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Comparative analysis of water deficit and salt tolerance mechanisms in *Silene*

A. Kozminska [a,b], M. Al Hassan [a,1], E. Hanus-Fajerska [b], M.A. Naranjo [a,c], M. Boscaiu [d,*], O. Vicente [b]

[a] *Universitat Politècnica de València, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), CPI, edificio 8E, Camino de Vera s/n, 46022, Valencia, Spain*
[b] *Institute of Plant Biology and Biotechnology, Unit of Botany and Plant Physiology, University of Agriculture in Krakow, 31-425 Krakow, 29 Listopada 54 Ave., Poland*
[c] *Tervalis S.L., 44002 Teruel, Spain*
[d] *Universitat Politècnica de València, Instituto Agroforestal Mediterráneo (UPV), CPI, edificio 8E, Camino de Vera s/n, 46022, Valencia, Spain.*

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A B S T R A C T

Comparative analyses of the responses to abiotic stress in related taxa with different degrees of tolerance can provide useful information to elucidate the mechanisms of stress tolerance in plants. This kind of study has been carried out in four *Silene* species, which were subjected to salt and water deficit treatments under controlled greenhouse conditions. Growth parameters and leaf levels of photosynthetic pigments, ions, osmolytes, malondialdehyde (MDA, an oxidative stress biomarker), total phenolic compounds and flavonoids, were determined in control and stressed plants. The degree of stress-induced growth inhibition allowed establishing the relative tolerance of the studied species, identifying *S. vulgaris* as the most tolerant to salinity and *S. sclerocarpa* to water deficit; these data correlated well with the characteristics of their natural habitats. All four species showed a high resistance to stress-induced leaf dehydration, and a good *negative* correlation was found between tolerance and the degradation of photosynthetic pigments. Salinity tolerance is mostly based on the inhibition of Na^+ transport to the leaves and the maintenance of relatively high leaf K^+ levels in the salt-treated plants. Proline is a reliable stress biomarker but does not appear to be involved in tolerance mechanisms in this genus, as it accumulates at higher levels in the more sensitive species. MDA contents did not increase significantly in response to the stress treatments – except in water-stressed *S. latifolia*, the species most affected by water deficit – suggesting that the plants were not affected by secondary oxidative stress under the experimental conditions used. Accordingly, the measured variations in the levels of total phenolic compounds and flavonoids were not statistically significant or did not correlate with the relative stress resistance of the studied species. Therefore, stress responses based on the activation of antioxidant systems do not seem to be relevant for abiotic stress tolerance in *Silene*.

1. Introduction

Soil salinity and drought are the most adverse environmental stress factors for agriculture, considering the damage they inflict on crop yields worldwide; they are also important because of their impact on the distribution of wild plant species in nature. Currently, more than 320 million hectares of land are affected by salinity (Munns and Tester, 2008; Rengasamy, 2010), and this area is expected to expand in the forthcoming years due to the foreseeable effects of global climate change. Climate change will also contribute to extending the surface of drought-affected areas, especially in arid and semiarid regions (IPCC, 2014). The most promising strategy to increase agricultural yields and food production under the present circumstances would be the genetic improvement of the tolerance to salt and water deficit of our major crops, by classical breeding techniques and/or genetic engineering (Fita et al., 2015). To reach this goal, a deep understanding of the molecular mechanisms of abiotic stress tolerance in plants is necessary, which explains why – apart from its academic interest – this is currently one of the most active research topics in plant biology.

The vast majority of wild plants and all major cultivated species, are highly sensitive to different abiotic stresses (Zhu, 2001; Lavor, 2013; Rejeb et al., 2015), notably to drought and salinity, although some (very few) wild taxa are adapted in nature to extremely harsh environments, such as arid (xerophytes) or saline (halophytes) habitats. It is well established that all plants, regardless of their tolerance to stress, activate the same series of basic, conserved reactions in response to abiotic stresses such as salinity or water deficit; these responses are

Abbreviations: Caro, total carotenoids; Chl a, chlorophyll a; Chl b, chlorophyll b; MDA, malondialdehyde; Pro, proline.

* Corresponding author.
E-mail addresses: akozminska@ogr.ur.krakow.pl (A. Kozminska), Mohamad.AlHassan@plantandfood.co.nz (M. Al Hassan), e.hanus@ogr.ur.krakow.pl (E. Hanus-Fajerska), mnaranjo@ibmcp.upv.es (M.A. Naranjo), mobosnea@eaf.upv.es (M. Boscaiu), ovicente@ibmcp.upv.es (O. Vicente).
[1] Present address: The New Zealand Institute for Plant & Food Research Limited (PFR), Mt. Albert Research Centre, Private Bag 92,169, Auckland, New Zealand, moalhas.

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based, for example, in the control of ion transport and ion homeostasis, the synthesis of specific compatible solutes for osmotic adjustment or the activation of antioxidant systems (Zhu, 2001; Flowers et al., 2010; Ariga et al., 2013; Flowers and Colmer, 2015; Song and Wang, 2015). This fact justifies the use of salt and drought-sensitive species, such as *Arabidopsis thaliana*, as models to explore the mechanisms of response to such abiotic stresses (Sanders, 2000; Zhu, 2001; Ariga et al., 2013; Rejeb et al., 2015). Yet, the relative efficiency of these responses varies widely among plant species, and the contribution of a particular response to the stress tolerance of a given species or group or related taxa remains generally unknown. Therefore, no single model can provide a general view of the mechanisms of abiotic stress tolerance in plants, the elucidation of which should be based on studies performed in different species.

Silene L. is one of the largest genera of flowering plants within the Caryophyllaceae family. The genus *Silene* comprises 43 sections and about 700 species, and the natural distributions of most of them lie throughout the northern hemisphere with two main biodiversity centres: the Mediterranean region, and the south-west Asian region. However, native species can also be found in North and South America, and in Africa (Bittrich, 1990; Kilic, 2009; Fawzi et al., 2010; Rautenberg et al., 2012). This genus has been traditionally included in many genetic and ecological studies and has a number of remarkable features. Firstly, *Silene* species vary widely in terms of their breeding systems and their ecology. Secondly, several members of this mainly Holarctic genus can be easily bred and have short life cycles, and are thus convenient for both, experimental and field studies; in fact, some species continue to be widely used in the fields of ecology and evolutionary biology. The genus has also been used for over a century as a model to understand the genetics of sex determination. Other studies carried out on *Silene* species include speciation, host-pathogen interactions, biological invasions, adaptation of some populations to heavy-metal-contaminated soils, metapopulation genetics, and organelle genome evolution (Bernasconi et al., 2009; Käfer et al., 2013; Fields and Taylor, 2014; Colzi et al., 2015; Hahn and Brühl, 2016). Notably, some members of the genus hold the distinction of harbouring the largest mitochondrial genomes ever identified (Rautenberg et al., 2012; Sloan et al., 2012a, 2012b). Genomic resources are now becoming increasingly available in *Silene*, which makes possible to undertake genetic, quantitative genetic and molecular studies in this genus. One of the strengths of *Silene* as a model system, compared with other classical model organisms, is the availability of a large number of previous ecological studies which encompass biotic interactions with sexually transmitted fungi, pollinators and herbivores (Bernasconi et al., 2009; Hahn and Brühl, 2016; Taiti et al., 2016). Even though the genus does not include halophytes or xerophytes, several *Silene* taxa stand out from other wild species and most crops for their relative resistance to abiotic stress; however, their mechanisms of adaptation to stress, at the physiological, biochemical and molecular levels, are still poorly understood. More specifically, information on the effects of salinity and water deficit on the growth and development of *Silene* plants is very limited.

In this study, we have analysed the responses to salinity and water deficit of four *Silene* species adapted in nature to different habitats. Our working hypothesis is that, when comparing related taxa, the more tolerant ones will activate more efficiently those specific stress responses that are relevant for the mechanisms of tolerance. Correlation of the relative tolerance to stress of the investigated species with the level in stressed plants of biomarkers characteristic of specific response pathways should allow distinguishing those responses that are important for tolerance, from those that are not. We have successfully used this strategy to investigate the mechanisms of tolerance to water deficit and salinity in other genera, such as *Limonium* (Al Hassan et al., 2017b; Monllor et al., 2018), *Juncus* (Al Hassan et al., 2016a, 2017a), *Plantago* (Al Hassan et al., 2014, 2016c) or *Phaseolus* (Al Hassan et al., 2016b; Morosan et al., 2017).

According to the ideas above, salt and water stress treatments were applied to the selected *Silene* species, under controlled greenhouse conditions. Growth parameters were determined in control and stressed plants, to estimate their relative degree of tolerance to each of the two stress factors – and the possible correlation with the characteristics of their natural habitats. We also measured the leaf contents of some biochemical markers associated with distinct stress responses: monovalent cations, photosynthetic pigments, osmolytes and antioxidant compounds, to establish the response reactions most important for tolerance to each type of stress, water deficit and salinity, in *Silene*.

2. Material and methods

2.1. Plant material

The four investigated *Silene* species were *S. vulgaris* (Moench) Garcke, *S. sclerocarpa* Dufour, *S. latifolia* Poiret, and *S. gallica* L. (Caryophyllaceae).

Silene vulgaris is an extremely variable species, occurring on sandy stands or in soils with a high percent of sand throughout Europe; it is frequent in the Mediterranean region, usually growing on coastal sands and rocks. *Silene sclerocarpa* is representative of the vegetation of semi-steppe shrublands in Middle Asia and the Mediterranean; the species is appropriate for xeriscaping, as it is relatively resistant to drought. *Silene latifolia* has a wide geographical range: Europe, including the whole Mediterranean region, West Asia, North Africa and North America, where it is an invasive species; it is present in foothills, mountains and subalpine areas, also in degraded zones and as a weed in cultivated land. *Silene gallica* is native to south and central Europe, it is present northwards up to Denmark, Poland and Russia, in western Asia and it can also be found in North Africa; in Australia, it is currently considered as an invasive species. *Silene gallica* usually grows in dry, non-saline habitats, such as dry meadows, but it is also present in wastelands or in arable land areas (Talavera, 1990).

Seeds of the aforementioned species, sampled in 'La Albufera' Natural Park, (Valencia, Spain), were provided by the 'Servicio Devesa-Albufera' of the city of Valencia, responsible for management of the Park.

2.2. Growth conditions and stress treatments

Seed germination, plant growth and stress treatments were performed under controlled greenhouse conditions: temperatures ranging between 17 °C and 23 °C, a long-day photoperiod (16 h light/8 h dark) with a light intensity of 130 μE m^{-2} s^{-1}, and humidity between 50 and 80%.

Seeds were sown in seed trays that contained a mixture of commercial peat and vermiculite (1:1). Seedlings were grown for four weeks before being transplanted into individual polyethene pots (Ø = 11 cm) with the same substrate and placed in plastic trays (12 pots per tray). During this period, plants were regularly watered with Hoagland's nutrient solution (Hoagland and Arnon, 1950). Water deficit and salt stress treatments were initiated one week after the plants were transplanted. Control plants were watered twice weekly with 1.5 L Hoagland nutrient solution per tray. Salt stress treatments were performed by adding NaCl to the nutrient solution, to final concentrations of 150 or 300 mM. Water stress treatments were initiated at the same time by completely ceasing irrigation; to follow the progressive decrease of soil water content, volumetric soil moisture in the pots was directly measured with a WET-2 sensor (Delta-T Devices, Cambridge, UK). Both stress treatments were extended for a three-week period.

2.3. Growth parameters

At the end of the treatments, all plants were harvested and the aerial parts were weighed individually on a precision balance. Since plants of the four species differed in size, to better compare the effects of salt