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## Effect of Temperature on Seed Germination of Some Steppe Species

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### ABSTRACT

Climate change is becoming more restrictive for growth and development of plants particularly in semi-arid and arid areas. This natural stress (drought) has changed the ecosystem stability and is largely the cause of desertification and land degradation as a consequence of vegetation. If the species is auto-ecologically suited to the site, its growth and development still require adaptation to local conditions of intra-and interspecific competition. In particular, it has been shown that temperature has a direct effect on the multiplication of species steppe (*Stipa tenacissima*, *Lygeum spartum*, *Retama retama*, *Atriplex halimus*) and their ecology. With regard to the practical results of this work, we can say that the plant species, found frequently in the steppe, may be included in programs for pastoral development.

**Keywords:** multiplication, Steppe, temperature, steppe, species.

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## INTRODUCTION

The dispersal is an internal factor of species propagation and expansion that is causing the distribution of living beings.

The dispersion is an important component of plant life. It reduces the competition between individuals, allows the reach of suitable habitat and takes into account the spatial and temporal variations of the environment, exchange of individuals between populations (gene flow) and to create new populations.

The dispersal patterns observed use most conventional dispersers agents: wind, water, mammals, birds, ants.

The objective of this study is to provide different temperatures for some studied species and see the response of each species at these temperatures.

## MATERIALS AND METHODS

### Materials

A trial evaluation; of species potentiality to be developed, through the appreciation of their germination rates; will decide on the ecological impact on the spread of these species.

The seeds of *Stipa tinaccessima*, *Lygeum spartum*, *Retama retama*, *Peganum harmala* and *Atriplex halimus*, were collected during the 2007-2008 dry season in Naama steppe, and belong to the semi-arid fresh bioclimatic level.

### Methods

All seeds were prepared in the same way (they were kept in plastic bags placed under natural conditions, in a temperature between 25 and 35°C). The germination tests were conducted in a germination chamber at different temperatures (20°C, 25°C and 30°C, all  $\pm 1^\circ\text{C}$ ); average temperature of the laboratory for germination, 30 to 90% relative humidity and light).

The germination tests were performed on whole fruits and intact seeds. The intact seeds, pericarp removed, served as controls for all experiments.

Through each test we take a batch of seeds, each containing 100 seeds.

### Pretreatment

- The seeds are soaked in a 4% acid chloride for a 4 min period.
- Seeds are re-soaked, for 8 hours in sterilized distilled water.

Seedlings were made in germination dishes 75 mm in diameter and 80 mm high lined with a double layer of filter paper moistened to saturation, each test is of 100 seeds of each species over 5 repetitions of 20 seeds for each.

The seeds were placed on cotton wool soaked with distilled water. The lots are germinated in a germination chamber.

### Appreciation and germination expression of the results

We chose to express the germination characteristics through the germination curves that give the evolution of cumulative percentages over time of germinated seeds. They visualize well the latency (percentage of seeds can germinate under certain conditions) and the duration of the germination period. These curves were smoothed by the moving means method.

For the comparison of several tests, it is easier to relate to the germination average time proposed by J. F. Harrington [3]:

$$\frac{N_1T_1 + N_2T_2 + \dots + N_nT_n}{N_1 + N_2 + \dots + N_n}$$

Or more precisely to its formula corrected by F. Douay [2] which reflects a germination ability which allows us to realize the differences between two tests even if the final rates of germination are clearly different; which is not the case with J. F. Harrington formula.

The germination ability is given by the following formula:

$$\frac{N_1T_1 + N_2T_2 + \dots + N_nT_n}{N_g + \frac{N_g}{N_t}}$$

Where

$N_1$ : Number of individuals germinated at time  $T_1$ .

$N_2$ : Number of germinated seeds between times  $T_1$  and  $T_2$ .

$N_g$ : Number of germinated seeds at the end of the experiment.

$N_t$ : Total number of individuals put in germination.

## RESULTS

At this point, it is possible to retain the following essential points:

It is reported that germination is a prerequisite for the preparation and production of juvenile plant material for the experimentation purposes. This study allowed us to examine the behavior of germinal *Peganum harmala*, *Lygeum spartum*, *Atriplex halimus*, *Retama retama* and *Stipa tenacissima* against the various thermal conditions.

The experiment was conducted in the laboratory germination chambers under different temperature conditions, adjustable with a germination chamber where temperatures are favored in by the vapor due to cooling or warming of existing water, which can help create certain humidity.

We conducted germination tests in the expressed range of temperature in an attempt to define to what extent it is possible for seeds to germinate and how the thermal factor can affect the seeds germination. These tests are conducted simultaneously on cotton soaked with distilled water in propagator in the germination chamber.

The counting of germinated seeds was performed daily for 15 days (Fig 1). During this period the cumulative percentages of germination were determined on a daily basis. The quantified parameters were based on the following:

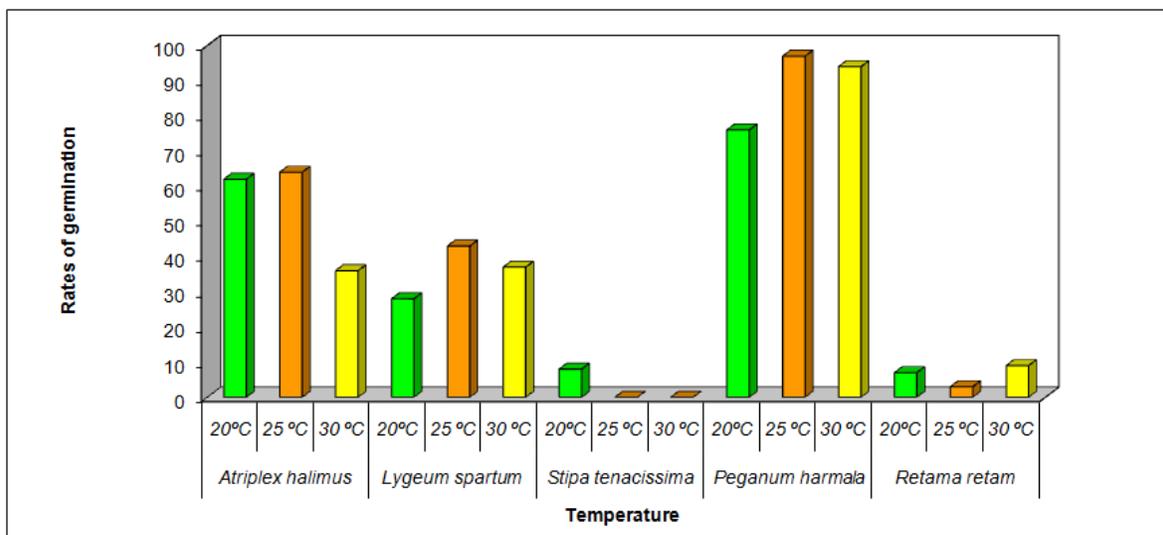


Figure 1: Evolution of the rate of germination seed studied as a function of temperature variations.

A seed has germinated when the radical had pierced the envelopes.

- The germination speed is the time required for seed germination after planting date.
- The germination capacity is the maximum germination percentage obtained under the conditions chosen by the experimenter.
- The latency is the time to obtain the first germinated seeds [6].

The results are expressed as the capacity of germination (CG) representing the maximum percentage of germinated seeds.

To compare the results for the studied species germination capacity, it seemed useful to analyze the statistical results. We used for that the correlation analysis. This latter seems appropriate to our study.

To better understand the ecological significance of germination behavior of the studied species, we have adopted the classification used by Neffati [7] and based on the value of the two main factors for germination (species and temperature), namely the speed of germination in relation with temperature to obtain the highest germination rate (Table I).

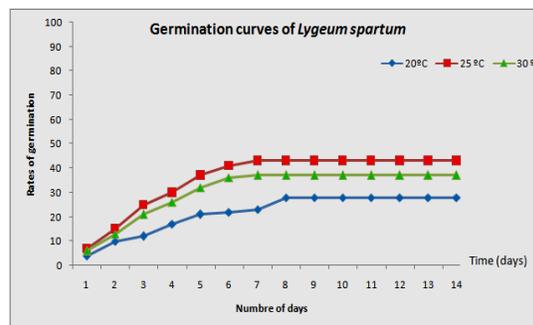
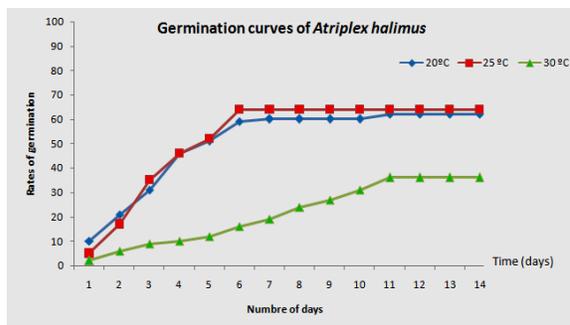
**Table I: Distribution of the studied species according to their rate of germination, germination speed at optimum temperatures (According to Neffati classification [7]).**

Temperature	Germination rate	Species	Germination speed	Species
20°C	Low ( $\leq 20\%$ )	- <i>Stipa tenacissima</i> - <i>Retama retama</i>	Slow $\geq 5$ days	- <i>Stipa tenacissima</i> - <i>Retama retama</i>
	High ( $>20\%$ )	- <i>Peganum harmala</i> - <i>Lygeum spartum</i> - <i>Atriplex halimus</i>	High $<5$ days	- <i>Atriplex halimus</i> - <i>Lygeum spartum</i> - <i>Peganum harmala</i>
25°C	Low ( $\leq 20\%$ )	- <i>Stipa tenacissima</i> - <i>Retama retama</i>	Slow $\geq 5$ days	- <i>Stipa tenacissima</i> - <i>Retama retama</i>
	High ( $>20\%$ )	- <i>Peganum harmala</i> - <i>Lygeum spartum</i> - <i>Atriplex halimus</i>	High $<5$ days	- <i>Atriplex halimus</i> - <i>Peganum harmala</i> - <i>Lygeum spartum</i>
30°C	Low ( $\leq 20\%$ )	- <i>Stipa tenacissima</i> - <i>Retama retama</i>	Slow $\geq 5$ days	- <i>Stipa tenacissima</i> - <i>Atriplex halimus</i> - <i>Retama retama</i>
	High ( $> 20\%$ )	- <i>Peganum harmala</i> - <i>Lygeum spartum</i> - <i>Atriplex halimus</i>	High $<5$ days	- <i>Peganum harmala</i> - <i>Lygeum spartum</i>

In the results presented above, we found that rates of germination are in inverse proportion to the germination speed increase at the different temperatures.

### Germination curves

For each temperature test, these curves (Fig 2) reflect the evolution of the cumulative percentage of germinated seeds over time (days) under thermal conditions. They also highlight other parameters (latency, average time of germination, germination capacity, etc.)



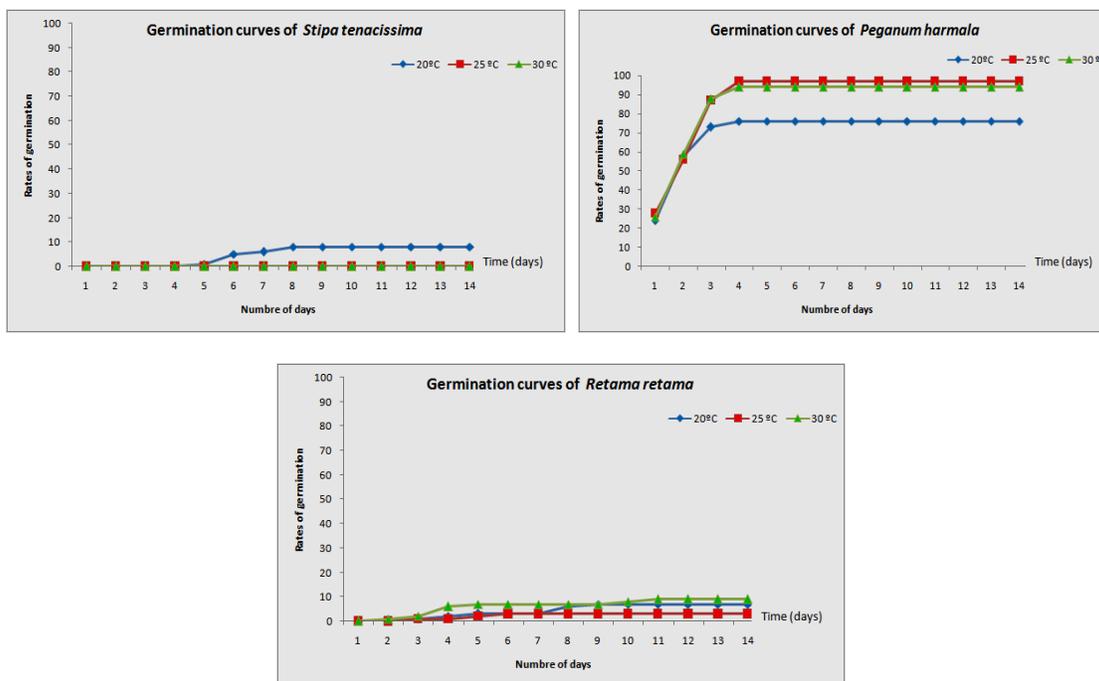


Figure 2: Evolution of seeds germination rate over time.

### Harrington Curves

Harrington curve expresses the germination ability of the studied germinated species according to temperature.

- Calculation of germination ability (X) (Table II)

#### Formula calculation germination ability (X)

$$X = \frac{N_1T_1 + N_2T_2 + N_3T_3 + \dots + N_nT_n}{N_g \cdot \frac{N_g}{N_t}} \quad [2]$$

Where:

- N1: Number of germinated seeds at time T<sub>1</sub>
- N2: Total number of germinated seeds at time T<sub>2</sub>
- Ng: Total number of individuals who sprouted
- Nt: Number of seeds set to germinate in early

Table II. Presentation of results on the ability of seeds germination under thermal condition

steppe species	T°	Nt	Ng	Ng/ Nt	Ng. Ng/Nt	$\sum N_1 T_1 + N_2 T_2 + \dots + N_n T_n$	X
<i>Atriplex halimus</i>	20°C	100	27	0.27	7.29	810	111.11
<i>Lygeum spartum</i>		100	28	0.28	7.84	750	95.66
<i>Stipa tenacissima</i>		100	8	0.08	0.64	141	220
<i>Peganum harmala</i>		100	76	0.76	57.76	663	11.48
<i>Retama retam</i>		100	7	0.07	0.49	137	279.59
<i>Atriplex halimus</i>	25°C	100	30	0.30	9	551	061.22
<i>Lygeum spartum</i>		100	43	0.43	18.49	964	52.14
<i>Stipa tenacissima</i>		100	-	-	-	-	-
<i>Peganum harmala</i>		100	97	0.97	94.09	789	8.39
<i>Retama retam</i>		100	3	0.03	0.09	31	344.44
<i>Atriplex halimus</i>	30°C	100	21	0.21	4.41	472	107.03
<i>Lygeum spartum</i>		100	37	0.37	13.69	834	60.92
<i>Stipa tenacissima</i>		100	-	-	-	-	-
<i>Peganum harmala</i>		100	94	0.94	88.36	784	8.87
<i>Retama retam</i>		100	9	0.09	0.81	246	303.7

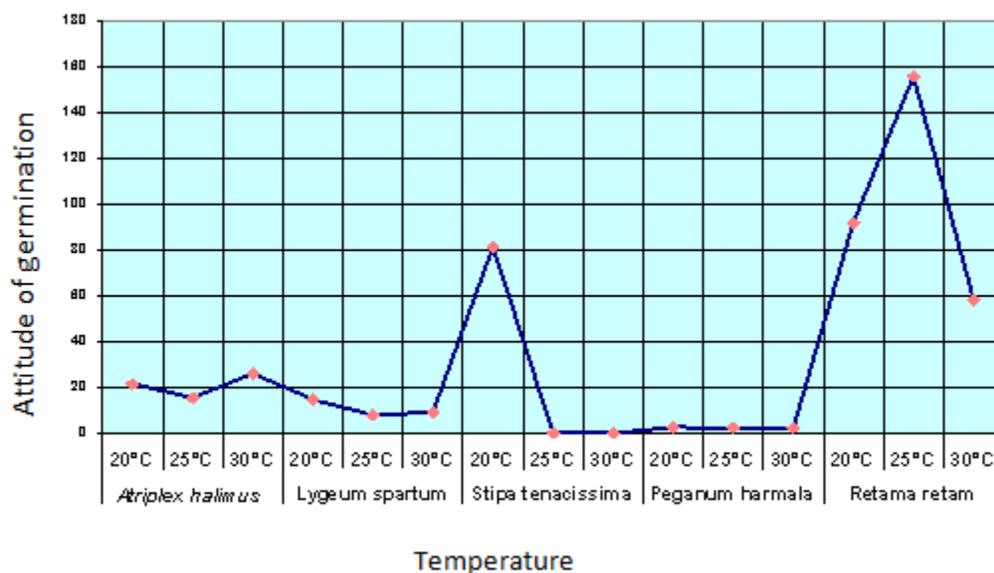


Figure 3: Germination ability of the species studied seeds as a function of temperature and species.

It turns out that most of the species studied are at low thermal optimum and slow germination. This corroborates our results at temperature 20°C, at which the studied species majority have a marked slow germination; and the only species, *Atriplex halimus*, which has a high thermal optimum (30°C), has also high speed germination. Otherwise at the temperature 25°C, most of the studied species seeds were able to rapid germination. Except for *Stipa tenacissima* (0) and *Peganum harmala* which went, respectively from 25°C to 30°C, from 2.24 to 2.16 (fig. 3 )



## DISCUSSION

Two factors are the cause of variation in germination behavior: the factor species and the temperature factor.

- Temperature plays an important role in seed germination, stimulates the enzymatic activities and also the speed of germination. The temperature controls the supply of oxygen to the embryo.

Thermal requirements vary from one species to another.

- Although reported differences between species that is expressed within the seed germination (time interval from sowing the first seeds germinated) the highest rates are recorded for seeds of *Peganum harmala* and *Lygeum spartum* they sprouted one day after sowing and at all temperatures. While the lag phase was more or less slow for *Retama retama* and *Atriplex halimus*, which varies from two to three days after sowing.

Germination is more or less extended for the seeds of *Retama retama*, *Atriplex halimus*, *Lygeum spartum*, *Stipa tenacissima* and *Peganum harmala* ranked respectively according to the required number of days for germination. This is according to the seeds membranes stiffness of the studied species, because the seed coat strongly hinders germination.

The phenomena that were considered by Neffati [7] in agreement with what is known about the thermal factor role in the activation of metabolic reactions are a great asset to the adaptation of species to arid conditions.

The rate of *Peganum harmala*, *Lygeum spartum* and *Atriplex halimus* seeds germination is over 20% at all tested temperature conditions.

On the other hand, the rate of germination of *Retama retama* and *Stipa tenacissima* is much less than 20%.

Neffati and Akrimi [8], with other authors, in results obtained for other species of southern Tunisia, find that the majority of these species is able to germinate at 20°C even if some others prefer to germinate at higher or lower temperatures. As for Ismail [4], Jordan and Haferkamp [5], Williams and Ungar [10] they showed that alternating temperatures cause a higher and faster germination.

Temperature 25°C is more favorable for germination of most of the studied species seeds at the temperature 20°C except for *Retama retama*. While *Stipa tenacissima* has not germinated at 25°C no rat 30°C.

From these results, the seed germination heterogeneity is due to several factors:  
- For *Stipa tenacissima* the cause may be due to germination conditions noting that this species does not endure atmospheric moisture which was favored in the germination chamber (the higher the temperature, the higher the evaporation). The non-germination of this species at

30°C may be related to the species tolerance at this temperature. Because; according to P. Mazliak [6]; 20°C is the optimum temperature for germination of the Alfa. Tazairtk [9] proved that of (*Stipa tenacissima*) regeneration is quite possible provided that one determines the right conditions for germination or the right treatment; in order to remove the inability to germination. He obtained then, at 27°C, a germination rate between 85% and 90%.

For the *Retama retama*, the cause may be due to tegument inhibitions or dormancy (embryonic) or their maturation was not reached because the shelf life after harvest was short (about one year). Or the inhibitions caused by fungi (existing in open air) in the germination chamber in view of favorable conditions to their multiplication (temperature humidity) during the days of germination that despite the pretreatment carried out before sowing seeds in germination. This probability may explain the behavior of seeds of *Retama retama* at 25°C.

For *Atriplex halimus*, the germination rate was more or less lower compared to the one the species tolerates, knowing that this species tolerates temperatures from below 0°C (-6°C) to 35°C. This is probably due to the valve of the seed, and appears to be related to phenomena of valves dormancy characteristics. According to the High Commission for Development of the Steppe (HCDS) technicians, this rate can be improved as a result of specific treatments.

The high rate of germination of *Peganum harmala* and *Lygeum spartum* may be due to the external seeds integument. With *Peganum harmala* seeds, the integument is reticulate, which helps to spread very quickly after their maturation. In the *Lygeum spartum* these large seeds are easy to germinate, despite the collar of hair they have, noting that these hairs help their release into the wild, because once detached from the parent plant, they are found in large quantities in clumps.

### CONCLUSION

The findings listed on the thermal influence on the studied steppe species germination seeds, showed certain heterogeneity.

This latter indicates that the inability of our studied species seed germination is due to poor germinating conditions. According to P. Cuissance [1] the success in germination depends on the quality of seeds, integumentary inhibitions, maturation is not reached or dormancy (embryonic). These differences may be due probably to the origin.

Such a result shows that it is difficult to relate the effect of temperature to the germination moment, to the species ecology or to its effect at the adult plant stage.

Despite this, these preliminary results are markers of interest to further elucidate the relationship between the temperature effect on the species and ecology of common species found in the steppe. This will develop a classification of tolerances to temperature, an important criterion for factors of the spread of species and the choice to be used in a drylands development program.

**APPENDICES**

**Annex A. Changes in seed germination of *Atriplex halimus*, *Lygeum spartum*, *Stipa tenacissima*, *Peganum harmala*, *Retama retam***

Temps (jours)	<i>Atriplex halimus</i>			<i>Lygeum spartum</i>			<i>Stipa tenacissima</i>			<i>Peganum harmala</i>			<i>Retama retam</i>		
	20°C	25 °C	30 °C	20°C	25 °C	30 °C	20°C	25 °C	30 °C	20°C	25 °C	30 °C	20°C	25 °C	30 °C
01	0	0	0	4	7	6	0	0	0	24	28	26	0	0	0
02	2	2	0	10	15	13	0	0	0	58	56	59	0	0	1
03	5	8	4	12	25	21	0	0	0	73	87	88	1	1	2
04	9	16	6	17	30	26	0	0	0	76	97	94	2	1	6
05	12	21	10	21	37	32	1	0	0	76	97	94	3	2	7
06	14	24	13	22	41	36	5	0	0	76	97	94	3	3	7
07	19	30	20	23	43	37	6	0	0	76	97	94	3	3	7
08	26	30	21	28	43	37	8	0	0	76	97	94	6	3	7
09	26	30	21	28	43	37	8	0	0	76	97	94	7	3	7
10	27	30	21	28	43	37	8	0	0	76	97	94	7	3	8
11	27	30	21	28	43	37	8	0	0	76	97	94	7	3	9
12	27	30	21	28	43	37	8	0	0	76	97	94	7	3	9
13	27	30	21	28	43	37	8	0	0	76	97	94	7	3	9
14	27	30	21	28	43	37	8	0	0	76	97	94	7	3	9

**Annex B. Presentation of results on the germination of seeds steppe under different thermal conditions**

Steppe species	T°	N <sub>t</sub>	N <sub>1</sub>	T <sub>1</sub>	N <sub>2</sub>	T <sub>2</sub>	N <sub>3</sub>	T <sub>3</sub>	N <sub>4</sub>	T <sub>4</sub>	N <sub>5</sub>	T <sub>5</sub>	N <sub>6</sub>	T <sub>6</sub>	N <sub>7</sub>	T <sub>7</sub>	N <sub>8</sub>	T <sub>8</sub>	N <sub>g</sub>
<i>Atriplex halimus</i>	20°C	100	2	2	3	5	4	9	5	12	6	14	7	19	8	26	10	27	27
<i>Lygeum spartum</i>		100	1	4	2	10	3	12	4	17	5	21	6	22	7	23	8	28	28
<i>Stipa tenacissima</i>		100	5	1	6	5	7	6	8	8									08
<i>Peganum harmala</i>		100	1	24	2	58	3	73	4	76	-	-	-	-	-	-	-	-	76
<i>Retama retam</i>		100	3	1	4	2	5	3	8	6	9	7							07
<i>Atriplex halimus</i>	25°C	100	2	2	3	8	4	16	5	21	6	24	7	30					30
<i>Lygeum spartum</i>		100	1	7	2	15	3	25	4	30	5	37	6	41	7	43			43
<i>Stipa tenacissima</i>		100																	00
<i>Peganum harmala</i>		100	1	28	2	56	3	87	4	97	-	-	-	-	-	-	-	-	97
<i>Retama retam</i>		100	3	1	5	2	6	3											03
<i>Atriplex halimus</i>	30°C	100	3	4	4	6	5	10	6	13	7	20	8	21					21
<i>Lygeum spartum</i>		100	1	6	2	13	3	21	4	26	5	32	6	36	7	37			37
<i>Stipa tenacissima</i>		100																	00
<i>Peganum harmala</i>		100	1	26	2	59	3	88	4	94	-	-	-	-	-	-	-	-	94
<i>Retama retam</i>		100	2	1	3	2	4	6	5	7	10	8	11	9					09

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