Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic ISSN 1842-4309

Not. Bot. Hort. Agrobot. Cluj 37 (2) 2009, 64-70

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Soil Requirements of Three Salt Tolerant, Endemic Species from South-East Spain

Antonio LIDÓN[1], Monica BOSCAIU[2], Francisco COLLADO[3], Oscar VICENTE[4]

[1] *Departamento de Química, Universidad Politécnica de Valencia, Spain; alidon@qim.upv.es*

[2] *Instituto Agroforestal Mediterráneo, CPI edificio 8E, Universidad Politécnica de Valencia Camino de Vera s/n, Spain; mobosnea@eaf.upv.es*

[3] *Oficina Técnica Devesa-Albufera, Ayuntamiento de Valencia, Spain; odevesa@valencia.es*

[4] *Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia, Spain; ovicente@ibmcp.upv.es*

Abstract

The edaphic requirements of three endemic species from SE Spain have been investigated. *Limonium dufourii* is a narrow endemic, critically endangered; *Thalictrum maritimum* and *Centaurea dracunculifolia* are SE Iberian endemics, considered vulnerable and near threatened, respectively. The three taxa inhabit the same type of saline habitats, salt marshes called "malladas". Several soil parameters (pH, electrical conductivity, soil chloride and sodium contents) were determined in sampling sites where plants of the selected taxa were present. In all the studied zones, soils are basic, with small differences in their pH. The presence of high amounts of chloride and sodium ions indicates sodic-alkaline soils, with NaCl of marine origin as the major salt. There are, however, clear differences in soil salinity, as revealed by electric conductivity (EC) measurements. The extension and size of the populations of the three taxa, as well as their degree of threat, correlate with the edaphic characteristics of their habitats. *Limonium dufourii*, by far the scarcest and most endangered species, was found only in the zones with the highest soil EC, where neither of the other two taxa were present. On the other hand, *C. dracunculifolia*, relatively frequent and with minimal risk of extinction, tolerates a wide range of variation of electric conductivity in the soil. *Thalictrum maritimum* shares some of the *C. dracunculifolia* habitats, those with relatively higher soil salinity, and shows higher salt tolerance under controlled greenhouse conditions.

Keywords: *Limonium dufourii, Thalictrum maritimum, Centaurea dracunculifolia*, halophytes, soil pH, electric conductivity, soil

Introduction

Salt tolerant taxa are known from many angiosperm families and are present in different types of ecosystems, from extreme dry to wetlands. A specific group of halophytes are those growing in salt marshes, in the past a well represented habitat along the coast in Valencia (SE Spain), but now very much affected by human activities, especially by urban development. The more frequent salt marshes in this region are the so-called "malladas", areas of variable size with a sandy superficial horizon but with a high amount of silt and clay in the deeper horizon, which retain water. They function as small endorheic basins, where salts accumulate after they are washed out from the neighbouring zones. This type of habitat shelters many halophytic plants, including endangered endemic species. Three of them, from different families and with different degrees of threat, were chosen for this study.

Limonium dufourii (Girard) O. Kuntze (family *Plumbaginaceae*) grows on calcareous rocks of marine cliffs and in coastal salt marshes. Its populations are disjunctive, small, with a very high risk of extinction. The species range was previously more extended, but some populations disappeared in the last decades and other suffered a drastic reduction (Crespo and Laguna 1993; Laguna *et al.,* 1994), and now it is found only in four geographic locations, one in the province of Castellón, and three in the province of Valencia (Navarro *et al.*, 2006). *Limonium dufourii* received the IUCN category of CR (critically endangered) and has been included in the Spanish catalogue of endangered plants (Crespo, 2004; VVAA, 2000).

Thalictrum maritimum Dufour (*Ranunculaceae*) is an endemic restricted to coastal areas in SE Spain, in the provinces of Castellón and Valencia, and is classified as VU (vulnerable) in the IUCN categories (Montserrat, 1986).

Centaurea dracunculifolia Dufour (*Asteraceae*) – considered by some authors (Bolòs *et al.*, 2000) at subspecies rank, as *Centaurea jacea* subsp. *dracunculifolia* (Dufour) A. Bolòs and O. Bolòs – is endemic in the SE Iberian Peninsula, with disperse populations in the region of Valencia, mostly in saline coastal grasslands. It is considered as NT (near threatened) according to the IUCN categories.

Given its critical status, *L. dufourii* has been recently the subject of diverse studies, including population censuses (Crespo and Laguna, 1993; Navarro *et al.*, 2006), *in vitro* cultures for *ex situ* conservation (Lledó *et al.*, 1993;

Martín and Pérez, 1995) or genetic studies (Palacios and González-Candelas, 1997; Palacios *et al*., 1999; Palop-Esteban, 2007). Several *in situ* conservation initiatives have been promoted, such as the declaration of two sites where it grows as flora micro-reserves, reintroduction of populations, or *ex situ* preservation of seeds in germplasm banks – for restoration and reinforcement of natural populations. However, there are no data on the soil requirements of this species, even though this is a fundamental aspect in management programmes of endangered plants. The other taxa, *T. maritimum* and *C. dracunculifolia*, as far as we know, have not been the subject of previous research.

Soil characteristics, such as pH or salt concentration, are extremely important for the growth and development of plant species. The pH exerts a strong effect on the physical, chemical and biological properties of the soil, and also on plant growth (Guigou *et al.*, 1989). In soils too acidic, there is a slowdown of biological activities (Spring *et al.*, 1993; Vidal, 1990), whereas in soils with a neutral or slightly basic pH the microbial activity is high; when the pH is too alkaline, some microbial processes are affected, such as nitrification, which is absent at pH above 9 (Chiang *et al*., 1983). The solubility of many compounds also depends on the pH; for example, phosphates are insoluble in very acidic or very basic soils, while metals such as Mn, Fe or Zn are more soluble at acidic pH. The pH can also affect the structure of the soils: when the ion Na^+ is very abundant and replaces Ca^{2+}, soils have a deficient structure with dispersion of colloidal material. All these effects have implications for plant growth, which leads to define an optimum pH for each species. Another important factor is the electrical conductivity, which depends upon the concentration of ions in the soil. All soils include many different soluble salts, in varying quantities; when the concentration of salts is too high and negatively affects plant growth, soils are considered "saline". Sodic soils are those with abundant Na^+ and alkaline those that have in addition a pH higher than 8.5.

The aim of this work was to study the edaphic requirements and the degree of salt tolerance of the three species mentioned above, in correlation with their different distribution patterns. Besides their scientific interest, the data obtained may provide useful information for the habitat restoration programmes that are currently under way in the area of study.

Material and Methods

Site description

The study area is located 15 km south of the city of Valencia, in the Natural Park of La Albufera, one of the most remarkable natural areas of the region, extending over 21,000 ha. La Albufera is the biggest lake in the Iberian Peninsula, with origin in an ancient marine gulf that was gradually closed by a littoral sandy strip, about 1200 m wide, known as "Devesa de La Albufera". Since 1990, the area is included in the list of wetlands of international importance of the Ramsar Convention; in 1991 it was declared as SPA (Special Protection Area) according to the EU directive on the Conservation of wild birds (79/409/CEE). In addition, the Park includes habitats and shelters species catalogued in the EU Directive Habitats (92/43/CEE) and is also included in the Geneva Protocol concerning specially protected areas in the Mediterranean.

The edaphic characteristics of "Devesa de La Albufera" are marked by three main factors: the gross granulometry, the saturation of the profile during the rainy seasons, and the high amount of salts. The combination of these factors or the predominance of one of them determines the presence of three types of soils: sandy soils Arenosols (Calcaric Arenosols or Gleyic Arenosols), Solonchaks gleyic and Gleysols calcareous (Sanchís *et al.*, 1986).

The coastal location and the absence of relief have a strong influence on the climate type, which belongs to the Mediterranean subtype (Csa) in the Köpen classification and is characterised by little climatic variation (Rubio *et al*., 1996). The yearly mean temperature is about 17.5°C; the warmest month of the year is August, with means of about 25°C, and the coldest month January, with means of 10°C. With thermal amplitude of about 12°C, the area belongs to the Thermomediterranean thermotype. The ombrotype is dry, with mean annual rainfall around 450 mm but very large variations among different years. The wettest season is autumn, especially October, and then spring (February to March), and the dry period extends over the summer months. The annual evapotranspiration is around 850 mm, which results in a negative hydric balance corresponding to a shortage of rains from May to September.

Soil sampling and plant populations

All soil samples were collected in the Natural Park of La Albufera, at sites where individuals of the species under study were present. In the case of *Limonium dufourii*, four defined populations were selected, and the number of individuals in each of them was counted. For the more abundant *Centaurea dracunculifolia* and *Thalictrum maritimum*, a total number of 21 collection sites were chosen, at points where *C. dracunculifolia* plants were growing, either alone or together with *T. maritimum* (no sites with *T. maritimum* alone were found). Identification of taxa and nomenclature follows Mateo and Crespo (2001).

Soil samples were collected using a 5-cm diameter auger, by punching at two depths: 0 – 10 cm and 10 – 20 cm. The soil was air dried and passed through a 2 mm sieve, previous to further analysis.

Soil analysis

Particle-size analysis was determined in the laboratory; sand particles were separated by sieving, and silt and clay using the differences in their settling velocities in the suspension, measured by a Bouyoucos hydrometer. The pH was measured in a soil:water (1:2.5) suspension, with a glass electrode pH-meter. Electrical conductivity (EC) was measured with a conductivity-meter in the saturation extract (Rhoades, 1982). Chloride in soil was determined in a 1:1 soil:water extract, after one hour in a rotating shaker, by automatic chloride titration with a Jenway Chloride Analyser. Sodium was determined in the saturation extract by flame photometry. Mean values of soil pH, conductivity, chloride and sodium content were calculated from five replicas for each sample. One way ANOVA was applied to test the significance of the differences between areas under study and between taxa.

In vitro growth of plants

Seeds of *Thalictrum maritimum* and *Centaurea dracunculifolia*, sampled in the wild in the studied area, were sown in seed trays, on a peat-based commercial gardening substrate, and maintained for two months in a growth chamber, with a photoperiod of 12 h, at 25°C (light) / 15°C (dark). Young plantlets were then transferred to plastic pots (9 cm diameter) and moved to the greenhouse, where treatments with NaCl solutions (75 mM, 150 mM and 300 mM) or water (for the controls) were started two weeks later. Salt treatments were prolonged for two additional months, and then plants were harvested and weighed on a precision balance. At the end of the treatments, soil pH and conductivity were determined in each pot of the sampled plants. *In vitro* experiments were not carried out with *Limonium dufourii*, since its "critically endangered" status precluded collection of seeds in the field.

Results and discussion

Limonium dufourii

The species was in the past abundant in the "Devesa de La Albufera". Information from the 50s and 60s indicate thousands to hundred of thousands of specimens in the area, where nowadays all studies indicate less than 100 plants (Navarro *et al.*, 2006), the most reduced and fragmented population of the species. In the present study, we have analysed four subpopulations of *L. dufourii*, localised in separated sites in the Natural Park, and determined the edaphic characteristics of each of these areas (Tab. 1.).

Zone "Cami Vell". This subpopulation consists of only five individuals, none of them in the juvenile stage; its size seems to be decreasing, since ten individuals were censed in 2005, and seven in 2006 (Navarro *et al.*, 2006). The plants are located at the border of a path, in the shadow of *Pinus halepensis*, surrounded by *Sporobolus pungens, Inula crithmoides* and *Pistacia lentiscus.*

Zone "Mallada del Fang". This is the best represented subpopulation, covering 8 m^2 and including about 40 plants, many of them in the juvenile stage – despite its location in the centre of a path. The community includes other halophytic species, such as Plantago crassifolia, Schoenus nigricans, Juncus maritimus and J. acutus.

Zone "Mallada El Saler". The plants, more than 20 individuals, cover about 8 m^2 and are situated at the transition between a saltmarsh ("mallada") – with *Limonium girardianum, Plantago crassifolia, Juncus acutus, Schoenus nigricans, Inula crithmoides, Elymus elongatus, Sporobolus pungens* – and scrubland – with *Pistacia lentiscus, Phillyrea angustifolia* and *Pinus halepensis.* More salt-resistant halophytic species, such as *Arthrocnemum macrostachyum* and *Juncus maritimus* are absent here, and the presence of shrubs and pines clearly indicates lower salinity than in the previous zone.

Zone "Racó de l'Olla". This is a new population established in 2002, by transplanting 176 individuals obtained by seed germination. In 2003 only 9 of these plants had survived, but the number increased to 16 in 2005 (Navarro *et al.*, 2006). At present, it extends over 2 m^2 and consists of more than 20 individuals, many of them juvenile. The plants are located in an ecotone area, at the transition between a grassland zone, dominated by *Phragmites australis*, *Juncus maritimus*, and *J. acutus*, and a saltmarsh with typical halophytic species, such as *Limonium girardianum, L. narbonense* and *Arthrocnemum macrostachyum.*

Although there is considerable variability in the different zones where *Limonium dufourii* was localised, in all of them the soil is clearly alkaline, with an average pH of 8.9 for the surface layer, or 9.2 in the second layer (10 – 20 cm deep). Electrical conductivity is also high in the root zone (0 – 20 cm), with average values of 8.5 dS·m^{-1} (0 –10 cm) and 18.5 dS·m^{-1} (10 –20 cm), indicating a high content of

Tab. 1. Soil characteristics (mean values) in the zones where the four selected subpopulations of *Limonium dufourii* are present. CE: soil coarse elements; EC_{se}: electrical conductivity in the saturation extract, at 25°C

Zone	Depth (cm)	CE (%)	Sands (%)	Silt (%)	Clay (%)	pH	EC_{se} (dS/m)	Cl^- (mg/kg)	Na^+ (mg/kg)
C. Vell	0 – 10	52.3	81.2	13.8	5.0	9.01	3.1	699	187
Fang	0 – 10	0.6	94.7	2.3	3.0	9.30	14.8	3456	1360
Fang	10 – 20	1.7	84.9	8.6	6.5	9.31	17.3	6007	1410
Saler	0 – 10	3.4	93.9	4.4	1.7	8.41	1.7	314	75
Saler	10 – 20	0.6	96.3	2.0	1.7	9.56	5.5	1099	364
R. Olla	0 – 10	4.5	95.0	3.0	2.0	8.86	14.3	4445	1310
R .Olla	10 – 20	24.5	70.9	21.6	7.5	8.59	32.8	5138	4090

Tab. 2. Characteristics of the sampling sites, and relative abundance of *Centaurea dracunculifolia* (*Cd*) and *Thalictrum maritimum* (*Tm*) plants

Mallada	Area (m²)	*Cd* + *Tm* plants (total nº)	Ratio *Cd:Tm*	"Only *Cd*" sites (nº)	"*Cd* + *Tm*" sites (nº)
1	14265	73	3.4 : 1	4	4
2	10990	32	7.3 : 1	1	2
3	8471	55	3.2 : 1	2	3
4	5539	64	11.5 : 1	2	–
5	4687	19	1.7 : 1	1	2

soluble salts, as it is also shown by Cl^- and Na^+ contents. As for the pH, the concentrations of these ions and the ratio sodium:chloride are higher in the deeper soil layer. Therefore, *L. dufourii* appears to be a salt-tolerant species, well adapted to alkaline and sodic soils. These data are in agreement with the degree of salt tolerance of other species accompanying *L. dufourii*: the most halophytic taxa were identified in those zones with higher EC_{se} ("Mallada del Fang" and "Racó de l'Olla"), while they are not present in "Mallada El Saler", the zone with the lower values of EC_{se} and ion contents.

Thalictrum maritimum and Centaurea dracunculifolia

The *T. maritimum* and *C. dracunculifolia* populations included in the present study were localised in five different, separated "malladas" in the Natural Park of La Albufera. These taxa have a much wider distribution than *L. dufourii*, with abundant specimens in the area under study; therefore, we do not detail the characteristics of each plant population, and refer only to the selected soil sampling sites. Tab. 2 shows the surface area of these "malladas" and, for each of them, the total number of *C. dracunculifolia* and *T. maritimum* plants counted at the sampling sites – from which the relative abundance of the two species (ratio *Cd:Tm*) was calculated – and the number of collection sites where *C. dracunculifolia* is present, either alone or together with *T. maritimum*.

The two species often grow together, but none of them was found in the zones where *L. dufourii* was localised. *Centaurea dracunculifolia* was present in all 21 selected sampling sites; *T. maritimum* was also present in 11 of them, but was not seen growing alone. In all collecting sites, *C. dracunculifolia* plants were more abundant than those of *T. maritimum*, although their relative numbers varied in the different zones, from 1.7-fold to 11.5-fold (Tab. 2.). Other taxa identified at the sites where *C. dracunculifolia* and *T. maritimum* grow together include typical halophytes with different degrees of salt tolerance (*Inula crithmoides*, *Aster tripolium*, *Schoenus nigricans*, *Juncus maritimus*, *J. acutus*), whereas in many sampling sites where only *C. dracunculifolia* was present, these halophytic species are absent.

The collected soil samples were used to determine several soil characteristics (pH, EC_{se}, Cl^- and Na^+ content). A summary of the results is shown in Fig. 1, which also

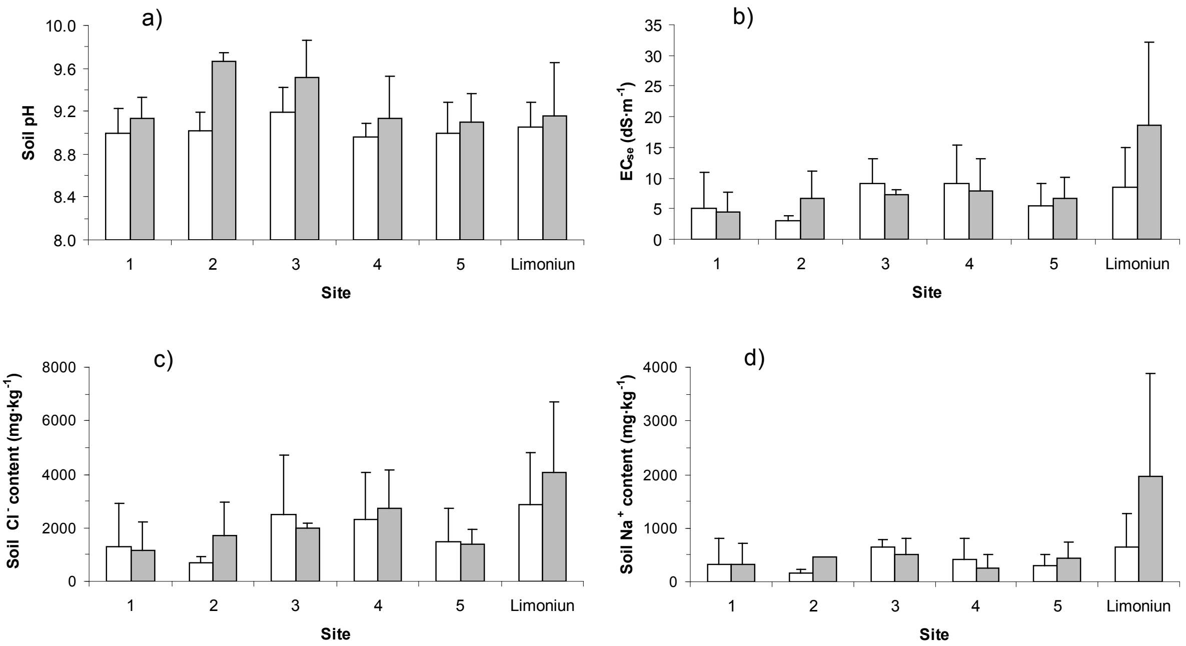

Fig. 1. Mean and standard deviation values of pH (a), electrical conductivity in the saturation extract (b), soil chloride content (c), and soil sodium content (d), in the studied areas, at two depths: 0 –10 cm (white bars) and 10 – 20 cm (shaded bars). In all panels, sites 1 to 5 correspond to the five "malladas" with *C. dracunculifolia* and *T. maritimum* plants, referred to in Tab. 2. The "*Limonium*" bars refer to the means (and SD) of all the samples collected at the *L. dufourii* sites referred to in Tab. 1.

includes, for comparison – and despite their high variability (see Tab. 1) – the means of the values obtained for *L. dufourii* zones.

Concerning soil pH measurements (Fig. 1a), analysis of variance revealed that there are not statistically significant differences between the sampling sites, neither at the surface, nor at the deeper layer, although the pH is always less basic at 0 –10 cm deep. In any case, all investigated zones have alkaline soils with relatively high pH, ranging from 8.59 to 9.88 and with average values of 9.0 to 9.3.

ANOVA of electrical conductivity data, on the contrary, showed that the zones inhabited by *Limonium dufourii* have significantly higher EC_{se} values than all the other sampling sites (Fig. 1b), especially at the subsurface layer (10 – 20 cm deep). Comparing the "malladas" with *C. dracunculifolia* and *T. maritimum* (sites 1 to 5), no significant differences in EC_{se} values were found at any of the two depths sampled. Very similar patterns of variation were observed when soil chloride and sodium contents were determined: the *L. dufourii* zone has significantly higher levels of both ions at the depth of 10 – 20 cm (Fig 1c, d), as compared to all the other sampling sites.

From these data, it can be concluded that the natural habitats of *Limonium dufourii* are more saline than those where *C. dracunculifolia* and *T. maritimum* grow; consequently, these two latter species could be considered as less salt-tolerant than the former. However, no direct comparison of salt tolerance between *C. dracunculifolia* and *T. maritimum* is provided by the results shown in Fig. 1. Therefore, mean values of the same soil parameters were calculated again, but this time grouping – on the one side – all the sampling sites, from the five "malladas", in which only *C. dracunculifolia* plants are present, and – on the other side – all those sites where both species were growing together. The results are shown in Fig. 2.

Here again, no differences in soil pH were found between the two groups of sampling sites, at any of the two depths analysed (Fig. 2a). However, electrical conductivity in the saturation extract (Fig. 2b), soil Cl^- content (Fig. 2c) and soil Na^+ content (Fig. 2d) are all significantly higher in those sites where *T. maritimum* is present, in the surface layer as well as in the deeper soil layer. These results confirm the wider range of soil salinity levels in the natural habitats of *C. dracunculifolia*, while *T. maritimum* is restricted to those zones with higher electrical conductivity and salt concentrations. Therefore, *T. maritimum* could be considered as more halophytic than *C. dracunculifolia*, as it appears to be adapted to higher salinity levels in nature.

Salt treatments under controlled conditions

To directly compare their sat tolerance levels, *C. dracunculifolia* and *T. maritimum* plants were grown from seeds, in a standard gardening substrate, and were treated in the greenhouse with increasing NaCl concentrations (0, 75, 150 and 300 mM). After two months, all plants treated with 300 mM NaCl have died; in the 150 mM NaCl treatments, survival rates were 83% for *T. maritimum* and 66% for *C. dracunculifolia*, while all plants survived the control

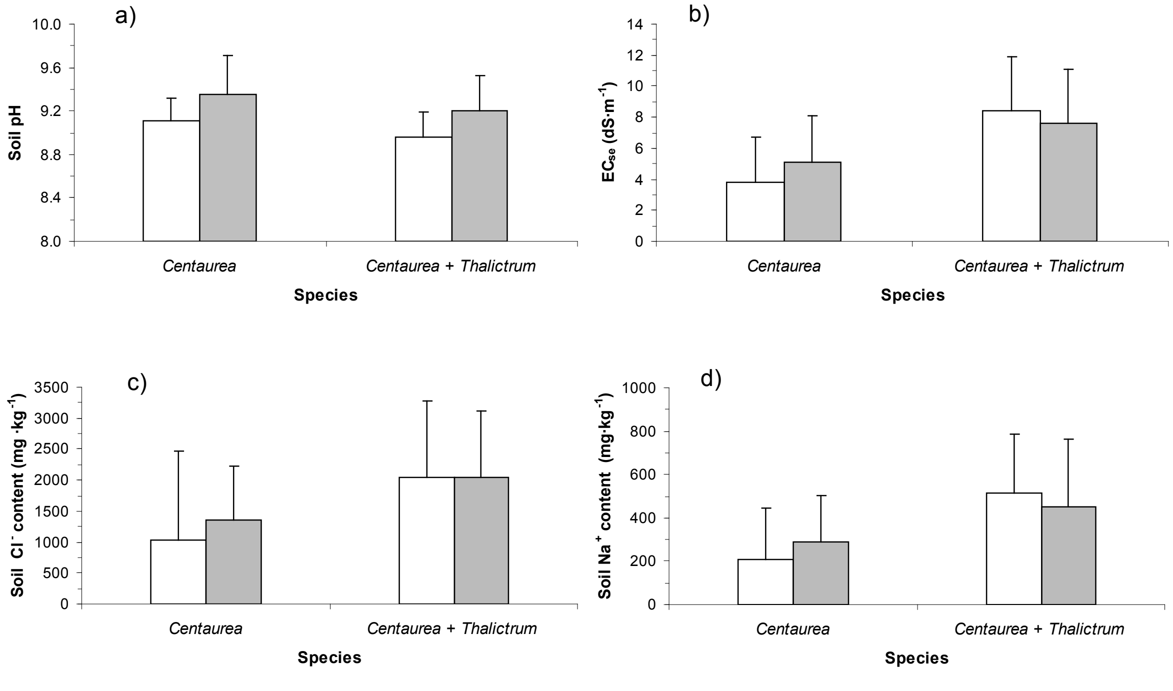

Fig. 2. Mean and standard deviation values of pH (a), electrical conductivity in the saturation extract (b), soil chloride content (c) and soil sodium content (d), at two depths: 0 –10 cm (white bars) and 10 – 20 cm (shaded bars). Bars labelled as "*Centaurea*" and "*Centaurea + Thalictrum*" refer to sampling sites where only *C. dracunculifolia* or both, *C. dracunculifolia* and *T. maritimum,* were present, respectively.

Tab. 3. pH and electrical conductivity in the saturation extract (EC_{se}) (means ± SD) in the pots where plants had been treated for two months with the indicated NaCl concentrations

NaCl (mM)	pH		EC_{se} (dS/m)	
	C. dracunculifolia	*T. maritimum*	*C. dracunculifolia*	*T. maritimum*
0	5.89 ± 0.10	5.82 ± 0.14	0.42 ± 0.07	0.44 ± 0.08
75	6.11 ± 0.11	5.98 ± 0.09	2.80 ± 0.13	2.78 ± 0.15
150	5.90 ± 0.13	5.93 ± 0.04	5.97 ± 0.17	4.90 ± 0.18
300	5.74 ± 0.01	5.86 ± 0.04	10.35 ± 0.30	9.76 ± 0.21

and the 75 mM treatments. At the end of the salt treatments, all remaining plants were collected and individually weighed in an analytical balance, to determine the effect of salt stress on plant growth (Fig. 3.), while pH and electrical conductivity were determined in the substrate left in the corresponding pots (Tab. 3.).

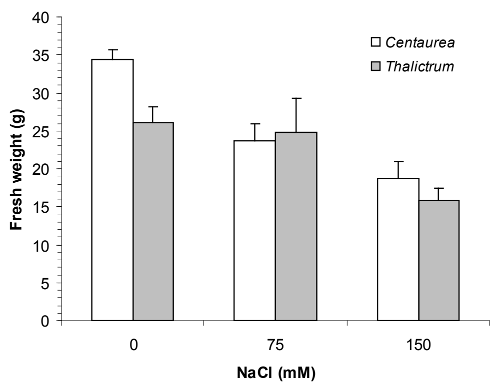

Fig. 3. Effect of salt treatments on plant growth. Fresh weight (means with SD) of *Centaurea dracunculifolia* (white bars) and *Thalictrum maritimum* (shaded bars) plants, after two month treatments with the indicated NaCl concentrations.

As shown in Fig. 3., both species grew optimally in the absence of salt, but their vegetative development was inhibited by more than 40% after two month treatments at 150 mM NaCl, as indicated by the decrease in plant fresh weight; this salt concentration generates in the pots electrical conductivities similar to the average EC_{se} values measured in the field (Tab. 3.). However, *Centaurea dracunculifolia* was significantly affected already by 75 mM NaCl, conditions which did not decrease the fresh weight of *Thalictrum maritimum*. These data, together with its higher survival rate at 150 mM NaCl, suggest that *T. maritimum* is more salt tolerant than *C. dracunculifolia*, thus supporting the results of the field work.

It is also worth mentioning that the commercial substrate used in the greenhouse has an acidic pH, around 6, much lower than the alkaline pH of the soil in the natural habitats of the studied taxa; this difference, however, does not seem to affect their growth.

Conclusions

The edaphic characteristics of the natural habitats of three endemic species from SE Spain (*Limonium dufourii*, *Thalictrum maritimum* and *Centaurea dracunculifolia*), have been investigated, in correlation with their distribution patterns and degree of vulnerability. Individuals of these taxa were localised in several salt marshes (called "malladas"), in the Natural Park of La Albufera, near the city of Valencia.

The soils in all the studied zones can be classified as saline and alkaline, considering their high pH (above 8.5) – rather constant in different sampling areas – and their high, but more variable, NaCl concentrations. Chloride and sodium ions accumulate in the soil due to the characteristics of the "malladas": hydromorphism, vicinity to the sea, and presence of underground waters at the interface freshwater–saltwater.

There is a clear correlation between soil salinity and the extension and size of the populations of the three taxa, as well as with their degree of threat. *Limonium dufourii*, by far the scarcest and the most endangered of the three species, is present in areas with the highest (average) levels of salinity. *Centaurea dracunculifolia*, relatively frequent and with the minimal risk of extinction, withstands the widest range of salt concentrations in the soil, although below those of *L. dufourii* habitats. Finally, *T. maritimum*, with intermediate levels of vulnerability, shares some areas with *C. dracunculifolia*, those with relatively higher salinity, but is not so abundant and was not found growing alone.

It appears that the reintroduction of these species in natural habitats – in the frame of restoration programmes of the "malladas", and regarding soil characteristics – will mostly depend on their degree of salt tolerance (*L. dufourii* > *T. maritimum* > *C. dracunculifolia*). However, other factors, such as species competition, may be critical to limit or exclude the presence of a taxon in a specific area. For example, *T. maritimum* is not present in the field below a certain salinity level, but does not necessarily require these salt concentrations since it grows optimally in the absence of salt under controlled greenhouse conditions.

Acknowledgments

This work has been partly supported by the Spanish Ministry of Science and Innovation, grant CGL2008-00438/BOS.

References

Bolòs, O., J. Vigo, R. Masalles and J. Ninot (2000). Handbook of Catalonian Flora (in Catalan). Editorial Pòrtic, Barcelona.

Chiang C., B. Soudi and A. Moreno (1983). Soil nitrogen mineralization and nitrification under Moroccan conditions. Nutrient balances and the need for fertilizer in semi-arid and arid regions. Proc. 17th Coll. Int. Potash Inst. Rabat and Marràqueix (Marroc) p. 129-139.

Crespo, M. B. (2004). *Limonium dufourii* (Girard) Kuntze, p. 352–353. *In:* A. Bañares-Baudet, G. Blanca, J. Güemes-Heras, J. C. Moreno-Saiz and S. Ortiz (Eds.). Atlas and Red Book of the Spanish vascular flora (in Spanish). Ministerio de Medio Ambiente, Madrid.

Crespo, M. B. and E. Laguna (1993). New localities of *Limonium dufourii* (Girard) O. Kuntze (*Plumbaginaceae*) (in Spanish). Anales del Jardín Botánico de Madrid 51:154-155.

Guigou B., B. Thonnelier, B. Duzan, and B. Félix-Faure (1989). Making use of soil analyses (in French) nº 134, p. 3-88. Purpan, Toulouse.

Laguna, E., A. Aguilella, J. L. Carretero, M. B. Crespo, R. Figuerola and G. Mateo (1994). Red Book of rare, endemic or endangered vascular flora of the Valencian Community (in Spanish). Conselleria de Medi Ambient, Generalitat Valenciana, Valencia.

Lledó, M. D., M. B. Crespo and J. B. Amo-Marco (1993). Preliminary remarks on micropropagation of threatened *Limonium* species (*Plumbaginaceae*). Botanical Gardens Micropropagation News 1:72-74.

Martín, C. and C. Pérez (1995). Micropropagation of five endemic species of *Limonium* from the Iberian Peninsula. Journal of Horticultural Science 70:97-103.

Mateo Sanz, G. and M. B. Crespo (2001). Handbook for identification of Valencian flora (in Spanish). 2nd ed. Gómez Coll, Valencia.

Montserrat, P. (1986). Genus *Thalictrum* (in Spanish) p. 387-401. In: S. Castroviejo (Ed.) Flora Ibérica vol. 1. Real Jardín Botánico de Madrid, Madrid.

Navarro, A. J., I. Ferrando and E. Laguna (2006). Census and extinction risk of the Valencian endemic *Limonium dufourii* (Girard) Kuntze (in Spanish). Toll Negre 8:38-50.

Palacios, C. and F. González-Candelas (1997). Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Molecular Ecology 6:1107-1121.

Palacios, C., S. Kresovich and F. González-Candelas (1999). A population genetic study of the endangered plant species *Limonium dufourii* (*Plumbaginaceae*) based on amplified fragment length polymorphism (AFLP). Molecular Ecology 8: 645-657.

Palop-Esteban, M., J. G. Segarra-Moragues and F. González-Candelas (2007). Historical and biological determinants of genetic diversity in the highly endemic triploid sea lavender *Limonium dufourii* (*Plumbaginaceae*). Molecular Ecology 16:3814-3827.

Rhoades, J. D. (1982). Cation exchange capacity, p. 149–157. In: A. L. Page (Ed.). Methods of soil analysis, part 2. 2nd ed. Agronomy Monograph nº 9. ASA and SSSA. Madison, WI.

Rubio, J. L., J. Sánchez and J. Forteza (1996). Soil map of the Valencian Community. Valencia (722) Project LUCDEME (in Spanish). Generalitat Valenciana, Consellería de Agricultura y Medioambiente. Valencia.

Sanchís, E., J. L. Rubio and J. Mansanet (1986). Soils and vegetation in Mount Dehesa de La Albufera (Valencia) (in Spanish). Revista de Agroquímica y Tecnología de Alimentos 26:435 -450.

Spring, J. L., P. Chapuis, C. Evéquez, G. Girardet, J. P. Ryser, C. Schmid, R. Terrethaz, M. Thentz and R. Vanetti (1993). Fertilisation of fruit trees, kiwies and shrubs. 1st part (directives) (in French). Rev. Suisse Vitic. Arboric. Hortic. 25 (3):189 -199.

Vidal, M. (1990). Soil acidity and whitewashing necessity (in Spanish). Agricultura: Revista agropecuaria 59:62-63.

VVAA (2000). Red list of the Spanish vascular flora (assessment according to UICN categories) (in Spanish). Conservación Vegetal 6 (Suppl.):11-38.