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Germination of *Atriplex halimus* Linnaeus, 1753 (Caryophyllales Chenopodiaceae) in North West Algeria

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ABSTRACT In arid and semi-arid ambients, soil salinity is a constraint for the development of plants and a threat for balanced diet. Current data in the Mediterranean basin report up to 16 million hectares of salt soil, 3.2 million of which in Algeria. Germination in vitro of seeds of *Atriplex halimus* Linnaeus, 1753 (Caryophyllales Chenopodiaceae) in both synthetic media (nutrient agar, and Mueller Hinton) reached rates of 80% at 25 °C and 50% at 5 °C. The taxon shows a good resistance to salt; because of high salinity treatments (500 to 600 meq/l), there is a delay in germination but not complete inhibition of the process.

KEY WORDS *Atriplex halimus*; germination, salinity; North West Algeria.

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INTRODUCTION

From the physiological point of view, germination is a process that translates the passage of the slow life of a seed to active life in the optimum conditions for germination. Several Authors (Côme, 1970; Mazliak, 1982; Suszka et al., 1994) divide the process of germination in two phases including:

- a first phase of entry of water into the seed (imbibition) which is accompanied by a resumption of intense metabolic activity;
- a second phase of turgidity of the seed which induces root elongation and therefore germination sensu stricto.

But all these steps occur only if certain intrinsic (conservation of the power of germination, lack of inhibition) and extrinsic (temperature, humidity, ventilation, and, sometimes, light) factors come together.

Given the importance of the germinal phase for the later stages of growth and development of any plant species, it is essential to study the germ behavior under various environments conditions. If some works have addressed the germination process of *Atriplex halimus* Linnaeus, 1753 (Caryophyllales Chenopodiaceae) (Belkhodja & Bidai, 2004), however little work has been done on the rootlets in synthetic culture media. This has led us to approach throughout this work:

- Seed germination of *Atriplex halimus* in different culture media
- Germination of seeds of *Atriplex halimus* and salt stress.

I. GERMINATION IN DIFFERENT CULTURE MEDIA

Material and methods

As plant material used in this experimental work, we employed seeds of *Atriplex halimus* collected in the fields.

The laboratory equipment was composed of Boxes of Petri dishes, oven set at 25 °C and 35 °C, sterile forceps, bleach, 95% ethyl alcohol, nutrient agar flask, flask of Mueller Hinton, cotton wool, distilled water.

Each culture medium was prepared from a dehydrated medium (20 g/l), incorporated in one litre of distilled water; all being heated to boiling. The medium was then put in autoclaved bottles for 20 minutes at 120 °C (Table 1).

Disinfection of the plant material is always difficult and uncertain. The degree of infection of tissue on the surface is highly variable.

The method of disinfection of seeds was done according to the following protocol:

- washing under running water,
- immersion in a 80% solution of chlorine bleach for four minutes,
- rinsing with sterile distilled water for thirty seconds,
- soaking of seeds in 95% ethyl alcohol for ten seconds,
- three washes with sterile distilled water.

Then culture medium was liquefied by bath-marie and poured (in supercooling) in Petri dishes between two benzene becs.

The boxes were kept open to prevent the formation of water droplets on the cover. After disinfection, ten seeds were placed in Petri dishes by sterilized pliers. The boxes were then closed to avoid contamination.

All manipulations took place under hood (in sterile conditions). As controls, seeds were seeded on a cotton ball moistened with distilled water.

The number of repetition was six for each medium. The boxes were then placed at three different temperatures: 5 °C (refrigerator), 25 °C (room temperature) and 35 °C (oven) to test the effect of temperature on germination.

Results and interpretations

Germinated seeds were counted per week regularly, taking as criteria of germination the envelopes pierced by the radicle; this allowed us to plot germination curves describing the course of germination, cumulative over time.

In our experience, we had a germination rate appreciable with a percentage of 80% (nutrient agar), 70% (distilled water and Mueller Hinton) at room temperature. But at cold temperature, germination percentage was lower reaching 40% (for Mueller Hinton), 50% (agar), and 70% (in distilled water) (see Tables 2, 3; Figs. 1, 2).

Nutrient agar		Mueller Hinton	
Peptone	15 g	beef infusion solids	300 g
meat extract	2 g	casein hydrolysate	10.9 g
NaCl	5 g	Starch	1.5 g
Agar Agar	15 g	Agar	17 g
Distilled water	1000 ml	Distilled water	1000 ml
pH	7.6-7.8	pH	7.4

Table 1. Chemical composition of two culture media.

II. GERMINATION AND SALT STRESS

Halophytes develop naturally in strongly saline environments and their seeds do appear to express a certain tolerance to salt at the germination stage (Binet, 1988).

In Halophytes seeds germination in saline conditions is variable and species specific (Ungar, 1978).

We have undertaken this work to determine the critical response to germination of *Atriplex halimus* in a saline environment since its seeds have a great potential for germination.

Material and methods

The Petri dish (es) used were sterile boxes of 19 cm in diameter and 3 cm thick. In each of the boxes, were placed ten seeds on cotton balls soaked in saline solutions at different concentrations. Each treatment included five boxes containing 10 seeds each. Petri dishes were kept with main parameters (temperature, photoperiod, humidity) helping to ensure a good environment relatively favourable to germination.

The seeds were selected based on size and health status. They were separated manually from fruit valves, then sterilized according to the following protocol:

- washing under running water,
- soaking in alcohol at 70% for 25 seconds,
- soaking in 80% bleach solution for 15 minutes,
- three washes with sterile distilled water, 10 minutes each.

	1st week		2nd week		3rd week		4th week	
	Number	%	Number	%	Number	%	Number	%
Distilled water	2	20	4	40	6	60	7	70
Nutrient agar	3	30	4	40	6	60	8	80
Mueller Hinton	2	20	3	30	5	50	7	70

Table 2. Number of *Atriplex halimus* seeds germinated at 25 °C.

	1st week		2nd week		3rd week		4th week	
	Number	%	Number	%	Number	%	Number	%
Distilled water	0	0	0	0	6	60	7	70
Nutrient agar	1	10	2	20	4	40	5	50
Mueller Hinton	0	0	1	10	2	20	4	40

Table 3. Number of *Atriplex halimus* seeds germinated at 5 °C.

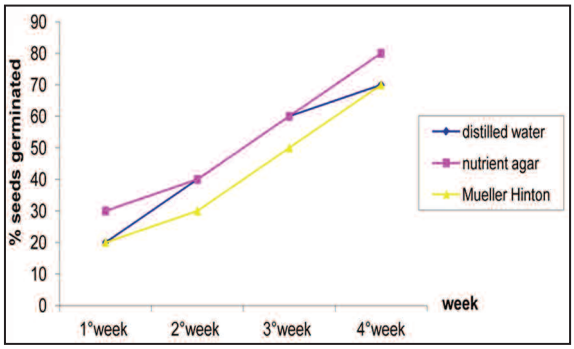

Figure 1. *Atriplex halimus* seed germination in various culture media at 25 °C.

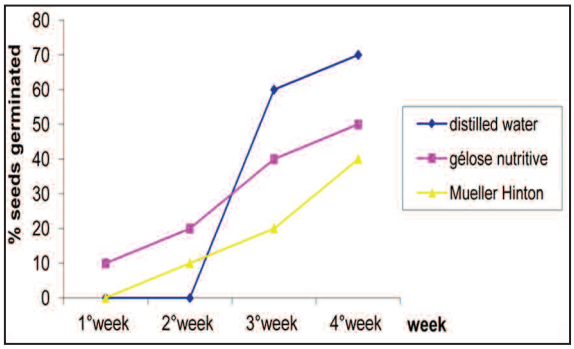

Figure 2. *Atriplex halimus* seed germination in various culture media at 5 °C.

By mixing equal volumes of two different salts: Sodium chloride (NaCl) and Calcium chloride ($CaCl_2$) ($VNaCl = VCaCl_2$), prepared in one litre of distilled water, we prepared six different concentrations (100, 200, 300, 400, 500, 600 meq.1^{-1}). Distilled water was employed as control.

Results and interpretations

Tolerance of plants to salt stress varies at different stages of development (Rev & Freeman, 1976, 1976). Germination appears to be a stage of high sensitivy to salt stress (Zid & Boukhris, 1977). Seeds of *Atriplex halimus* have a great ability to germinate under high salinity conditions.

For treatments at 100 meq./l, 200 meq./l and 300 meq./l there is a respective decrease in germination of 10, 30 and about 40% compared with the control. For treatment at 400 meq./l, 500 meq./l and 600 meq./l, the germination was seriously affected,

ranging from 4 to 22% of germination rate. Germination occurred two days later for treatments at 300 meq./l, 400 meq./1 and 500 meq./1, and four days later for treatment with solution at 600 meq./1

Therefore salinity of waters and soils not only results in inhibition of germination, but also in a very net delay of the process itself.

DISCUSSION

Germination appears to be a stage of high sensitivity to salt stress (Zid & Boukhris, 1977). Changes in salinity degree strongly affect on germination, growth and cellular anatomy of plants. Inhibition of germination of the seeds of *Atriplex halimus* is caused by the presence of high concentrations of sodium chloride. Stroconov (1964) confirms by his works that the response of seed to salinity is an indicator of tolerance of the plant.

Salinity can affect germination in two ways:

- decreasing the input speed of the amount of water absorbed by seed, the increase in the osmotic pressure of the water where the inhibition is too high.
- increasing the penetration of the ions that can accumulate in the seed at doses that become toxic Riyad (1987).

Atriplex halimus support concentrations of

meq.1^{-1}	**100**	**200**	**300**	**400**	**500**	**600**
NaCl (mM)	100	200	300	400	500	600
g/l	5.84	11.68	17.53	23.37	29.22	35.06
$CaCl_2$ (mM)	100	200	300	400	500	600
g/l	5.54	11.08	16.64	22.19	27.74	33.29

Table 4. Composition of saline solution.

Box / Treatment	B1	B2	B3	B4	B5	average deviation	standard deviation
Witness	8	7	6	7	8	7.6	0.94
T1	6	5	7	7	6	6.2	0.83
T2	5	4	3	5	5	4.4	0.89
T3	3	3	2	4	4	3.2	0.83
T4	2	2	3	2	2	2.2	0.44
T5	1	1	1	0	1	0.8	0.20
T6	1	0	0	1	0	0.4	0.42

Table 5. Number of sprouts in different salinity conditions. B1-B5: Boxes or Petri dishes; Treatments: 100 meq/l (T1), 200 meq/l (T2), 300 meq/l (T3), 400 meq/l (T4), 500 meq/l (T5), 600 meq/l (T6).

Differences between averages	calculated t	Tests of significance
M1-M2	1.4	*
M1-M3	3.2	*
M1-M4	4.6	*
M1-M5	5.4	**
M1-M6	6.8	**
M1-M7	7.2	**
M2-M3	1.8	*
M2-M4	3.2	*
M2-M5	4	*
M2-M6	5.4	**
M2-M7	5.8	**
M3-M4	1.4	*
M3-M5	2.2	*
M3-M6	3.6	*
M3-M7	4	*
M4-M5	0.8	NS
M4-M6	2.2	*
M4-M7	2.76	*
M5-M6	1.4	*
M5-M7	1.8	*
M6-M7	0.4	NS

Table 6. Pairwise comparison between germination averages (M1–M7, in %); t = standard deviation.

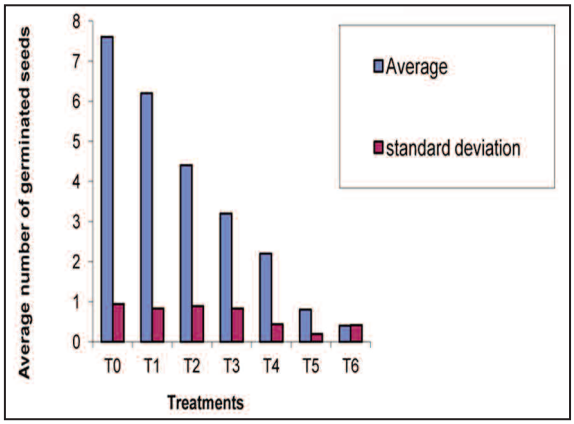

Figure . Average number of *Atriplex halimus* germinated seeds at different salinity conditions.

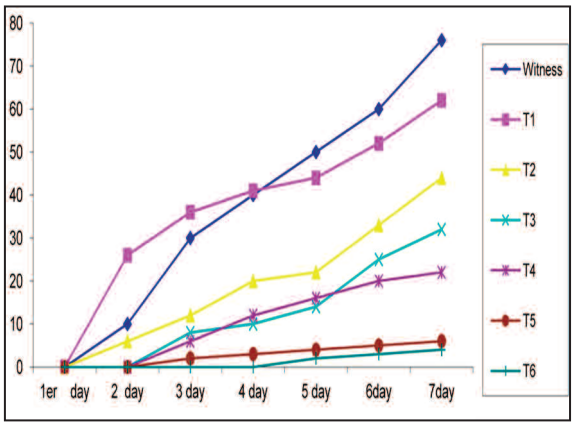

Figure 4. Percentages of germinated seeds at different salinity conditions.

Treatement / Time germination	Witness	T1	T2	T3	T4	T5	T6
1 day	0	0	0	0	0	0	0
2 day	10	26	6	0	0	0	0
3 day	30	36	12	8	6	2	0
4 day	40	41	20	10	12	3	0
5 day	50	44	22	14	16	4	2
6 day	60	52	33	25	20	5	3
7 day	76	62	44	32	22	6	4

Table 7. Percentages of *Atriplex halimus* seed germination in different salinity conditions.

sodium chloride similar as that found in seawater (Ben Ahmed et al., 1996).

This is consistent with our results where the high salinity treatments at 500 meq./l and 600 meq./l, cause a delay in germination, but not a complete inhibition. It would be necessary much higher concentrations to really observe inhibition of germination of the seeds of *Atriplex halimus*.

Clemens et al. (1983) showed that at different daline concentrations there are differences in the germination rate of the seeds. These differences would likely come from the exogenous NaCl effect in general, which causes a reduction of the germination process.

The final stage of germination or inability of seeds to germinate seems to mean that with increasing salt concentration, the toxicity effect dominates due to the accumulation of sodium in the embryo by installing an osmotic inhibition (Guerrier, 1983; Bliss et al., 1986). Tolerance of *Atriplex halimus* to salt stress is often attributed to the presence of trichomes, on the surface of the leaves. The osmotic pressure of cell content is very high which is due to the massive mineral salts (sodium) accumulation or to the synthesis of large amount of organic substances.

CONCLUSIONS

This study was carried out to highlight the germination capability of *Atriplex halimus* in various synthetic media. Germination in vitro in two synthetic media (nutrient agar and Mueller Hinton) and different temperatures (5 °C and 25 °C) shows that *Atriplex halimus* are characterised by a considerable germination rate.

According to our results, seeds seem to support a variable temperature range noting that cold induced retardation of germination, without however affecting the final germination percentage. *Atriplex halimus* has a resistance to saline stress, despite the observed delay, there was a germination rate that varies between 8 and 22% at a concentration of 600 meq./l. As already well underlined by Belkhoudja & Bidai (2004), our results confirm that confirm that *Atriplex* support high salinity conditions which allows to cultivate it on soils and waters generally considered unsuitable for agriculture.

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