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RESEARCH PAPERS

Modulation of Morphological and Several Physiological Parameters in *Sedum* under Waterlogging and Subsequent Drainage[1]

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Abstract—A comparative study was undertaken to investigate the morphological and physiological differences between tolerant *Sedum spectabile* Boreau "Carl" and susceptible *S. spectabile* "Rosenteller" in response to simulated waterlogging for 36 days and subsequent drainage for 12 days. Although the stress induced visible leaf stress symptoms in both cultivars, symptoms occurred earlier and severe in the susceptible cultivar. In the *S. spectabile* "Carl", adventitious roots emerged earlier from leaf scar. Waterlogging induced more acute decrease of plant height, canopy, leaf area, root length, shoot/root biomass in *S. spectabile* "Rosenteller". Leaf chlorophyll, relative water content and water soluble carbohydrate concentration were comparatively more salient in *S. spectabil*e "Carl". The activities of SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase) increased in both accessions after suffering of the stress, and all activities of them were more pronounced in *S. spectabile* "Carl". In addition, the lower MDA content of *S. spectabile* "Carl" was lower than that of *S. spectabile* "Rosenteller" demonstrated that less oxidative damage was induced by waterlogging and drainage. All these results suggest that the greater relative waterlogging tolerance to withstand waterlogging stress up to 36 days and better recovery capacity after soil drainage of *S. spectabile* "Carl" appears to depend on the combination of morphological and metabolic responses.

Keywords: Sedum spectabile, waterlogging, drainage, lipid peroxidation, antioxidant enzyme

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INTRODUCTION

Unpredictable and time unsuitable rainfall or poor drainage often cause waterlogging or flooding. Overabundant moisture alters soil aeration status with gases in soil pores are replaced by water. Low diffusion rate of oxygen induces oxygen deficiency of soil environment resulting in limitation of oxygen availability for plant roots and soil microorganisms [1]. Waterlogging engenders oxygen deprivation stress in plant cells includes three different states: hypoxia, anoxia and reoxygenation, which initiate a series of harmful effects on tissues with root being particularly vulnerable, such as disarrange normal metabolism and physiology [1–4]. Hypoxic or even anoxia condition causes the shift aerobic metabolism to the low ATP-yielding anaerobic fermentation, triggering off a fast depletion of carbohydrate reserves and accumulation of phytotoxins [5, 6], resulting in limitation of water uptake and nutrient absorption, closure of stomata and destruction of chlorophyll followed by decline in photosynthesis, alterations in hormone balance, growth retardation, reduction in leaf size, wilting and necrosis, and decrease of ornamental quality [1, 3].

Expose to waterlogging, the reaction of plants varies with species, some species such as rice are particularly tolerant [7] whereas others, such as cucumber (*Cucumis sativus* L.) are extremely sensitive [8]. The ability of plants to survive waterlogging is associate with the evolutionary developed resistance to stress factor, which is consist of two major classes of adaptations.

Anatomical adaptations contain modification of tissue structures, including formation of aerenchyma, adventitious root, hypertrophied lenticels and radial oxygen-loss barrier [3, 9] to create spaces for ventilation and to minimize oxygen demand. As well as facilitating oxygen capture and diffusion from shoots to roots, they also encourage water and nutrient uptake and the removal of toxic products [3, 9, 10].

Biochemical adaptations comprise induction of pathways of anaerobic metabolism and protective enzymes for the elimination of phytotoxic by-products [4], which are essential for plant survival under water-

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Abbreviations: APX—ascorbate peroxidase; CAT—catalase; SOD—superoxide dismutase.

logging conditions. Water soluble carbohydrates (WSCs) are the primary fermentation substrates in higher plants. When suffer to waterlogging, in order to response the altered balance between photosynthesis and carbohydrate metabolism, the WSCs reserves may be reduced [11], and the induced change of WSCs is considered as one of the pertinent factors that affected the fermentation rate, then influenced survival of some species [2, 12].

It has been reported that excessive generation of reactive oxygen species (ROS) is characteristic for hypoxia/anoxia and especially for re-oxygenation [6]. In this conditions, an impairment of redox balance may easily initiate oxidative damage to many different cellular components including lipids, proteins, carbohydrates and nucleic acids, causing peroxidation of unsaturated fatty acids in membranes, proteins denaturation followed by enzyme deactivation, membrane fluidity change and genomic damage, even irreversible metabolic dysfunctions leading to cell death [5, 13]. To cope with oxidative stress, plants may counterattack the deleterious effect of ROS enhancing in endogenous antioxidant defense system, comprised of antioxidative enzymes and non-enzymatic antioxidants to scavenge ROS and alleviate oxidative damages [1, 13]. The distinction of ROS formation, the ability of induction in antioxidant defenses, the collaboration between enzymes, and antioxidants localization, synthesis and transport are contributing to the activity [6, 14]. Malondialdehyde (MDA) is a product of lipoperoxidation of cell membranes, which is used as a marker of oxidative lipid injury under waterlogging stress [1]. Higher MDA depicts lower antioxidative ability pondering lower resistance to waterlogging stress.

Sedums have characteristics of effective aesthetic, low maintenance and wide adaption, particularly in periodic or prolonged drought and exposed conditions. Several researches of sedums have been actualized for the selection of appropriate vegetation for extensive green roofs [15], estimate of the performance with different substrate types and depths [16], irrigation regimes [17, 18]. It was found that *Sedum* is an ideal genus for the surrounding conditions [15] and some Sedum spp. survived even after four months without water and maintained active photosynthetic metabolism [17].

Although several reports describe the differences found in tolerance and the responses to drought among sedum species, published information about the physiological or anatomical responses of *Sedum* spp. under waterlogging and restoration of aerobic conditions have yet not been possessed. Genetic variation for waterlogging tolerant has been observed in practical landscape applications and the accession perennials *S. spectabile* "Carl" and *S. spectabile* "Rosenteller" contrast markedly from one another with respect to their waterlogging tolerance. The former appears to be tolerant, but the latter is susceptible. Therefore, this study was conducted to investigate the anoxic and post-anoxic responses linked to growth and physiological differences to reveal the mechanisms responsible involved in waterlogging tolerance in *Sedum*, and help to develop sedum future breeding programs as a key element on climate change mitigation.

MATERIALS AND METHODS

Plant material and experimental conditions. Cuttings of two sedum species *Sedum spectabile* Boreau "Carl" (tolerant) and *S. spectabile* "Rosenteller" (susceptible), propagated at Beijing Forestry University nursery (40°00′ N, 116°19′ E). Two weeks later, cuttings were transported to plastic pots (15 × 15 cm) filled with a mixture of peat, vermiculite and sandy soil in 2 : 2 : 1 ratio (v/v/v) as substrate and two plants per replication. After two months of growth, plantlets were selected for uniformity and then randomly imposed to waterlogging treatments. Waterlogging treatment was given by placing pots in plastic troughs, filled with water to maintain a 2–3 cm above soil surface in pots. The water level was checked daily and water was added as necessary. Treatments consisted of six waterlogging treatment (0, 7, 14, 21, 28 and 36 days of waterlogging), and recovery of 36-day waterlogged plants after 12 days of termination of treatment. Control plants were provided normal moisture throughout the experiment. The experiment was carried out as a completely randomized split-plot with three replications.

Morphological characteristics investigation. At the end of each waterlogging treatment, combined with the samples obtained for physiological characteristics analysis, survival, ornamental quality, plant height, canopy, stem diameter and leaf area were also investigated. Ornamental quality was visually rated as an integral of foliage color, morphological victim characteristics and growth rate on a scale from 1 (healthy, green leaves) to 6 (obvious atrophy and loss of lamina or death). At the end of waterlogging treatment, aboveground biomass and root were harvested, washed under tap water to remove soil, and root length was measured. Then, dry biomass of each part was determined by weighting after drying at 105°C for 0.5 h and then 65°C for 72 h until constant weight was reached.

Estimation of relative water content (RWC) and chlorophyll content. The RWC was determined according to the method of Garnier et al., [19]. The 4th fully expanded and exposed leaves from an apex were collected to obtain fresh weight (WF). Then, those leaves were incubated in distilled water for 12 h to obtain turgid weight (WT). Dry weight (WD) was determined by weighing after drying at 105°C for 0.5 h and then 65°C for 72 h. The RWC was obtained using equivalents:

$$\mathrm{RWC\%} = \frac{(\mathrm{WF} - \mathrm{WD})}{(\mathrm{WT} - \mathrm{WD})} \times 100. \qquad (1)$$

Chlorophyll extraction (200 mg leaf fr wt) was performed according to the method of Knudson et al. [20].

Approximately 200 g of deveined leaf tissue was taken and cut into small pieces in 95% ethanol for 24 h in darkness until the chlorophyll were sufficiently extracted. Then it was centrifuged at 4000 rpm for 10 min. The absorbance of supernatant was taken at 645 and 663 nm.

Estimation of MDA (malondialdehyde) content and water soluble carbohydrates. Membrane lipid peroxidation was determined in terms of malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method [21]. Briefly, 200 mg of freeze dried sample was homogenized in 5 mL of 50 mM chilled sodium phosphate buffer (pH 7.8). Following centrifugation at 10000 rpm for 20 min, 1 mL supernatant was mixed with 1 mL of 20% (w/v) trichloroacetic acid containing 0.5% (w/v) TBA and incubated in boiling water for 30 min. Then, the mixture immediately chilled on ice. After centrifugation at 4000 rpm for 10 min, the absorbance of the supernatant was read at 440, 532 and 600 nm. The MDA content was calculated as follows:

$$\begin{aligned} &\text{MDA}\ [\mu\text{mol/g fr wt}] \\ &= [6.45(A_{532} - A_{600}) - 0.56A_{450}] \times V/W, \end{aligned} \tag{2}$$

where A_λ is the absorbance at wavelength λ nm, V is the volume of extracted liquid, W is the mass of sample.

The content of water soluble carbohydrates in leaves was determined by the anthrone colorimetric method with tiny modify [22]. 200 mg of sample was extracted in boiling water for 60 min and the sugar levels determined using the anthrone reagent with glucose as standard. The absorbance was read at 630 nm, and the sugar content was determined using a glucose standard curve.

Estimation of enzyme activity. The frozen tissues (200 mg) were homogenized in a mortar with liquid nitrogen and chilled sodium phosphate buffer (50 mM, pH 7.8) with ethylenediaminetetraacetic acid (EDTA, 0.1 mM), 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM dithiothreitol (DTT). Subsequently, the homogenate was centrifuged at 10000 rpm for 10 min at 4°C. The supernatants were collected and kept at 4°C for analysis.

SOD activity was determined based on the method of Giannopolitis-Ries [24]. The reaction mixture composed of 50 mM sodium phosphate buffer (pH 7.8), 130 mM L-methionine, 0.75 mM NBT, 0.1 mM EDTA-Na_2, 0.02 mM riboflavin and enzyme extract was illuminated at 4000 Lx for 20 min. Then the absorbances were recorded at 560 nm. 1 U of SOD was defined as sufficient to inhibit the photo-reduction of NBT by 50%. Catalase (CAT) activity was determined in a 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 μL enzyme extract to induce a linear decrease in absorbance at 240 nm for 40 s. APX activity was assayed by tracking the consumption of ascorbate at 290 nm for 4 min [23], in a 3 mL assay mixture of 50 mM sodium phosphate buffer (pH 7.0), 0.3 mM H_2O_2, 0.15 mM ascorbate and sufficient extract.

Statistical analysis. Statistical analysis was performed using the analyses of one-way variance (ANOVA). Means were separated by Duncan's test ($P < 0.05$). Pearson correlations were made for determining the relationship between parameters. Means and standard errors based on three replications are indicated.

RESULTS

Growth Inhibition and Morphometric Response to Waterlogging

Waterlogging induced significant growth inhibition (Tables 1, 2) and dramatic morphological changes in the root system with disappearance of basal roots and formation of lateral adventitious roots (data not shown). Generally, *S. spectabile* "Rosenteller" presented more severe reduction of plant height, plant canopy, leaf area, root length, aboveground dry biomass and root dry biomass (12, 34, 32, 72, 87, 86% reduction at 36 days of waterlogging, compared to the respective control plants, respectively) which progressed with stress prolongation, whereas waterlogging-induced in *S. spectabile* "Carl" seemed to show no limitation in height and canopy (102% of the control, both) and less decline in root length, aboveground dry biomass, and root dry biomass (57, 28, 29% of the untreated control). It is notable that the leaf area of both cultivars increased rapidly to a maximum (by 1.2-flod in *S. spectabile* "Rosenteller" on day 7 and 1.3-flod in *S. spectabile* "Carl" kept on day 14) during the early stages of waterlogging.

In addition, behavior and perceptible variations of adventitious roots had emerged from leaf scar above the water surface after 15 days waterlogging in *S. spectabile* "Carl" while only a few adventitious roots have been observed until the end of treatment in 20% of *S. spectabile* "Rosenteller" waterlogged plants. Suffered for 36 days of waterlogging, only 50% of *S. spectabile* "Rosenteller" survived while no one plant of *S. spectabile* "Carl" died (Table 2). After 12 days of waterlogging withdrawal, the recovery performance of *S. spectabile* "Carl" was better than *S. spectabile* "Rosenteller". Up to the end of treatment, the stem diameter of both cultivars did not show any significant deviation among control (Table 1).

Symptoms of Premature Senescence in the Plants

Ornamental quality was reduced by waterlogging and there were significant differences between the test accessions (Table 1). At the end of waterlogging treatment, ornamental quality of *S. spectabile* "Carl" and *S. spectabile* "Rosenteller" was 2.2- and 5.8-flod of the untreated control, respectively. The major symptoms of waterlogging injury including leaf chlorosis, leaf rot

Table 1. Ornamental quality, height, canopy, stem diameter and leaf area of *Sedum spectabile* "Carl" and *S. spectabile* "Rosenteller" under control and waterlogging conditions

Species	Treatment	OQ	H, cm	PC, cm	StD, mm	LA, cm^2
"Carl"	CK-6	1.00	19.27a	13.67a	6.57a	17.17a
	T-6	2.20	19.57a	14.00a	6.79a	12.78b
"Rosenteller"	CK-6	1.00	20.83a	14.10a	7.10a	18.52a
	T-6	5.60	18.43a	9.30b	6.03a	12.51b
"Carl"	CK-12	1.00	21.50a	13.98a	6.70a	16.91b
	R-12	2.00	20.13a	14.23a	6.83a	13.29c
"Rosenteller"	CK-12	1.00	21.47a	14.30a	7.11a	19.12a
	R-12	5.60	19.57a	10.90b	6.39a	7.69d

OQ—ornamental quality, H—height, PC—canopy, StD—stem diameter, LA—leaf area, CK—control, T—waterlogging treatment, R—recovery, the followed number represent plants waterlogged for 36 days, respectively and recovery for 12 days, successively. The data represents mean of three replicates. Means within the same treatment followed by the same letter in the column are not different based on Duncan's multiple range test at 0.05 probability.

Table 2. Survival, aboveground dry biomass, root dry biomass and root length of *Sedum spectabile* "Carl" and *S. spectabile* "Rosenteller" at the end of waterlogging conditions

Species	Treatment	Survival, %	ADB, g	RDB, g	RL, cm
"Carl"	CK	100	2.85b	1.85a	16.80a
	T	100	1.61c	0.52b	4.81b
"Rosenteller"	CK	100	4.60a	1.67a	14.37a
	T	50	1.28c	0.21c	2.03b

ADB—aboveground dry biomass, RDB—root dry biomass, RL—root length, CK—control; T—waterlogging treatment. The data represents mean of three replicates. Means within the same treatment followed by the same letter in the column are not different based on Duncan's multiple range test at 0.05 probability.

and defoliation during waterlogging. The symptoms of premature senescence occurred earlier and serious in *S. spectabile* "Rosenteller" within 7 days of stress and evident stem base rot was observed just after 10 days (data not shown), which became pronounced with prolongation of stress, while in *S. spectabile* "Carl" a minor leaf yellowing was observed only after 20 days of waterlogging (Fig. 1).

In waterlogged plants the content of total chlorophyll content diminished with the duration of waterlogging stress (Fig. 2). Significant decline of chlorophyll content was observed in *S. spectabile* "Rosenteller" at 21 days while dramatic decrease in waterlogged plants of *S. spectabile* "Carl" was seen a week later, at the 28th day. Under 36 days of waterlogging, the chlorophyll content decreased by 53% for *S. spectabile* "Rosenteller" and 29% for *S. spectabile* "Carl" with comparison of respective control. After re-oxygenation for 12 days, the chlorophyll content in *S. spectabile* "Carl" recovered relative faster than in *S. spectabile* "Rosenteller", although there was still a certain distance to recover that of control (Fig. 2).

Relative Water Content Response to Waterlogging

Significant decline of RWC were observed in both sedum cultivars in comparison to control when given waterlogging treatment (Fig. 2). RWC reduction increased with the duration of waterlogging ($P < 0.05$) and the reduction of *S. spectabile* "Rosenteller" was more than *S. spectabile* "Carl". *S. spectabile* "Rosenteller" exhibited a dramatic decline of RWC even in the 14 day of waterlogging while *S. spectabile* "Carl" maintained higher RWC even after 21 days of waterlogging. In addition, RWC of "Rosenteller" decreased almost 39% at 36 day of waterlogging with comparison of control. However, that of *S. spectabile* "Carl" was reduced by 11% after 36 days of waterlogging treatment. Drainage of the soil succeed to increase significantly the RWC and *S. spectabile* "Carl" showed a better performance than *S. spectabile* "Rosenteller", and the RWC was comparable to that of control after recovery.

Accumulation of Oxidative Damage in the Leaves

MDA content was significantly higher in waterlogged plants of *S. spectabile* "Rosenteller" than

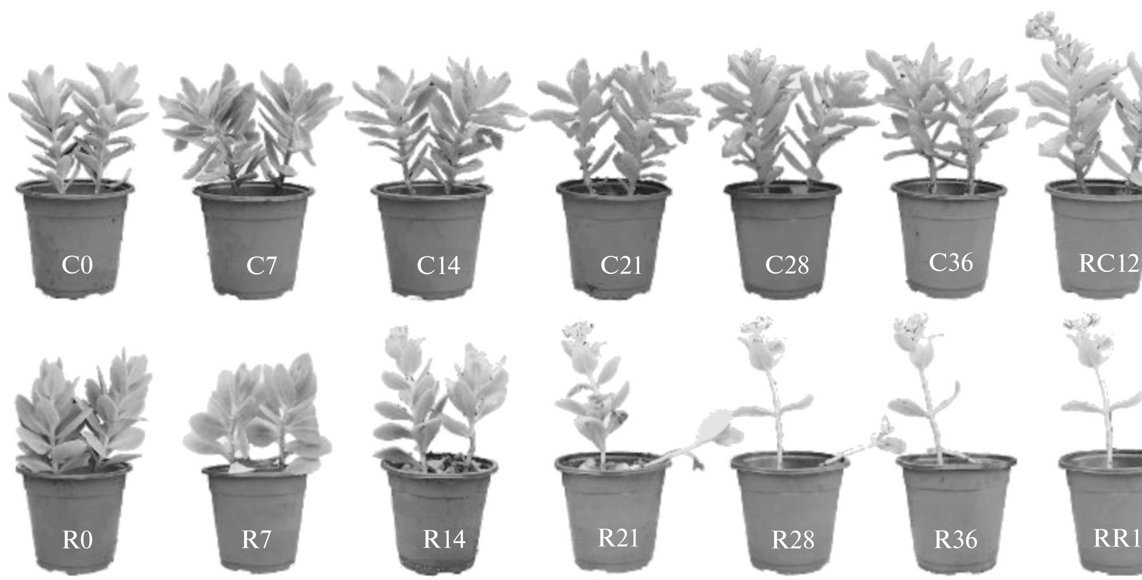

Fig. 1. Morphological responses of tolerant *Sedum spectabile* "Carl" and susceptible *S. spectabile* "Rosenteller". C0, C7, C14, C21, C28, C36 and RC12 – *S. spectabile* "Carl" plants waterlogged for 0, 7, 14, 21, 28 and 36 days, respectively, and recovery for 12 days; R0, R7, R14, R21, R28, R36 and RR12 – *S. spectabile* "Rosenteller" plants waterlogged for 0, 7, 14, 21, 28 and 36 days, respectively, and recovery for 12 days.

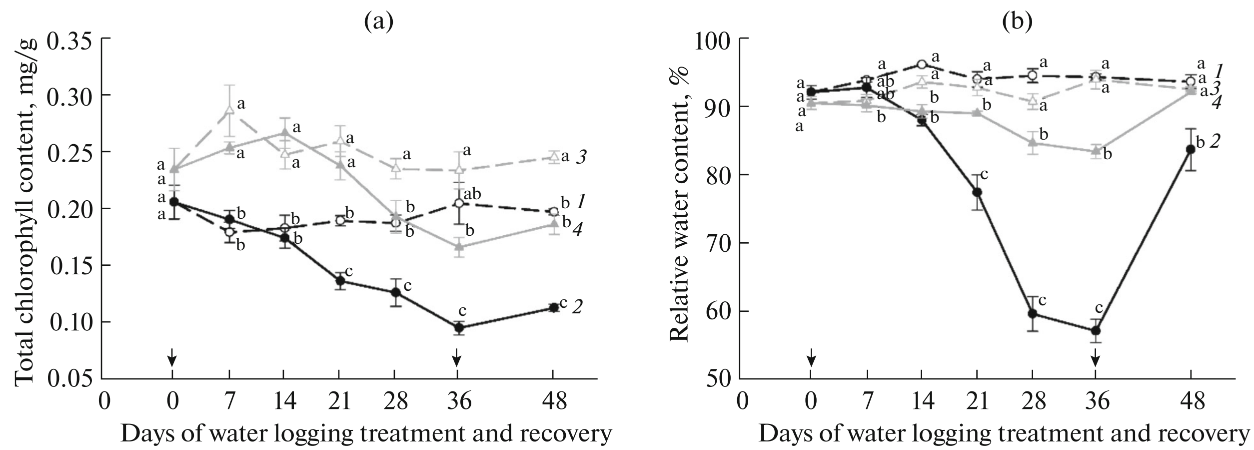

Fig. 2. Total chlorophyll content (a) and relative water content changes (b) in *Sedum spectabile* "Carl" and *S. spectabile* "Rosenteller" during 36 days of waterlogging treatment and recovery for 12 days (shown by arrows). *1*—control of *S. spectabile* "Rosenteller", *2*—treatment of *S. spectabile* "Rosenteller", *3*—control of *S. spectabile* "Carl", *4*—treatment of *S. spectabile* "Carl". Means and standard errors based on three replications are indicated. Means in the same time period with same letter are not different based on Duncan's multiple range test at 0.05 probability.

plants in non-waterlogged, increasing by about 5.3-fold (day 7) (Fig. 3). Subsequently, it decreased in day 14 and kept relative lower levels (1.9- and 1.8-flod, respectively) until 28 days. However, *S. spectabile* "Carl" did not show any significant change in MDA content until 21 days of continuous stress. Anyway, the rise rate of *S. spectabile* "Rosenteller" was still over 2.8-fold higher than that in *S. spectabile* "Carl" on the 36 days of waterlogging stress. After 12 days recovery, *S. spectabile* "Carl" exhibited better ability than *S. spectabile* "Rosenteller" and the content of MDA almost closed to the control demonstrated that lesser oxidative damage than *S. spectabile* "Rosenteller".

Metabolic Responses to Waterlogging

Waterlogging prompted significant changes in SOD, CAT, APX activity across timings (Fig. 4). The two test accessions showed a similar pattern of SOD induction. However, the increase of SOD activity in waterlogged plants of *S. spectabile* "Carl" was greater than in *S. spectabile* "Rosenteller". Interestingly, the

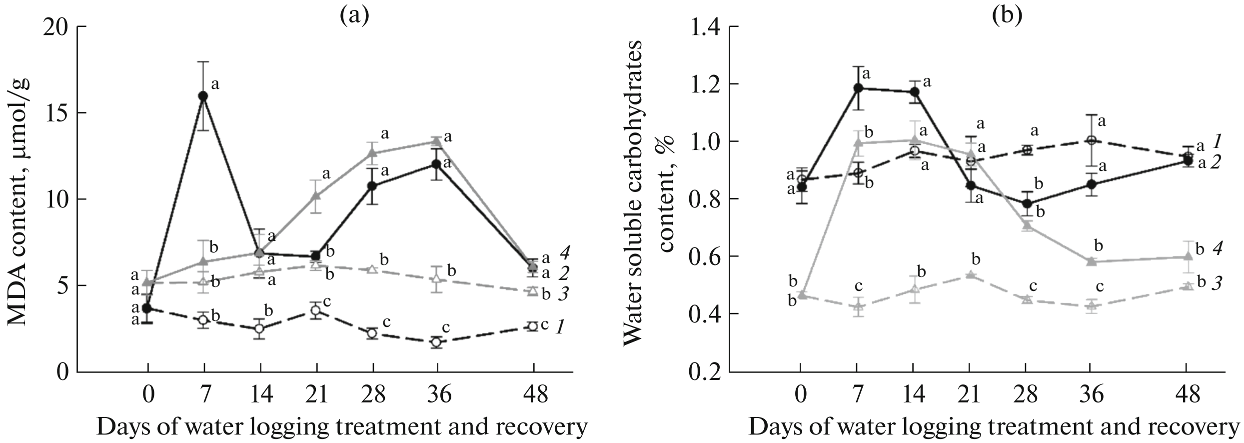

Fig. 3. MDA content (a) and water soluble carbohydrates (b) changes in *Sedum spectabile* "Carl" and *S. spectabile* "Rosenteller" during 36 days of waterlogging treatment and recovery for 12 days. *1*—control of *S. spectabile* "Rosenteller", *2*—treatment of *S. spectabile* "Rosenteller", *3*—control of *S. spectabile* "Carl", *4*—treatment of *S. spectabile* "Carl". Means and standard errors based on three replications are indicated. Means in the same time period with same letter are not different based on Duncan's multiple range test at 0.05 probability.

two cultivars did not show any decline when after withdrawal of stress and *S. spectabile* "Rosenteller" exhibited higher activity than continuously 36 days flooded plants (Fig. 4).

As for CAT, APX, the temporal pattern of their expression exhibited a different pattern in both cultivars (Fig. 4). Significant and persistent increases were recorded until the 14 and 21 days of the experiment (in *S. spectabile* "Carl" and *S. spectabile* "Rosenteller", respectively). The CAT activity in *S. spectabile* "Rosenteller" peaked at 13.1-fold of their background level by day 21. After peaking, a rapid decline was observed in the rest time of treatment. On the contrary, *S. spectabile* "Carl" maintained a relative stable activity up to the end of waterlogging stress and showed rapid recovery while *S. spectabile* "Rosenteller" still keep higher activity of CAT over controls after 12 days of release of waterlogging stress.

Under waterlogging, leaves accumulated significantly higher levels of APX than in control conditions which are similar to the change trend of CAT (Fig. 4). *S. spectabile* "Rosenteller" maintain a higher activity with comparison to control up to 21 days, and then the activity decreased progressively. In contrast, the increase was greater in *S. spectabile* "Carl" than *S. spectabile* "Rosenteller" and also keep higher activity with respect to control until the end of treatment. Anyway, the increase in APX activity was more pronounced in *S. spectabile* "Carl" than in *S. spectabile* "Rosenteller".

In both cultivars, the WSC content increased rapidly to a peak on day 7 by 1.4-flod in *S. spectabile* "Rosenteller" and 2.1-flod in *S. spectabile* "Carl" during the early stage of waterlogging, and then decreased progressively (Fig. 3). It was known that the decline rate was less in *S. spectabile* "Carl", especially during the period from day 7 to day 21, while rapid decline was detected in *S. spectabile* "Rosenteller" and the level was lower than that in control plants after less than 21 days of waterlogging. Overall, the two cultivars differed from one another relating to the provoked level of this parameter, with the decline was 7% in *S. spectabile* "Rosenteller" but the raise was 35% in *S. spectabile* "Carl" when compared to non-waterlogged plants after 36 days of waterlogging.

DISCUSSION

Waterlogging dramatically affected the growth, development and survival of plants not adapted to excess water in soil environment [1, 4]. Moreover, stress release does not seem to improve plant performance but may enhance the incidence of oxidative damage owing to the restoration of normal oxygen tension withdrawal from an anoxic period [24]. In our research, the responses of both cultivars in anoxic and post-anoxic was assessed in terms of growth, morphometrics, relative water content, oxidative damage and antioxidant enzyme capacity. The results presented in this paper show that typical symptoms damage associated with hypoxia and anoxic stress, such as leaf yellowish, senescence, wilting and necrosis were observed earlier and severe in sensitive cultivar *S. spectabile* "Rosenteller" but later and mild in the tolerant cultivar *S. spectabile* "Carl" (Fig. 1).

Both cultivars responded similarly to waterlogging stress with substantial root growth inhibition, diminution in leaf chlorophyll and relative water content (Tables 1, 2; Figs. 1, 2). More severe decrease of the above parameters presented in *S. spectabile* "Rosenteller" compared to *S. spectabile* "Carl". Moreover, *S. spectabile* "Carl" presented a higher capacity to recover after release. These results suggest that less

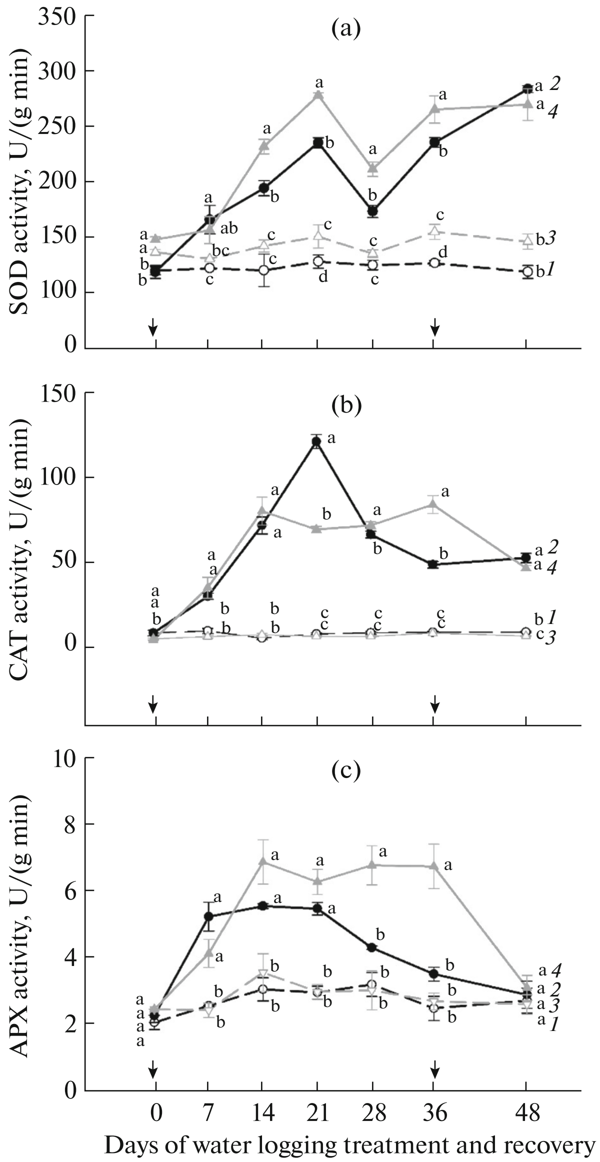

Fig. 4. The effect of waterlogging on the activity of SOD (a), CAT (b) and APX (c) in *Sedum spectabile* "Carl" and *S. spectabile* "Rosenteller" during 36 days of waterlogging treatment and recovery for 12 days (shown by arrows). *1*—control of *S. spectabile* "Rosenteller", *2*—treatment of *S. spectabile* "Rosenteller", *3*—control of *S. spectabile* "Carl", *4*—treatment of *S. spectabile* "Carl". Means and standard errors based on three replications are indicated. Means in the same time period with same letter are not different based on Duncan's multiple range test at 0.05 probability.

injury suffered by waterlogging most probably because of a more biomass roots as a result of more growth, which implies greater gas diffusion in a more aerated root environment that allowed plants of *S. spectabile* "Carl" to recover faster. Similar loss phenomenon of chlorophylls were observed in other *Sedum* spp. [25] and *Mentha* [26], which could be explained rather by adjustment of the photosynthetic apparatus to reduce photosynthetic activity under waterlogging, reported in different plant species [2, 24]. Additionally, tolerant cultivars had higher RWC than sensitive ones within the same treatment, which is in agreement with previous research in *Sedum* spp. [25]. Sufficient water content is required to stabilize subcellular structures and facilitate cell recovery from stress, which is responsible for the higher capacity of *S. spectabile* "Carl" to endure and recover associated with waterlogging and subsequent drainage.

The formation of adventitious roots is a mechanism to waterlogging tolerance [10], which functionally replace basal roots when the original root system becomes unable to supply required water and mineral to shoot [27]. Adventitious roots commonly emerged from the base of the stem or in the region where lenticels are abundant and the growth of them is lateral and parallel to the water–soil surface. In waterlogged *S. spectabile* "Carl" plants, the development of adventitious roots expressing its adaptability towards hypoxia, while there was limited the growth of these roots in *S. spectabile* "Rosenteller". Although *S. spectabile* "Carl" did not developed a large adventitious root system from the leaf scar, the high waterlogging tolerance let us to believe that there are other mechanisms involved in ameliorate oxygen capture and diffusion to alleviate the hypoxic conditions and contribute to the survival of plants during late stages of waterlogging.

Reactive oxygen species are produced at the transition when plant either suffers for hypoxia/anoxia from normoxic conditions or return to aerobic environment [13]. Either an overload of ROS and/or a decrease in the activity of the scavenging enzymes can touch off a shortfall in ROS scavenging. MDA has been used as a biomarker to assess oxidative damage. Phukan et al. [26] reported that waterlogging induced a dramatic increase of MDA content in *Mentha*. Under our experimental conditions, neither continuously waterlogged or drainage showed same pattern in MDA level over control (Fig. 3). Additionally, higher level of lipid peroxidation puts an injurious effect on plants which is absolutely evident from morphological response of *S. spectabile* "Rosenteller" showing its sensitivity towards waterlogging. On the contrary, the MDA content of *S. spectabile* "Carl" was lower than that of *S. spectabile* "Rosenteller", implying a greater ability to detoxify ROS by stimulating SOD, APX and CAT activity, which is in concordance with results obtained in sesame [10].

SOD constitutes the first line of defense against ROS, which plays a crucial role in cellular defense against oxidative stress by directly modulate the amounts of O_2 and H_2O_2. APX and CAT are mainly involved in detoxify H_2O_2 [1]. The tolerant accessions *S. spectabile* "Carl" sustained higher levels of SOD, APX and CAT than *S. spectabile* "Rosenteller", which allowed it to maintain a fine tuning between ROS production and scavenging (Fig. 4). CAT and APX activity accumulated rapidly in the early stages of stress (day 14) and maintained a higher level until the end of treatment in the tolerant *S. spectabile* "Carl" (Fig. 4), which inferred that CAT and APX play an important role in H_2O_2 scavenging for the present tolerant cultivars against waterlogged stress. These findings are consistent with previous reports such as in sesame, chrysanthemum [10, 28] and sedums under drought stress [18]. Differences are especially noticeable in CAT activity, where the activation in the sensitive cultivars at 21 days of treatment becomes evident with an significant increased free radical scavenging activity, and the same phenomenon was found in sensitive citrus genotype "Felinem" [14], which agrees with the hypothesis that CAT is described as an earlier response in the antioxidant system and also that acclimatizing has been shown to be dependent on the activation of such enzyme. In addition, it is notable that SOD significantly increased and CAT kept a high level in drainage *S. spectabile* "Rosenteller" plants, which indicate that withdrawal of stress is not always positive for plants, but seems more deleterious for plants due to the sudden oxygen burst after a long anoxic period causes more oxidative damage and need to scavenge. A similar increase pattern in SOD and CAT activity over post-anoxic period have been obtained previously in *Citrus* [24] and *Prunus* [14]. The correlation ($r = 0.79$, $r = 0.61$, $P < 0.01$, respectively) between SOD and CAT, APX activity implied that coordinated involvement of these three enzymes in alleviating waterlogging induced oxidative stress damage.

In conclusion, our study showed that *S. spectabile* "Carl" presented the greater relative waterlogging tolerance to withstand waterlogging stress up to 36 days and better recovery capacity after soil drainage in comparison to *S. spectabile* "Rosenteller" appears to depend on the combination of morphological and metabolic responses. The capacity to form adventitious roots is an important ways of promoting rapid post-stress recovery and for adaptation to more long-term period of waterlogging. In addition, *S. spectabile* "Carl" was adapted to the waterlogging might be closely related to its more effective antioxidant enzyme system (SOD, CAT and APX) to cope with ROS. To the best of our knowledge, this is the first report on the morphological and physiological response of *Sedum* species to waterlogging and subsequent drainage.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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