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Effects of temperature, drought and salinity on seed germination of Fabaceous species (*Anthyllis henoniana*)

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Abstract. This study was conducted at germination stage on *Anthyllis henoniana,* a Fabaceae species, which grows under arid lands in Tunisia. The effects of temperature, salt and drought stresses at the same water potentials induced by NaCl and polyethylene glycol (PEG 6000), were investigated on seed germination. Results indicated that a temperature between 15 and 25°C was the most favorable at germination stage. An increase in PEG and NaCl potentials progressively decreased both the percentage and the mean germination time. Germination under salt and water stresses showed the differences occurred; salt stress had less inhibitory effect on germination than drought stress. The germination behavior of this species would therefore imply adaptive mechanisms to colonize the arid lands threatened by desertification in Tunisia.

Keywords. Arid lands – *Anthyllis henoniana* – Germination – Temperature – Water stress – Salt stress.

Effet de la température, du stress hydrique et du stress salin sur la germination des graines d'une espèce de* fabaceae *(*Anthyllis henoniana*)

Résumé. *Cette étude concerne le comportement germinatif d'*Anthyllis henoniana *de la famille des Fabaceae, collectée dans des zones arides de la Tunisie. Les effets de température, du sel et du stress hydrique aux mêmes potentiels hydrique induits par NaCl ou le PEG 6000, ont été examinés sur la germination des graines. Les résultats indiquent qu'une température entre 15 et 25°C était la plus favorable à la germination des graines. Une augmentation du PEG et des potentiels du NaCl diminuent progressivement le pourcentage et le temps moyen de germination. La germination sous l'effet du sel et du stress hydrique a montré des différences entre les traitements. L'effet inhibiteur du stress salin sur la germination est moins marqué que celui du stress hydrique. Le comportement germinatif de cette espèce impliquerait donc des mécanismes adaptatifs pour coloniser des zones menacées par la désertification en Tunisie.*

Mots-clés. *Zones arides –* Anthyllis henoniana *– Germination – Température – Stress hydrique – Stress salin.*

I – Introduction

Desertification is a serious threat to arid and semiarid environments that cover 40% of the global land surface. Tunisia is one of the most affected countries. On account of desertification problems and soil erosion affecting large areas of the Mediterranean region, it's necessary to create programs leading to revegetation, to improve arid ecosystems and also to preserve natural resources.

Several species of *Fabaceae* family have a high interest due to their high adaptation to arid and semi-arid environments, nitrogen fixing capacity and ability to grow in poor soils (Ibanez and Passera, 1997). They are of great importance worldwide, especially in countries under Mediterranean climate like Tunisia. They contribute to soil fertility and to the prevention of soil erosion. A successful germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time as it determines whether or not the populations will successfully establish (Tlig *et al.*, 2008). The germination is adversely affected by unfavorable moisture conditions due to lack of rain. Seed germination rates generally decrease with decreases in soil water potential, which is always associated with failure of plant emergence (Willenborg *et al*., 2005).

Salinity is another major constraint to seed germination. Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions. Because of the increase of expanded saline areas throughout the world, that makes a dangerous trend of 10% per year, many studies were carried out to determe salt effects on this stage (Abari *et al*., 2011). Severe drought and high salinity are rapidly increasing and could promote the desertification and salination of lands. In this regard understanding seed germination under various levels of water stress, salt stress, and temperatures is essential for successful plant establishment and revegetation.

The aim of this study was to evaluate, during germination, the adverse effects of temperature, salinity, and drought on *Anthyllis henoniana,* an endemic species of East Algeria, south of Tunisia, and west Libya, grown under conditions of the arid environments of Tunisia.

II – Materials and methods

This experimental study was carried out under laboratory conditions. Seeds of *Anthyllis henoniana*, collected by Arid Land Institute Medenine, were used without any type of pretreatment. Seeds were then surface sterilized in aqueous solution of 0.1% mercuric chloride for 60s to prevent fungal attack and rinsed in several changes of sterile distilled water. Germination experiments were conducted in incubators set at 5, 10, 15, 20, 25, 30, and 35°C in complete darkness (four repetitions of 25 seeds). The effect of drought and salt stress were conducted at the optimal temperature, according to the temperature experiment. It was investigated on seeds placed in Petri dishes with 5 ml of aqueous solutions of polyethylene glycol (PEG 6000) or NaCl to create water stress or salt stress, respectively, with iso-osmotic potentials: 0 (distilled water), -0.1, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa.

Final germination percentage (FGP) and median germination time (MGT) or the time to 50% germination were calculated. Final germination percentage was calculated as the cumulative number of germinated seeds at termination of the experiment:

$$FGP = \frac{\sum n}{Nt}.100$$

where *n* is the number of germinated seed at each enumeration interval, and *Nt* is the number of seeds in each experiment;

$$MGT = \frac{\sum ni, ti}{N}$$

where *ni* is the number of seeds germinated at day *i*, *ti* is the incubation period in days and *N* the total number of germinated seeds in the treatment.

Statistical analyses were performed using SAS software (version 8.2, USA). A one-way analysis of variance (ANOVA) was performed on all results.

III – Results

ANOVA showed significant effects ($p < 0.001$) of temperature on mean germination time and final germination percentage (Fig. 1).

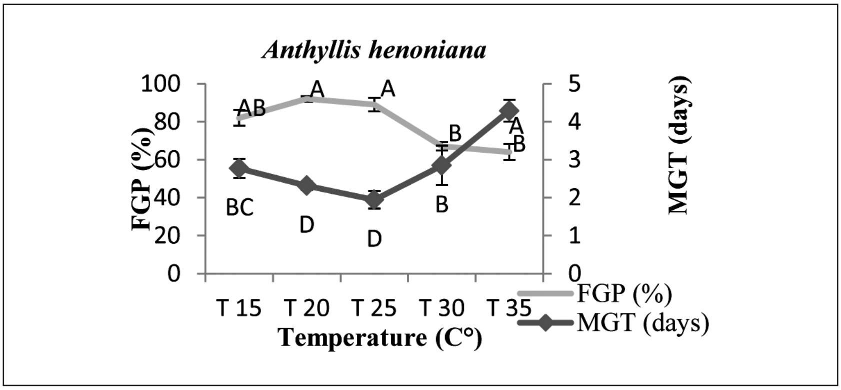

Fig. 1. Variation of the final germination percentages (FGP) and the mean germination time (MGT, days) of *Anthyllis heoniana* seeds at different temperatures. Values of each parameter (mean ± 95% confidence limits, n = 4), having the same letter are not significantly different (P>0.05) each other (Duncan test).

Optimal germination occurred at 20°C in *Anthyllis heoniana* seeds. This species showed relatively higher records of germination percentages at the treatments between 15° and 25°C, but it showed a reduction at the highest temperature regime (T35).

The effects of water stress (PEG treatment) on seed germination are reported in Table 1.

Table 1. Effect of PEG 6000 on final germination percentage (FGP) and mean germination time (MGT) of *Anthyllis heoniana* at the optimal temperature (T = 20°C)

	Water stress (PEG 6000, MPa)					
	0	**-0,2**	**-0,4**	**-0,6**	**-0,8**	**-1**
FGP	89 ± 1,49^{A}	91 ± 1,10^{A}	83 ± 1,49^{AB}	70 ± 1,32^{B}	57 ± 1,79^{DC}	51 ± 1,49^{D}
MGT	2,74 ± 0,22^{D}	3,05 ± 0,16^{D}	4,13 ± 0,25^{C}	4,76 ± 0,18^{B}	5,03 ± 0,19^{B}	5,77 ± 0,22^{A}

Means (± s.e.: n = 4) with the same letter across rows and columns were not significantly different ($P > 0.05$).

Water stress induced by PEG had a significant effect (P < 0.001) on final germination percentage (FGP) and mean germination time (MGT) of the species. Final germination percentage and speed decreased with a decrease of water potential, this decrease was most significant at lower PEG level (-1MPa). The potential of -0.2MPa indicated the highest records among all treatments (91%). Also, germination percentage exceeded 50 % at -1 MPa.

The results of ANOVA showed that salt stress (NaCl treatments) had a significant effect (P < 0,001) on germination percentage and mean germination time (Table 2). The germination was significantly reduced by high NaCl levels and there were no great differences in final germination percentage between 0 and -0,4 MPa, so germination percentage was reduced with increasing NaCl to levels above -0,4 MPa (Duncan test, 1%).

The germination speed, expressed by MGT, decreased with the decrease in NaCl potentials.

Table 2. Effect of NaCl on final germination percentage (FGP) and mean germination time (MGT) of *Anthyllis heoniana* at the optimal temperature (T°20)

	Salt stress: NaCl (MPa)					
	0	-0,2	-0,4	-0,6	-0,8	-1
FGP	90 ± 3,06^{A}	87 ± 1,54^{A}	89 ± 1,03^{A}	74 ± 0,95^{AB}	63 ± 0,85^{BC}	53 ± 1,10^{C}
MGT	1,65 ± 3,06^{C}	2,18 ± 0,08^{C}	2,35 ± 0,96^{BC}	3,13 ± 0,09^{B}	4,32 ± 0,27^{A}	4,27 ± 0,62^{A}

Means (± s.e.: n = 4) with the same letter across rows and columns were not significantly different ($P > 0.05$).

IV – Discussion

Seed germination behavior in relation to thermal stress is very important to determine the colonization capacity of species (Ungar, 1982). In the present study germination was significantly (P < 0.001) inhibited by either an increase or decrease in temperature from thermal optimum (Fig. 1). Seeds of this species germinate better at temperatures between 15 and 25°C. The behavior of the species is a typical strategy of Mediterranean plants with optimal temperatures ranging between 15 and 25°C (Baskin and Baskin, 1998). In the present study, 51% of *Anthyllis heoniana* seeds germinated at the lowest water potential (-1 MPa). This suggested that this species easily grows under low water availability. Other study demonstrated that even desert species like *Ziziphus lotus* presented less than 5% of germinated seeds at -1 MPa (Maraghni *et a*l., 2010). Ibanez and Passera (1997) found that *Anthyllis cystoides* seeds were able to germinate at -1,12 MPa (48%). Also at growth stage, Ourcival and Berger (1995) confirmed the high resistance of *Anthyllis heoniana* plants which continued at -1,9 MPa in May. Similar results were reported for other *Fabaceae* species like *Acacia tortilis* which had no germinated seeds at -0,8 MPa (Jaouadi *et al*., 2010). This limit of tolerance (-0,8 MPa) was also observed in other *Fabaceae* and desert species like *Retama raetam* (Youssef, 2009).

The results of this study showed that increases in salinity caused a decrease in germination percentage and speed, it also delayed germination in all studied species. Generally, salt stress affected the germination capacity and speed of *Anthyllis heoniana* seeds. These results were confirmed by several other research (Lachiheb *et al*., 2004).

In our study, the seed germination was higher in NaCl than in PEG at the same water potential. In this regard some studies have demonstrated that NaCl and PEG adversely affected germination but NaCl had a less inhibitory effect on seed germination than an iso-osmotic of PEG (He *et al*., 2009). In contrast, Katembe *et al.* (1998) found that higher concentrations of NaCl (-1MPa) were more inhibitory to germination of two *Atriplex* species than iso-osmotic PEG solutions.

Considering the percentage of seeds that germinated in the -1 MPa, we can conclude that this *Fabaceae* species is well adapted to germinate under conditions of water and salt stresses. These features are typical of the environments where it grows and lives. Arid lands of Tunisia are widely affected by desertification which is caused particularly by the degradation of vegetation cover, deforestation, and drought. The high ability of *Anthyllis heoniana* to germinate over a wide range of environmental conditions provides an opportunity to contribute to future reforestation programs.

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