant Physiology* 23: 40-47.

- [19] Davey, M. W., Stals, E., Panis, B., Keulemans, J., Swennen, R. L. (2005): High-throughput determination of malondialdehyde in plant tissues. – *Analytical Biochemistry* 347: 201-207.
- [20] De Faria, A. P., Lemos-Filho, J. P., Modolo, L. V., França, M. G. C. (2012): Electrolyte leakage and chlorophyll a fluorescence among castor bean cultivars under induced water deficit. – *Acta Physiologiae Plantarum* 35: 119-128.
- [21] Deans, R. M., Brodribb, T. J., Busch, F. A., Farquhar, G. D. (2018): Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis. – *New Phytologist* 222: 382-395.
- [22] Dehury, B., Sarma, K., Sarmah, R., Jagajjit, S., Smita, S., Mousumi, S., Priyabrata, S., Mahendra, K. M., Gauri, D. S., Manabendra, D. C., Madhumita, B. (2012): In silico analyses of superoxide dismutases (SODs) of rice (*Oryza sativa* L.). – *Journal of Plant Biochemistry and Biotechnology* 22: 150-156.
- [23] Diez, J. M., D'Antonio, C. M., Dukes, J. S., Grosholz, E. D., Olden, J. D., Sorte, C. J., Cascade, J. B. S., Dana, M. B., Bethany, A. B., Regan, E., Inés, I., Sierra, J. J., Joshua, J. L., Miller, L. P. (2012): Will extreme climatic events facilitate biological invasions? – *Frontiers in Ecology and the Environment* 10: 249-257.
- [24] Dionisio-Sese, M. L., Tobita, S. (1998): Antioxidant responses of rice seedlings to salinity stress. – *Plant Science* 135: 1-9.
- [25] Elton, C. C. (1977): The Ecology of Invasions by Animals and Plants. – *Journal of Range Management* 47: 1601.
- [26] Fan, X. W., Li, F. M., Song, L., Xiong, Y. C., An, L., Jia, Y., Fang, X. W. (2009): Defense strategy of old and modern spring wheat varieties during soil drying. – *Physiologia Plantarum* 136: 310-323.
- [27] Feizabadi, A., Noormohammadi, G., Fatehi, F. (2020): Changes in Growth, Physiology, and Fatty Acid Profile of Rapeseed Cultivars Treated with Vermicompost Under Drought Stress. – *Journal of Soil Science and Plant Nutrition* 21: 200-208.
- [28] Gao, J. M., Xiao, Q., Ding, L. P., Chen, M. J., Yin, L., Li, J. Z., Zhou, S. Y., He, G. Y. (2008): Differential responses of lipid peroxidation and antioxidants in *Alternanthera philoxeroides* and *Oryza sativa* subjected to drought stress. – *Plant Growth Regulation* 56: 89-95.
- [29] García-Sánchez, F., Syvertsen, J. P., Gimeno, V., Botía, P., Perez-Perez, J. G. (2007): Responses to flooding and drought stress by two citrus rootstock seedlings with different water-use efficiency. – *Physiologia Plantarum* 130: 532-542.
- [30] Ge, Y., He, X., Wang, J., Jiang, B., Ye, R., Lin, X. (2014): Physiological and biochemical responses of *Phoebe bournei* seedlings to water stress and recovery. – *Acta Physiologiae Plantarum* 36: 1241-1250.
- [31] Gill, S. S., Tuteja, N. (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – *Plant Physiology and Biochemistry* 48: 909-930.
- [32] He, Q., Zhao, S., Ma, Q., Zhang, Y., Huang, L., Li, G., Hao, L. (2014): Endogenous Salicylic Acid Levels and Signaling Positively Regulate Arabidopsis Response to Polyethylene Glycol-Simulated Drought Stress. – *Journal of Plant Growth Regulation* 33: 871-880.
- [33] He, F., Sheng, M., Tang, M. (2017): Effects of *Rhizophagus irregularis* on Photosynthesis and Antioxidative Enzymatic System in *Robinia pseudoacacia* L. under Drought Stress. – *Frontiers in Plant Science* 8: 183.
- [34] Hemmati, K., Ebadi, A., Khomari, S., Sedghi, M. (2018): Influence of ascorbic acid and 24-epibrassinolide on physiological characteristics of pot marigold under water-stress condition. – *Journal of Plant Interactions* 13: 364-372.
- [35] Hojati, M., Modarres-Sanavy, S. A. M., Karimi, M., Ghanati, F. (2010): Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. – *Acta Physiologiae Plantarum* 33: 105-112.

- [36] Jalil, S. M., Movahhedi, D. M., Salehi, A., Bahreininejad, B. (2018): Physiological and yield responses of purple coneflower (*Echinacea purpurea* (L.) Moench) to nitrogen sources at different levels of irrigation. – *Physiology and Molecular Biology of Plants* 25: 177-187.
- [37] Kercher, S. M., Zedler, J. B. (2004): Flood tolerance in wetland angiosperms: a comparison of invasive and noninvasive species. – *Aquatic Botany* 80: 89-102.
- [38] Komatsu, S., Yanagawa, Y. (2013): Cell wall proteomics of crops. – *Frontiers in Plant Science* 4: 17.
- [39] Lawlor, D. W. (2012): Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. – *Journal of Experimental Botany* 64: 83-108.
- [40] Lewis, J. D., Griffin, K. L., Thomas, R. B., Strain, B. R. (1994): Phosphorus supply affects the photosynthetic capacity of loblolly pine grown in elevated carbon dioxide. – *Tree Physiology* 14: 1229-1244.
- [41] Li, Y., Zhang, S., Jiang, W., Liu, D. (2012): Cadmium accumulation, activities of antioxidant enzymes, and malondialdehyde (MDA) content in *Pistia stratiotes* L. – *Environmental Science and Pollution Research* 20: 1117-1123.
- [42] Li, W., Wang, Y., Zhang, Y., Wang, R., Guo, Z., Xie, Z. (2020): Impacts of drought stress on the morphology, physiology, and sugar content of Lanzhou lily (*Lilium davidii* var. *unicolor*). – *Acta Physiologiae Plantarum* 42: 127.
- [43] Liu, C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y., Yang, R. (2011): Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. – *Environmental and Experimental Botany* 71: 174-183.
- [44] Liu, D., Hu, L. Y., Ali, B., Yang, A. G., Wan, G. L., Xu, L., Zhou, W. J. (2016): Influence of 5-aminolevulinic acid on photosynthetically related parameters and gene expression in *Brassica napus* L. under drought stress. – *Soil Science and Plant Nutrition* 62: 254-262.
- [45] Mack, R. N., Simberloff, D., Mark, L. W., Evans, H., Clout, M., Bazzaz, F. A. (2000): Biotic invasions: causes, epidemiology, global consequences, and control. – *Ecological Applications* 10: 689-710.
- [46] Malik, A. I., Colmer, T. D., Lambers, H., Setter, T. L., Schortemeyer, M. (2002): Short-term waterlogging has long-term effects on the growth and physiology of wheat. – *New Phytologist* 153: 225-236.
- [47] Miao, Y., Zhu, Z., Guo, Q., Ma, H., Zhu, L. (2015): Alternate wetting and drying irrigation-mediated changes in the growth, photosynthesis and yield of the medicinal plant *Tulipa edulis*. – *Industrial Crops and Products* 66: 81-88.
- [48] Mitton, J. B., Grant, M. C., Yoshino, A. M. (1998): Variation in allozymes and stomatal size in pinyon (*Pinus edulis*, Pinaceae), associated with soil moisture. – *American Journal of Botany* 85: 1262-1265.
- [49] Moore, J. P., Vicré-Gibouin, M., Farrant, J. M., Driouich, A. (2008): Adaptations of higher plant cell walls to water loss: drought vs desiccation. – *Physiologia Plantarum* 134: 237-245.
- [50] Pasala, R. K., Md, I. R. K., Paramjit, S. M., Farooq, M. A., Sultana, R. (2016): Can plant bio-regulators minimize crop productivity losses caused by drought, salinity and heat stress? An integrated review. – *J Appl Bot Food Qual* 89: 113-125.
- [51] Pei, Z. F., Ming, D. F., Liu, D., Wan, G. L., Geng, X. X., Gong, H. J., Zhou, W. J. (2009): Silicon Improves the Tolerance to Water-Deficit Stress Induced by Polyethylene Glycol in Wheat (*Triticum aestivum* L.) Seedlings. – *Journal of Plant Growth Regulation* 29: 106-115.
- [52] Pintó-Marijuan, M., Munné-Bosch, S. (2013): Ecophysiology of invasive plants: osmotic adjustment and antioxidants. – *Trends in Plant Science* 18: 660-666.

- [53] Pintó-Marijuan, M., Cotado, A., Fleta-Soriano, E., Munné-Bosch, S. (2016): Drought stress memory in the photosynthetic mechanisms of an invasive CAM species, *Aptenia cordifolia*. – *Photosynthesis Research* 131: 241-253.
- [54] Praxedes, S. C., DaMatta, F. M., Loureiro, M. E., Ferrão, M. A. G., Cordeiro, A. T. (2006): Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. kouillou) leaves. – *Environmental and Experimental Botany* 56: 263-273.
- [55] Quinet, M., Descamps, C., Coster, Q., Lutts, S., Jacquemart, A-L. (2015): Tolerance to Water Stress and Shade in the Invasive *Impatiens parviflora*. – *International Journal of Plant Sciences* 176: 848-858.
- [56] Reddy, A. R., Chaitanya, K. V., Vivekanandan, M. (2004): Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. – *Journal of Plant Physiology* 161: 1189-1202.
- [57] Ruprecht, E., Fenesi, A., Nijs, I. (2013): Are plasticity in functional traits and constancy in performance traits linked with invasiveness? An experimental test comparing invasive and naturalized plant species. – *Biological Invasions* 16: 1359-1372.
- [58] Sasidharan, R., Mustroph, A., Boonman, A., Akman, M., Ammerlaan, A. M. H., Breit, T., Schranz, M. E., Voeselek, L. A. C. J., Tienderen, P. H. (2013): Root transcript profiling of two *Rorippa* species reveals gene clusters associated with extreme submergence tolerance. – *Plant Physiology* 163: 1277-1292.
- [59] Shukla, P. S., Gupta, K., Agarwal, P., Jha, B., Agarwal, P. K. (2015): Overexpression of a novel SbMYB15 from *Salicornia brachiata* confers salinity and dehydration tolerance by reduced oxidative damage and improved photosynthesis in transgenic tobacco. – *Planta* 242: 1291-1308.
- [60] Smaoui, A., Jouini, J., Rabhi, M., Bouzaien, G., Albouchi, A., Abdelly, C. (2011): Physiological and anatomical adaptations induced by flooding in *Cotula coronopifolia*. – *Acta Biologica Hungarica* 62: 182-193.
- [61] Taheri-Garavand, A., Rezaei, N. A., Fanourakis, D., Fatahi, S., Ahmadi, M. M. (2021): Employment of artificial neural networks for non-invasive estimation of leaf water status using color features: a case study in *Spathiphyllum wallisii*. – *Acta Physiologiae Plantarum* 43: 1-11.
- [62] Valliyodan, B., Nguyen, H. T. (2006): Understanding regulatory networks and engineering for enhanced drought tolerance in plants. – *Current Opinion in Plant Biology* 9: 189-195.
- [63] Vince, G. H., Bendszus, M., Schweitzer, T., Goldbrunner, R. H., Roosen, K. (2004): Spontaneous regression of experimental gliomas - An immunohistochemical and MRI study of the C6 glioma spheroid implantation model. – *Experimental Neurology* 190: 478-485.
- [64] Wang, Z. L., Gao, J. F. (2006): Practical soil moisture monitoring and forecasting technology. – China Water Power Press, Beijing. (A Chinese book).
- [65] Wang, H. F., Zhu, Y. H., Sun, H. J. (2006): Determination of drought tolerance using root activities in *Robinia pseudoacacia* 'Idaho' transformed with *mtl-D* gene. – *Forest Ecosystems* 8: 75-81.
- [66] Wang, N., Yuan, M. L., Chen, H., Li, Z. Z., Zhang, M. X. (2019): Effects on drought stress and rewatering on growth and physiological characteristic of invasive *aegilops tauschii* seedlings. – *Acta Prataculturae Sinica* 28: 70-78. (In Chinese).
- [67] Williams-Linera, G., Berry, Z. C., Díaz-Toribio, M. H., Espejel-Ontiveros, X. (2021): Drought responses of an exotic tree (*Eriobotrya japonica*) in a tropical cloud forest suggest the potential to become an invasive species. – *New Forests* 52: 1-15.
- [68] Wu, X., Yuan, J., Luo, A., Chen, Y., Fan, Y. (2016): Drought stress and re-watering increase secondary metabolites and enzyme activity in *dendrobium moniliforme*. – *Industrial Crops and Product* 94: 385-393.

- [69] Xu, L., Islam, F., Ali, B., Pei, Z., Li, J., Ghani, M. A., Zhou, W. (2017): Silicon and water-deficit stress differentially modulate physiology and ultrastructure in wheat (*Triticum aestivum* L.). – *Biotech* 7: 273.
- [70] Yang, L., Zhao, Y., Zhang, Q., Cheng, L., Han, M., Ren, Y., Yang, L. (2019): Effects of drought-re-watering-drought on the photosynthesis physiology and secondary metabolite production of *Bupleurum chinense* DC. – *Plant Cell Reports* 38: 1181-1197.
- [71] Zhang, S. M., Li, Z. X., Wang, Q., Chen, C., Jiang, X. P., Sun, H. K. (2009): A newly recorded species *Rorippa amphibia* (L.) Besser from China. – *Journal of Tropical and Subtropical Botany* 17: 176-178. (In Chinese).
- [72] Zhang, X., Yang, Z., Li, Z., Zhang, F., Hao, L. (2020): Effects of drought stress on physiology and antioxidative activity in two varieties of *Cynanchum thesioides*. – *Brazilian Journal of Botany* 43: 1-10.