Salinity and germination of annual *Melilotus* from the Guadalquivir delta (SW Spain)

T. MARAÑÓN, L. V. GARCÍA and A. TRONCOSO

Instituto de Recursos Naturales y Agrobiología, CSIC, Apdo. 1052, E-41080 Sevilla, Spain

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Abstract

The germination response to NaCl treatments has been studied in *Melilotus* seed populations collected from saline and non-saline soils in the Guadalquivir delta.

The rank orders for salt tolerance and seed weight were the same in the three *Melilotus* species living in this area: *Melilotus messanensis* > *M. segetalis* > *M. indica*.

Within the species, differences in germination response to salinity were found in *M. indica* (6 populations) and *M. segetalis* (8 populations). The relationship between salt tolerance during germination and salinity of maternal habitat is discussed.

Introduction

When seeds are moistened with a saline solution their germination is affected by the high internal concentration of toxic ions and the low external water potential (Redmann, 1974). These interactions of specific and osmotic effects induce both a reduction in the number of seeds germinated and a retardation in the speed of germination (see review by Ungar, 1978).

Salt tolerance during the germination stage is critical for a plant growing in a saline soil. The heritability of this trait has been demonstrated in several species: for instance Allen *et al.* (1985), using mass selection in alfalfa (*Medicago sativa*), obtained an increase from 3% to 86% in germination at -1.3 MPa (310 mM) NaCl, after 5 generations.

Seed populations from plants living in saline soils would be expected to exhibit salt tolerance during the germination stage, as a result of natural selection (*e.g.* Bazzaz, 1973). These naturally selected salt-tolerant genotypes have a potential value as plant genetic resources for saline areas.

In this paper we study the effects of NaCl treatments on the germination of seeds from 15 popu-

lations of three *Melilotus* species: *M. segetalis* (8), *M. indica* (6) and *M. messanensis* (1), sampled on a salinity gradient in the Guadalquivir delta (S.W. Spain).

Materials and methods

Salt marsh environment

The Guadalquivir salt marsh was formed by fluvial sediments filling a coastal lake during the Holocene; currently it occupies an area of *ca.* 1,500 km². The northern part has been transformed into rice fields, whereas the southern area (*ca.* 300 km²) is protected within the boundaries of the Doñana National Park. Because of its importance as a reserve of plant genetic resources, it has been included as a salt-marsh site in the European network of Biogenetic Reserves (Dikjema, 1987).

The climate is Mediterranean, with average annual rainfall of 600 mm and average annual temperature of 18°C. The soils, developed from clays and silts, are calcareous, saline-alkaline, with sodium and chloride ions dominant. The water and salinity regime is highly seasonal: the marsh is

flooded, and salinity low, during winter and spring, but dry and hypersaline in summer.

Plants are grazed, often heavily, by free range livestock and wild herbivores: red deer, fallow deer, hare and goose.

Three *Melilotus* species (*M. segetalis*, *M. indica* and *M. messanensis*) are frequent in "vetas" (upper sites of the salt marsh), and river banks, and in grasslands and roadsides in the drained areas.

Indehiscent fruits containing 1 or 2 seeds are borne in racemes. They were collected from different sites in the Guadalquivir delta during May–June 1987, air-dried and stored at room temperature until the germination experiments were carried out. Average seed weight was determined for each population weighing individually a sample of 50 seeds.

Germination experiments

Scarified seeds were placed in Petri dishes, on filter paper moistened with 5 ml of the treatment solution, and randomly located, in the dark (wrapped in aluminium foil), in a growth chamber with alternating temperatures: 25°C (16h) and 15°C (8h). The treatment solutions were: distilled water (control), 10, 50, 100 and 200 mM NaCl, that simulate the gradient of salinity in the soil saturation extract (Table 1). Four replicates of 25 seeds were used for each treatment.

Germinated seeds (radicle having emerged from seed coat) were counted every 2 days until the 20th day. The germination experiments were carried out

throughout August/November 1987 and April/May 1988.

A mixed between-within factorial design ANOVA (Dixon, 1985) was applied, using two grouping factors (population and treatment), one trial-factor (time) and one dependent variable (relative germination percentage from control). When the ANOVA null hypothesis was rejected, the Duncan multiple range test was used to compare means.

Soil analysis

Soil samples (depth 0–10 cm) were collected from each site and analyzed according to standard procedures for saline soils (Richards, 1954). Salinity (sodium, calcium, chloride and sulphate ions), electrical conductivity (EC) and pH were measured in saturation extracts (Table 1). The osmotic potential (OP) of saturation extracts was estimated from Campbell's equation (Campbell, 1948). The condosity (NaCl concentration having the same EC that the soil saturation extract at 25°C) was spline-interpolated from Weas (1973). Particle size distributions were determined by the hydrometer method (Gee and Bauder, 1986).

Results and discussion

Habitats

Seed populations of *Melilotus* were collected

Table 1. Habitat characteristics of *Melilotus* populations in the Guadalquivir delta

Site no.	Habitat type	Soil saturation extract			Ionic ratios		Texture
		E.C. (dS/m)	O.P. (MPa.10 ⁻³)	Condosity (mol.10 ⁻³)	Na ⁺ /Ca ²⁺	Cl ⁻ /SO ₄ ²⁻	
1	Roadside	0.93	–33	8	2.14	0.96	Clay
2	Roadside	0.95	–34	8	0.43	0.32	Clay
3	Upper-marsh grassland	1.22	–44	10	2.02	0.40	Silty clay
4	Upper-marsh grassland	1.44	–52	12	3.85	0.66	Sandy loam
5	Upper-marsh grassland	1.91	–69	17	1.56	3.09	Loam
6	Marsh grassland	3.24	–117	29	2.13	3.57	Clay
7	Marsh grassland	4.94	–178	45	1.42	3.55	Silty clay loam
8	Roadside, ditch	9.04	–325	84	3.26	0.22	Clay
9	Shell-sand deposit	9.39	–338	88	1.16	16.30	Sandy loam
10	Marsh grassland	13.43	–483	126	5.71	10.66	Clay
11	River bank	26.01	–936	257	7.78	4.28	Silty clay loam

from a variety of habitats in the Guadalquivir delta (Table 1). In six sites, where the soil conductivity was over 3 dS/m (up to 26 dS/m), plants were living under saline conditions. Three of these saline sites were located in marsh grasslands, one in the tidal-fluvial zone, one in a shell-sand elevation ("veta") and one in the soil removed from a ditch. The other five sites were non-saline, upper grasslands or gravel roadsides.

Sodium is the main cation in the saline soils (sites 6–11), except in site 9, where the seashell-enriched sediments have a high calcium and magnesium content. The sandy texture of this soil (72% sand fraction) contrasts with the predominantly fine textured soils (less than 5% of sand) in the marsh grassland (Table 1).

Seed size

The rank order of seed size was: *M. messanensis* > *M. segetalis* > *M. indica* (Table 2). All *M. indica* fruits examined were single-seeded, whereas 1–44% double-seeded fruits were found in *M. segetalis* and *M. messanensis* populations. Estimated seed loss (predated by weevils) was lower in *M. indica* (0–10% empty pods) than in *M. segetalis* (7–65%) or *M. messanensis* (27–44%).

No significant within-species difference (using the t-test) was found between the average seed weight from non saline (sites 1–5) and saline (sites 6–11) habitats for *M. indica* or *M. segetalis* (Table 2).

Table 2. Seed weight mean (N = 50) and standard error (between brackets) of *Melilotus* populations from non saline (NS) and saline (S) habitats in the Guadalquivir delta. Site numbers as in Table 1

Site	Seed weight (mg)		
	<i>M. indica</i>	<i>M. segetalis</i>	<i>M. messanensis</i>
1 (NS)		1.61 (0.05)	4.31 (0.32)
2 (NS)	1.45 (0.05)	2.54 (0.07)	
3 (NS)		1.79 (0.05)	
4 (NS)	1.12 (0.03)		
5 (NS)	0.87 (0.03)		
6 (S)		1.60 (0.06)	
7 (S)		2.06 (0.08)	
8 (S)	1.57 (0.05)	2.10 (0.04)	
9 (S)	1.49 (0.04)		
10 (S)	1.27 (0.04)	1.54 (0.05)	
11 (S)		2.10 (0.06)	

Germination response to NaCl

a) *Among species.* The general germination response to increasing NaCl concentrations is retardation and reduction in the germination percentage (Figure 1 and Table 3).

Of the three *Melilotus* species, *M. messanensis* is the most salt-tolerant during the germination stage, with no significant reduction from control when moistened with 200 mM NaCl. *M. segetalis* seeds tolerate 100 mM NaCl but their germination is reduced by 200 mM NaCl. Seed populations of *M. indica* are sensitive to 50 mM or even (2 populations) to 10 mM NaCl (Table 3).

The three *Melilotus* species can be ordered in a rank of salt tolerance during germination — *M. messanensis* > *M. segetalis* > *M. indica* — that parallels the order for seed weight (Table 2). González-Murúa *et al.* (1985) suggested that a greater amount of stored food reserves (in herbaceous legume seeds) might contribute to osmoregulation and protection from salt toxicity.

A significant positive correlation was obtained between average seed weight and germination percentage (relative to control) after 20 days at 200 mM NaCl, for the whole data set ($n = 15$, $r = 0.82$, $P = 0.00$), however, no significant correlations were found within the species, *M. indica* ($n = 6$, $r = -0.18$, $P = 0.68$) and *M. segetalis* ($n = 8$, $r = 0.31$, $P = 0.40$).

Sano (1977) has estimated 90% self fertility for small flowered (2–2.5 mm) *M. indica* and 20% for large flowered (5–7 mm) *M. segetalis*, using hand pollination in a greenhouse (corolla size from Valdés *et al.*, 1987). Field pollination in the study area has been observed only in the latter species (T.M. pers. obs.). A higher outcrossing rate in *M. segetalis* might speed up the salt tolerance differentiation.

b) *Within species.* Differences in the germination response to NaCl have been shown among populations of *M. indica* seeds (Table 3). According to the Duncan multiple comparison test, three response types can be distinguished: 1) sensitive to even low values of salinity, 10 mM NaCl (populations from sites 2 and 4, average EC = 1.2 dS/m); 2) tolerant of low values but sensitive to 50 mM NaCl (populations from sites 5, 8 and 10, average EC = 8.1 dS/m); 3) moderately tolerant to 50 mM

Fig. 1. Germination response of *Melilotus* species to NaCl treatments.

Table 3. Germination response of 15 *Melilotus* populations to 10, 50, 100 and 200 mM NaCl, expressed as relative percentage to control (absolute number between brackets). Populations are named by species initial and site number (Table 1). Identical letters at the right of the percentage indicate non-significant difference ($P < 0.05$) by the Duncan multiple comparison test

Population	NaCl concentration (mM)				
	0 (control)	10	50	100	200
Mi-2	100(96)c-f	54.6b	16.5a	22.7a	17.0a
Mi-4	100(65)c-f	40.0b	10.8a	10.8a	9.0a
Mi-5	100(94)c-f	78.7b-e	28.7a	8.5a	25.0a
Mi-8	100(82)c-f	89.0c-f	22.0a	4.9a	9.0a
Mi-9	100(57)c-f	98.3c-f	52.6b	28.1a	29.0a
Mi-10	100(70)c-f	74.3b-d	31.4a	15.7a	21.0a
Ms-1	100(86)c-f	76.7b-e	90.7c-f	72.1b-d	37.2ab
Ms-2	100(82)c-f	114.6ef	100.0c-f	95.1c-f	45.5b
Ms-3	100(69)c-f	97.1c-f	108.7ef	88.4c-f	42.7b
Ms-6	100(82)c-f	98.8c-f	95.1c-f	63.4b	50.5b
Ms-7	100(56)c-f	96.4c-f	116.1f	105.4d-f	61.5b
Ms-8	100(71)c-f	108.5ef	85.9c-f	104.2d-f	41.4b
Ms-10	100(91)c-f	83.5c-f	67.0bc	52.8b	36.4a
Ms-11	100(86)c-f	97.9c-f	104.2d-f	83.3c-f	43.8b
Mm-1	100(67)c-f	88.1c-f	85.1c-f	76.1b-d	82.0b-e

NaCl (populations from sites 5, 8 and 10, average EC = 8.1 dS/m); 3) moderately tolerant to 50 mM NaCl, although sensitive to 100 and 200 mM NaCl (population from site 9, EC = 9.4 dS/m).

Seed populations of *M. indica* exhibiting the most sensitive response come from non-saline soils with sandy texture (sites 2 and 4). On the other hand, the seed population with a moderate salt tolerance was collected from an isolated saline habitat (site 9), with a mixture of sodium, calcium and magnesium ions (Table 1).

found at 10 and 50 mM NaCl treatments (Table 3). Higher NaCl concentrations (100 and 200 mM) produced a more general reduction in germination percentage for the six *M. indica* populations.

Bazzaz (1973) found differences in the germination response to salinity among three ecotypes of *Prosopis farcta* (legume) from habitats varying in soil salinity. In general, Ungar (1978) considers that salt tolerance in seed germination is closely related to the salinity conditions in which plant populations are growing. A differentiation in salt tolerance during germination can also be hypothesized for *Melilotus indica* populations living in saline habitats in the Guadalquivir delta.

On the other hand, *M. segetalis* seed populations

did not show a significant reduction in germination for either 10 or 50 mM NaCl, and only two of them (from sites 6 and 10) were sensitive to 100 mM NaCl. This less tolerant populations correspond to saline habitats (average EC = 8.3 dS/m), whereas the other, more tolerant, six populations come from saline (3) and non-saline (3) habitats (average EC = 7.2 dS/m).

The response of *M. segetalis* populations to salt treatments is more uniform than in *M. indica*, and there is no clear relation with habitat salinity. Is it a case of a pre-adapted salt tolerant genotype of wide distribution?

The only *M. messanensis* population tested was tolerant (not significantly different from control) to 10, 50, 100 and 200 mM NaCl.

The germination response to NaCl concentrations is a useful, fast laboratory test for evaluation of salt tolerance, but it must be borne in mind that salt tolerance in field conditions depends upon many plant, soil, water and environmental variables (Maas and Hoffman, 1977). In fact, salt tolerance during plant growth is frequently not correlated to germination (e.g. West and Taylor, 1981) and needs to be evaluated in these *Melilotus* populations.

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