Scientia Horticulturae 243 (2019) 235–242

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Responses of succulents to drought: Comparative analysis of four *Sedum* (*Crassulaceae*) species

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ARTICLE INFO

Keywords:
Succulence
Water deficit
Osmolytes
Antioxidants
Growth response

ABSTRACT

The increasing frequency and intensity of drought periods is a serious threat for agriculture, prompting research to select and develop crop species and cultivars with enhanced water stress tolerance. Drought responses were studied in four ornamental *Sedum* species under controlled greenhouse conditions, by withholding watering of the plants for four weeks. Determination of growth parameters (stem length, fresh weight) allowed establishing the relative degree of tolerance of the selected species as *S. spurium* > *S. ochroleucum* > *S. sediforme* > *S. album*. The levels of photosynthetic pigments (chlorophylls a and b and total carotenoids), oxidative stress [using malondialdehyde (MDA) as a marker], non-enzymatic antioxidants (total phenolic compounds and total flavonoids) and putative osmolytes [proline (Pro) and total soluble sugars] were measured in leaves of control and stressed plants, to correlate drought tolerance with the activation of specific response mechanisms. The results obtained indicate that a higher tolerance to water deficit in *Sedum* is associated with: **a)** relatively lower stress-induced degradation of chlorophylls and carotenoids, especially of the latter (which did not decrease in water-stressed plants of *S. spurium*, the most tolerant species, whereas it was reduced to about 40% of the control in *S. album*, the most sensitive); **b)** no increase in MDA levels, reflecting the lack of drought-induced oxidative stress; and **c)** higher Pro contents in the non-stressed controls of the taxa most resistant to drought, which could be the basis of constitutive mechanisms of tolerance. However, Pro contribution to drought tolerance in *Sedum* must be based on an 'osmoprotectant' role, as its concentrations, below 16 μmol g^{-1} DW in all cases, are too low to have any significant osmotic effect. The identification of these biochemical markers of drought tolerance should help to develop rapid and efficient screening procedures to select *Sedum* taxa with enhanced tolerance when comparing different species within the genus, or different cultivars within a given species.

1. Introduction

One direct effect of global warming is an increased evapotranspiration, producing more frequent and more intense drought periods throughout the world. By the end of this century, extreme dry land will increase by 30%, affecting many different areas. Increased summer droughts are predicted for Asian mid-continental areas. Vulnerable areas such as southern Europe will suffer from increased effects of drought, and reduction of precipitation rates are predicted for Northern Africa and the south-western parts of South Africa (IPCC 2014). The frequency and intensity of drought periods has dramatically increased in the last decades in the Mediterranean Basin (Giannakopoulos et al., 2009; Cai et al., 2015; Lopez-Nicolas et al., 2018; Ortega-Gómez et al., 2018). Drought adversely affects the physiological and biochemical status of plants (Chaves and Oliveira, 2004; Osakabe et al., 2014), and is one of the most challenging stress factors currently encountered by global ecosystems (Bartlett et al., 2012; Sandoval et al., 2016). Even a slight destabilisation in cell water balance may negatively affect

Abbreviations: Caro, total carotenoids; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; MDA, malondialdehyde; Pro, proline; TSS, total soluble sugars; TPC, total phenolic compounds; TF, total flavonoids

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https://doi.org/10.1016/j.scienta.2018.08.028
Received 6 March 2018; Received in revised form 27 July 2018; Accepted 17 August 2018
Available online 25 August 2018
0304-4238/

physiological processes associated with plant growth and, for cultivated species, with crop yields (Mathur et al., 2014; Nxele et al., 2017). Drought, similarly to other abiotic stresses, causes cell dehydration and the enhanced generation of reactive oxygen species (ROS). ROS are continuously produced in plants as by-products of aerobic metabolism, but under stress conditions their concentrations largely increase leading to oxidative stress (Ahmad et al., 2010; Talukdar, 2013; You and Chan, 2015). To survive drought and other abiotic stresses, plants activate a series of conserved mechanisms to minimise possible injuries caused by those stress factors. One of these general responses to water deficit involves the maintenance of cellular osmotic balance, based on the synthesis and accumulation in the cytoplasm of compatible solutes or 'osmolytes', to avoid cellular dehydration (Szabados and Savoure, 2010; Talukdar, 2013; Per et al., 2017). Osmolytes, such as soluble sugars or some amino acids, play also the role of osmoprotectants in the responses to stress, maintaining the fluidity of plasma membranes and the proper activity of enzymes (Blum, 2011; Rabbani and Choi, 2018) by directly stabilising macromolecular structures as low-molecular-weight chaperons. Another common reaction to water stress is the activation of antioxidant systems, both enzymatic and non-enzymatic, to counteract oxidative damage caused by ROS (Apel and Hirt, 2004; Chen et al., 2007; Das and Roychoudhury, 2014). Among non-enzymatic antioxidants, phenolic compounds, including flavonoids, have been shown to be synthesised in many plant species as a response to water deficit (Sánchez-Rodríguez et al., 2011; Bautista et al., 2016).

Comparing information on the reaction of different species to water stress regarding growth parameters and biochemical markers allows distinguishing genotypes that are resistant or susceptible to this stress (Ji et al., 2012; Nxele et al., 2017). Furthermore, such comparative studies, correlating the activation of specific stress responses and the relative tolerance of genetically related taxa (for example, different species of the same genus or different varieties or cultivars of the same species) provide insight into the mechanisms of tolerance to abiotic stress in plants (Gil et al., 2013; Sandoval et al., 2016; Cicevan et al., 2016; Al Hassan et al., 2017)

Sedum L. is one of the largest species-rich genus of the stonecrop family (Crassulaceae DC., Crassuloidae, Saxifragales), comprising about 450 species assigned to thirty sections (Thiede and Eggli, 2007; Thorne and Reveal, 2007; The Plant List, 2013), or when subgenus *Sedum* is delineated – about 320 species (Nikulin et al., 2016). This genus mostly consists of branched, multi-stem perennial succulent herbs, occasionally sub-shrubs, which are not considered invasive. *Sedum* species have a wide geographic distribution; they are primarily growing in arid environments on shallow soils on stony or gravel stands, mainly in temperate to subtropical regions. Thus, the diversity of the genus representatives is high in the Mediterranean region, followed by America (mainly Central America), the Himalayas, and East Asia (Stephenson, 1994; Thiede and Eggli, 2007). Phytochemical screening has shown that sedums are an excellent source of a variety of secondary metabolites, including condensed tannins, alkaloids, flavonoids, free sugars, cyanogenic compounds, and triterpenoids, which are considered to play a major role in plant tolerance to biotic and abiotic stresses (Stevens et al., 1994; Han and Zhao, 2005; Al-Qudah et al., 2012; Xu et al., 2015).

Species belonging to this genus are distinctive in growing in dense clumps, with succulent stems and leaves, and rose, yellowish or creamy to white tiny flowers arranged in showy inflorescences. Numerous cultivars are well-known ornamental plants due to their attractive appearance and hardiness. They are ideal for sunny environments that get too little water, and are frequently used as ground covers or rock gardens because of their limited height (Stephenson, 1994). Moreover, *Sedum* are slow-growing plants that survive long periods without water, yet they can grow faster when water is available (Durhman et al., 2006; Carter and Butler, 2008; Nektarios et al., 2015). The economic importance and horticultural interest in this genus has increased in recent years due to its suitability to be used in the so-called 'green roofs', which represent a sustainable and economically sound strategy to mitigate environmental problems in urban areas, namely to counteract air pollution and the effect of urban heat islands (Getter and Rowe, 2006; Van Mechelen et al., 2014; Vijayaraghavan, 2016; Vahdati et al., 2017). Sedums are generally considered among the best plant species for extensive green roof technology due to their growth habit, shallow root system and drought tolerance, although different taxa may differ in their adaptability to this special environment (Damas et al., 2010; Nagase and Dunnett, 2010; Starry et al., 2014). Therefore, a comparative evaluation of drought responses in various *Sedum* species would facilitate selection of the most suitable genotypes to be used as ornamentals for 'green roofs' under conditions of limited water availability.

In this study, we have analysed the responses to water stress, under controlled greenhouse conditions, of four species: *Sedum spurium*, *S. ochroleucum*, *S. album* and *S. sediforme*, belonging to different taxonomic subgroups within the genus, with different geographical origins and distribution, but all possessing valuable ornamental traits. Drought-induced inhibition of growth was used as a criterion to establish the relative degree of tolerance of the investigated species, which we hypothesised would be dependent on the relative efficiency of the mechanisms activated to maintain osmotic balance and to counteract oxidative stress under water deficit conditions. Furthermore, we expected to correlate differences in tolerance with the species' geographical distributions and the ecological characteristics of their natural habitats.

Therefore, the main aim of the work was to obtain information on the physiological and biochemical mechanisms underlying drought tolerance in *Sedum* and, specifically, to establish which responses to water stress are the most relevant for tolerance. For this, we have determined the levels in control and stressed plants of several metabolites associated to specific stress responses: proline and total soluble sugars (as putative osmolytes involved in maintenance of cellular osmotic balance), malondialdehyde (MDA, a reliable marker of oxidative stress), and total phenolic compounds and favonoids, as examples of non-enzymatic antioxidants. Additionally, these experiments should allow identifying reliable biochemical markers, to be eventually used for the rapid and efficient screening of a much larger number of *Sedum* taxa, to select genotypes with enhanced drought tolerance.

2. Material and methods

2.1. Plant material, experimental design and stress treatments

Seeds of the selected species: *Sedum spurium* M. Bieb. [syn. *Phedimus spurius* (M. Bieb.)´t Hart], *S. ochroleucum* Chaix, *S. album* L. and *S. sediforme* (Jacq.) Pau, were purchased from the company B&T World Seeds (Paguignan, France).

Seeds were sown directly into a moistened mixture of peat (50%), perlite (25%) and vermiculite (25%) in 1 L pots (Ø = 11 cm). The substrate was kept moderately moist, using Hoagland's nutrient solution (Hoagland and Arnon, 1950). Two months after seedling emergence, a water stress treatment was initiated by stopping watering the plants. Control plants grown in parallel were watered twice a week (125 mL per pot). The experiments were performed twice, using five biological replicas (five individual plants) per species and per treatment. Plant samples (the aerial part of all plants) were collected after four weeks of treatment. Experiments were conducted in a growth chamber with controlled environment, under the following conditions: long-day photoperiod (16 h of light, obtained by supplementing natural light with artificial light), temperature of 23 °C during the light period and 17 °C during the dark period. Air relative humidity ranged between 60% and 70% during the course of the experiment.