

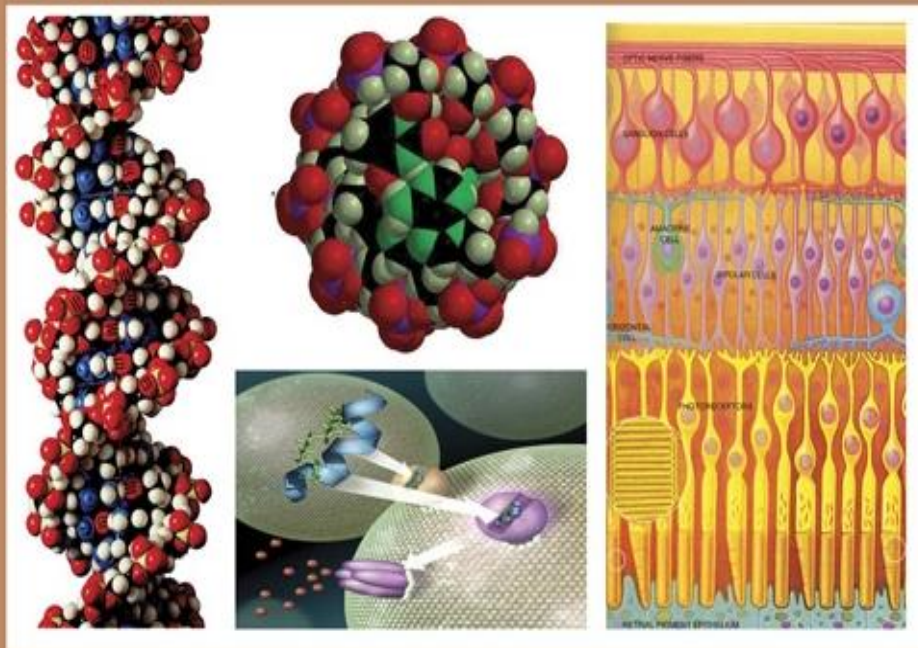


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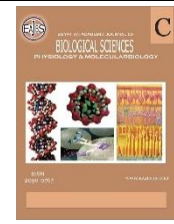
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**Comparative Study on Caryopses Germination of *Stipa tenacissima* L. From Two Habitats of Western Algeria Under Different Controlled Conditions of Salinity and Water Potential.**

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**ABSTRACT**

The Alpha grass steppes (*Stipa tenacissima*) are one of the most representative steppes in the Mediterranean arid ecosystems. They are in continuous regression due to a strong climatic and anthropic pressure without forgetting the difficulties of natural regeneration. Our work is based on the conservation of this species, it consists of carrying out a comparative study of the germinative behavior of Alpha grass caryopses from the coast and the steppe (Western Algeria) under the effect of salt and water stress. For each provenance, each germination trial was conducted at the optimum temperature of 20°C and involved four batches of caryopses with 25 caryopses per batch. The effect of salt stress was tested under different salt concentrations prepared from sodium chloride: 0, 1, 2, 4, 6 and 8 g.L<sup>-1</sup> corresponding to the molar concentrations of 0, 17.11, 34.22, 68.44, 102.66 and 136.89 mM. The effect of water stress was evaluated under different concentrations of polyethylene glycol (PEG<sub>6000</sub>) corresponding to water potentials of: - 0.04; - 0.2; - 0.4; - 0.8 and - 1.6 MPa. The results obtained showed that the salinity and the water potential directly affect the germination capacity of the caryopses of both provenances. However, coastal caryopses are more resistant to salinity than steppe caryopses with a tolerance threshold corresponding to 8g.L<sup>-1</sup> (136.89 mM) of NaCl. Also, the coastal caryopses are much more resistant to water deficit than the steppe caryopses, they are able to germinate at a low water potential (- 1.6 Mpa).

**INTRODUCTION**

The Alpha (*Stipa tenacissima* L.) is one of the most important species of the steppe; it is a xerophilous perennial *Poaceae*, endemic to the Mediterranean basin (Cortina & Amghar, 2012). It has ecological importance since it is considered one of the bulwarks against the advancing desert thanks to its highly developed root system stabilizing the soil (Zeriahene, 1987), and, economically the richness in the cellulose of its leaves is used for the manufacture of paper pulp (Mabrouk *et al.* 2012; Labidi *et al.* 2019).

The wide distribution range of this species in the Mediterranean Basin can be explained by the capacity to tolerate a variety of climatic conditions (Boussaid *et al.* 2010; Krichen *et al.* 2017) but the alpha steppes are continuously regressing and we are witnessing a continuous loss of vegetation cover and consequently low plant diversity.

This regression is due to the restrictive climatic conditions, soil salinity (Belkhodja & Benkabilia, 2010), the increasing anthropic pressure and excessive overgrazing to the difficulties of natural regeneration that this species knows, in particular by sowing (Aidoud & Touffet, 1996; Mehdadi *et al.* 2004), to seed predation by ants (Shöning *et al.* 2004) but also to the biological ignorance of this species, in particular on its germinative behavior.

Some works have highlighted the existence of ecotypes in alpha grass. Raffaelli & Ricceri (1989), on the basis of morphological characterization, described three new species in Tunisia that belong to the *S. tenacissima* complex. Nedjraoui (1990), through a study on the adaptation of esparto to water constraints and climatic factors limiting the environment, has demonstrated the existence of two ecotypes, one characteristic of semi-arid regions and the other of pre-Saharan regions. Guemou (2010), and Boussaid *et al.* (2012) on the basis of morphological characterization have contributed to an assessment of the genetic diversity of Alpha in the region of Tiaret.

Moreover, the work of Kadi-Hanifi

(2003) carried out on the biological and phytogeographic diversity of *S. tenacissima* formations in Algeria, shows the existence of a real rise of steppe species towards the North, which explains the installation of this grass in various regions of the northwest of the country including some areas of the coastline according to our personal observations in the field.

In this context and as a complement to these works whose objectives aim at a better understanding of the physiology and biology of alpha plant, we undertook the present study which consists in studying and comparing the germinative behavior of the caryopses of *S. tenacissima* of the littoral and the steppe of the west of Algeria under the effect of saline and hydric stress.

#### MATERIALS AND METHODS

The caryopses studied were taken, on panicles of alpha clumps of two stations of the Algerian west (alpha of steppe: station of Ras El Ma; alpha of the Coastal: station of BéniSaf), whose characteristics are summarized in Table 1.

The collected caryopses were stored in paper bags in the absence of moisture until they were used.

**Table 1:** Characteristics of caryopsis sampling stations.

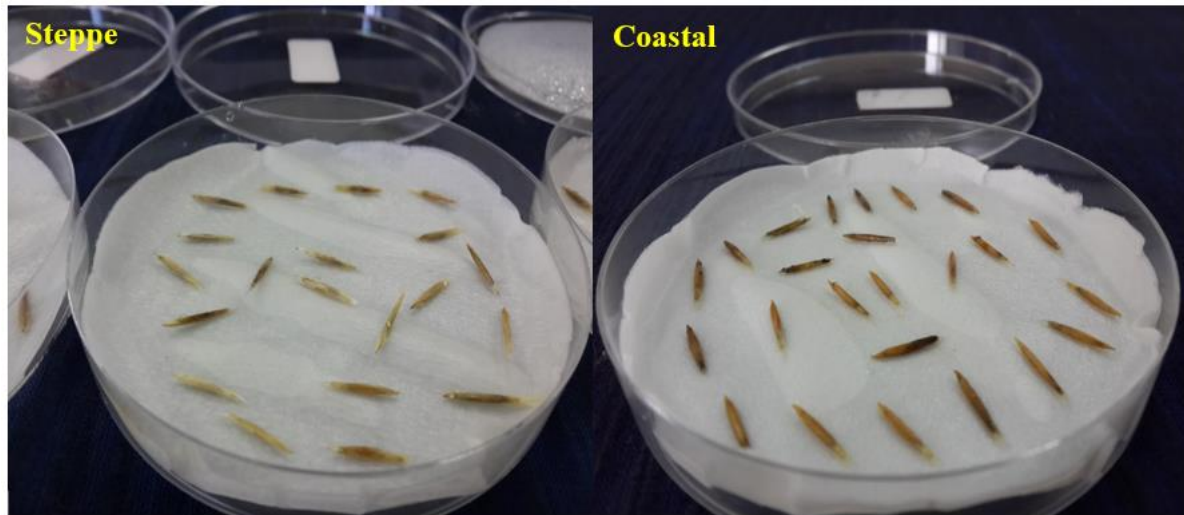
Stations	Bioclimatic stage	GPS coordinates
Steppe of Ras El Ma (Province of Tlemcen)	Arid (ONM, 2020)	N: 34°31'538" W: 000°54'882" Altitude: 1113m
Coastline of Beni-Saf (Province of Temouchent)	Semi-arid superior (ONM, 2020)	N: 35°19'667" W: 001°19'617" Altitude: 533m

ONM: National Office of Meteorology.

#### Preparation of Caryopses:

The caryopses used are sorted, and only the healthiest ones with no apparent morphological anomalies are tested. The caryopses are previously disinfected with 2% sodium hypochlorite and then rinsed with distilled water to remove all traces of chlorine. The tests were conducted in glass

Petri dishes. For each provenance, each germination trial listed below involved four batches of caryopses with 25 caryopses per batch (four replicates) (Fig.1). The germination criterion used is the breakthrough of the envelopes by the radicle (Calone *et al.* 2020).



**Fig.1:** Preparation of the caryopses of the two provenances.

### **Salt Stress Effect:**

This part of the experiment is conducted to determine the tolerance limits of this species to salinity at the germination stage. The caryopses are germinated in a Memmert-type oven in the dark at the continuous optimum temperature of 20°C (Moulessehou, Mehdadi, 2015) under different salt concentrations, prepared from sodium chloride (NaCl):0 (control), 1, 2, 4, 6 and 8 g.L<sup>-1</sup> corresponding to molar concentrations of 0, 17.11, 34.22, 68.44, 102.66 and 136.89 mM. For the control trial, the caryopses were sprayed with distilled water. The duration of the follow-up germination trials was 30 days.

### **Effect of Water Stress:**

Germination tests were conducted under different levels of water potentials by using polyethylene glycol with a molar mass of 6000 (PEG<sub>6000</sub>) (Lu *et al.* 1998).

The effect of water stress was also tested in the dark and at the continuous optimum temperature of 20 °C under different concentrations of PEG<sub>6000</sub>: 0 (control), 5, 10, 20, 40 and 80 g.L<sup>-1</sup> which correspond respectively to water potentials of: 0, - 0.04; - 0.2; - 0.4; - 0.8 and - 1.6 MPa. These water potentials were evaluated according to the equation of Michel *et al.* (1973).

### **Expression of Results and Data Processing:**

The results of the germination tests were expressed by germination capacity (GC) which represents the final percentage of germination and the germination speed or velocity coefficient (VC) (Jones & Sanders, 1987).

The effect of salinity and water deficit on the average of GC and VC was verified by analysis of variance (ANOVA I) using the IBM SPSS Statistics 20 software package.

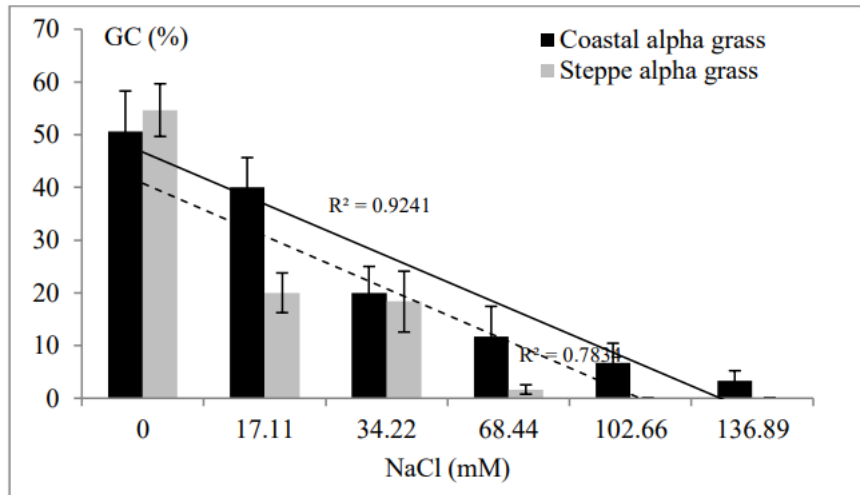
In addition, the relationship showing the evolution of GC and VC with different molar concentrations of NaCl and different water potentials was demonstrated by evaluating the coefficient of determination R<sup>2</sup> using linear regression analysis.

## **RESULTS**

### **Effect of Salinity on GC and VC:**

Figure 2 shows that the GC of caryopses from both provenances gradually decreases with increasing NaCl concentration in the environment. The best GC is registered in the control trials (50.66% for coastal caryopses and 54.66% for steppe caryopses). While the lowest are noticed at 68.44 mM (11.66% for coastal caryopses and 1.66% for steppe caryopses) to be cancelled at 136.89 mM.

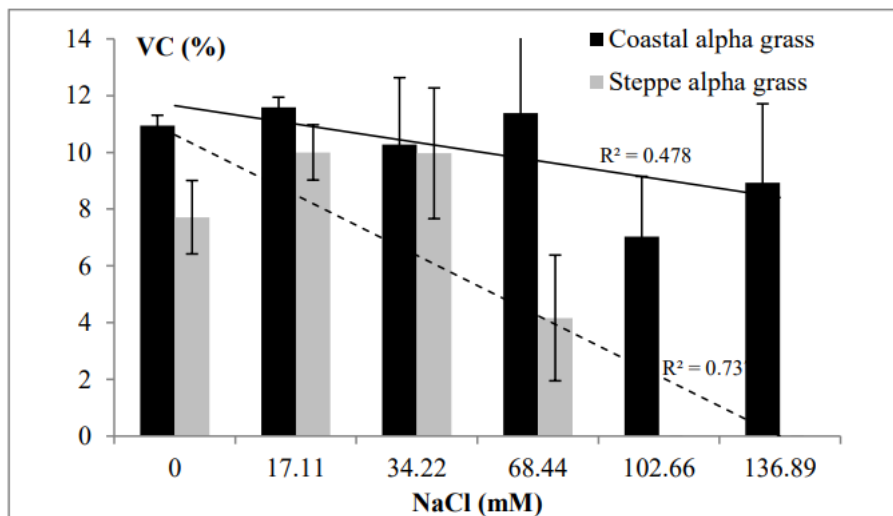
The highly significant effect of salinity on GC and VC was confirmed by analysis of variance (p< 0.001).



**Fig 2.** Effect of different molar concentrations of NaCl on germination capacity.

Figure 3 shows variability in the VC for caryopses from each provenance at the different concentrations tested ( $p < 0.05$ ). For caryopses from the steppe region, it seems that the VC is stimulated by low NaCl concentrations (17.11 and 34.22 mM); it

starts to decline gradually at 68.44 mM to reach the lowest value (4.16%) and is canceled at 102.66 mM. However, the VC of littoral caryopses seems to be uncorrelated with different NaCl concentrations, in that this parameter shows a sawtooth evolution.



**Fig 3.** Effect of different molar concentrations of NaCl on velocity coefficient.

From figures 2 and 3, it appears that coastal caryopses are more tolerant to salinity than steppe caryopses. The tolerance threshold for coastal caryopses is 136.89 mM while that of steppe caryopses is about 68.44 mM.

Analysis of variance revealed significant variability in the average LT characterizing germination of caryopses of

both provenances depending on the salt concentrations tested ( $p < 0.05$ ). The longest latency time is registered in caryopses from the steppe station at 68.44 mM ( $8 \pm 1.3$  days), while for the littoral station it is about 7 days at 138.89 mM.

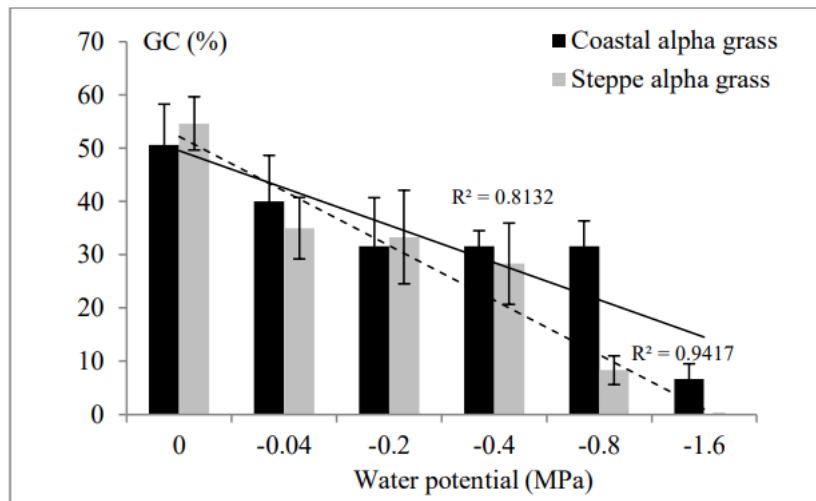
A high relationship between the different molar concentrations of NaCl and GC was demonstrated by the analysis of the

linear regression for the caryopses of the two provenances (coastal caryopses:  $R^2 = 0.92$ ; steppe caryopses:  $R^2 = 0.78$ ). However, for VC, this correlation is important for caryopses of the steppe ( $R^2 = 0.73$ ), while it is weak for those of the littoral ( $R^2 = 0.47$ ).

#### Effect of Water Stress on GC and VC:

Germination capacity was

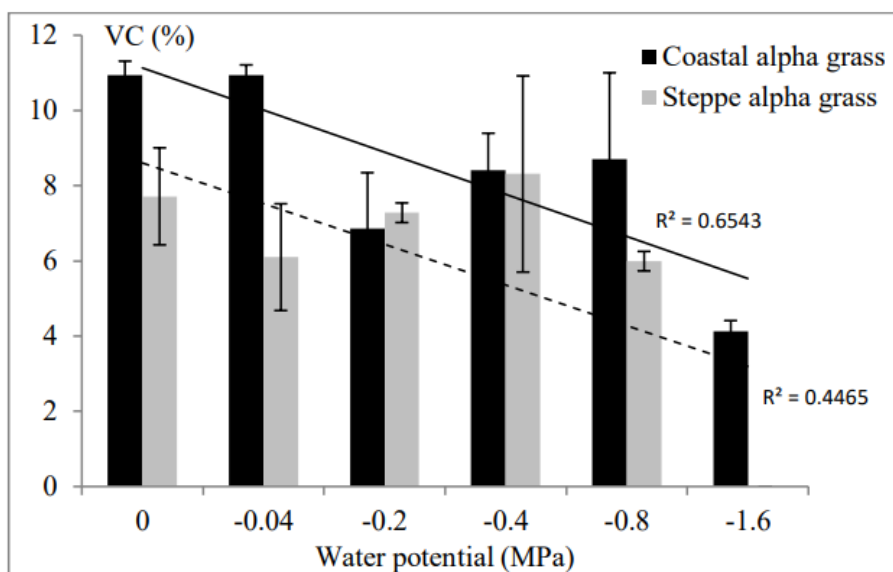
significantly affected and reduced by water stress in caryopses of both provenances ( $p < 0.001$ ). The osmotic potential of  $-1.6$  Mpa corresponds to the threshold beyond which the steppe caryopses no longer germinate, those from the coast are able to germinate at this potential with a low germination percentage (6.66%) (Fig. 4).



**Fig 4.** Effect of different water potentials on germination capacity.

Analysis of variance showed a highly significant difference between the average GC of the caryopses of the two provenances ( $p < 0.001$ ) except for the osmotic potentials of  $-0.2$  and  $-0.4$  Mpa, where this difference was not significant ( $p >$

$0.05$ ). Statistical analysis revealed variability in the VC of caryopses from each provenance ( $p > 0.05$ ). It appears that the VC of steppe caryopses are much more affected by water stress (Fig. 5).



**Fig 5.** Effect of water potential on velocity coefficient.

For the different water potentials tested, the analysis of variance revealed highly significant differences between the caryopses of the two provenances ( $p < 0.001$ ) except for the potentials - 0.2 and - 0.4 Mpa.

The GC values are strongly correlated with the different water potentials with  $R^2 = 0.81$  and  $0.94$ , respectively for the coastal and the steppe caryopses. Whereas for VC,  $R^2$  is moderate to weakly correlated ( $R^2 = 0.65$  and  $0.44$ ).

### DISCUSSION

Salinity can be edaphic, atmospheric, or both. In different ecosystems, in addition to moisture and temperature, salinity can play a crucial role during seed germination (Khan & Gulzar, 2003).

Seed germination is both slowed and inhibited by excess salt in the soil solution because, in addition to the toxic effect of NaCl on tissues, salinity acts by lowering water and osmotic potential, affecting carbohydrate metabolism, and increasing the need for water in this phase (Prado *et al.* 2002).

The results obtained show that salinity affects and reduces the germination capacity and the velocity coefficient of alpha grass caryopses. This agrees with the results obtained by Lamara *et al.* (2016) and Nedjimi *et al.* (2014) on *Lygeumspartum* L., *Poaceae* living in association with *Stipa tenacissima* L.

The comparative study of the germinative behavior of the caryopses of the two provenances reveals that the caryopses of the coastal region are particularly tolerant and are able to germinate at a molar concentration of 136.89 mM of NaCl, registering an average germination capacity of 3.33%, while germination of caryopses from the steppe region is inhibited above a molar concentration of 68.44 mM of NaCl. The inhibitory effect of NaCl begins to manifest itself above the molar concentration of 17.11mM and becomes more and more accentuated at 136.89 mM. The salinity becomes very probably lethal or toxic from this concentration for the most tolerant

caryopses (the caryopses of the littoral region).

The toxic effect of salt is manifested following the accumulation of  $\text{Na}^+$  in the embryo installing an osmotic inhibition (Bliss *et al.* 1986) by acting on a hormonal activity like that of the particular abscisic acid, a key hormone in the passage of the seed from the slowed life to the active life. This enzyme inhibits the synthesis of germination-specific enzymes, limits water uptake and regulates cell osmotic pressure (Luttge *et al.* 2002; Hopkins, 2003).

During salt stress, metabolites resulting from the degradation of reserves, and particularly carbohydrate compounds, would constitute potential osmotic regulators (Larheret al.1982), the embryo can pass to the germinative state if the salinity is not very accentuated. When salinity is high, the slowing down of germination is more important. For some authors, this relationship characterizes both glycophytes and halophytes. Salinity tolerance changes with species and phenological stages (Hopkins, 2003).

Our investigation revealed that caryopses from the coastal region (Béni-Saf) are more tolerant to salinity than caryopsis from the steppe region (Ras El Ma). Several authors like Khalid *et al.* (2001), Lamara *et al.* (2016) presented similar results regarding the variability of salinity tolerance threshold in different genotypes. For example, Khalid *et al.* (2001) showed that for two chickpea genotypes under salt stress, germination capacity decreased compared to the control by more than 60% in seeds of both provenances. Moreover, the work of Lamara *et al.* (2016) on two provenances of *Lygeumspartum* L. (coastal and steppe) of western Algeria revealed that the caryopses of Ain Skhona and Maamora (steppe region) tolerate salinity better than the caryopses of Sidi Djeloul (littoral region).

The delay in germination caused by increasing salt concentrations in the irrigation water would result from a difficulty in hydration of the caryopses as a

result of high osmotic potential and can be explained by the time required for the caryopses to set up mechanisms allowing them to adjust its internal osmotic pressure. This supposition has been certified by several authors (Smaoui *et al.* 1986; Dadach *et al.* 2021).

It has been shown that the salinity/temperature combination should be considered in the interpretation of the effect of salinity on seed germination because the toxic effect of NaCl is increased when the temperature is higher (Ignaciuk & Lee, 1980).

The more or less important tolerance of the caryopses of the coastal region to salinity can be explained by the fact that the clumps of alpha grass on which these caryopses were taken, grow on a substrate with a higher electrical conductivity than the one we measured on the soil of the steppe region. In this sense, Neffati (1994) points out that knowledge of salinity tolerance at the time of germination is useful but not sufficient information to explain the distribution of species and their development in saline environments. This difference may also be related to genetic variability as demonstrated in the work of Boussaid *et al.* (2012).

In addition to temperature and salinity, water stress affects the germination of alpha grass caryopses of both provenances. For this species, soil water affects germination more than any other parameter. Indeed, water represents the initial factor for the imbibition of caryopses and their germination, especially in arid and semi-arid regions (Cavalcante & Perez, 1995).

The results obtained in this study showed that with increasing osmotic pressure, the germination speed decreased. The response of caryopses of the two provenances to stress is different. Indeed, at -0.8 MPa germination is strongly reduced and then completely inhibited at -1.6 MPa in caryopses from the steppe region. For the same water potential, germination is still possible but very reduced in caryopses from

the coastal region.

Similar results were revealed by the work of Krichen *et al.* (2014, 2017) regarding the effect of water stress on the germination of *S.tenacissima* L. caryopses from five provenances in western Tunisia. Several other works on the *Stipa* genus confirm our results, for example, those of Neffati *et al.* (1997) on *Stipa lagascae*, Bonvissuto, Busso (2007) on *Stipa neaei*; and Hu *et al.* (2013) on *Stipa bungeana*, all recorded a strong reduction in the germination capacity as the osmotic potential increases in the environment.

Also, the same results were recorded in the work of Zemour (2014) on eight different genotypes of durum wheat (*Triticum durum* Desf.) and Oukarroum (2007) on the vitality of barley plants (*Hordeum vulgare* L.) under water and thermal stress conditions. The different studies on the effect of water stress on germination in other species like *Acacia tortilis* L. (Jaouadi *et al.* 2010) and *Marrubium vulgare* L. (Dadach *et al.* 2018) also revealed similar results where the germination rate is reduced in the high osmotic potential environment.

The decrease in the water potential of the germination environment induced a very pronounced slowing down of the VC in the caryopses of both provenances. The delay recorded in the report of germination and the reduction of its rate would result from a reduction in the acuity of water intake and which would be compensated by an osmotic adjustment resulting from the release of the reducing sugars resulting from the degradation of starch. These results confirm many works (Hardegre & Emmerich, 1994) which show that the inhibition of seed germination would result in particular from a difficulty of hydration of the tissues, which is reflected in the process of elongation of the radicle and difficulty of the penetration of the water in the seed, so that osmotic adjustment is not carried out in a favorable way.

The difference in water stress tolerance thresholds between the caryopses



of the two provenances can be attributed to the difference in the power of metabolite accumulation and osmotic adjustment for the maintenance of cell turgor and physiological activities. An increase in cells by maintaining osmotic force balance by keeping turgor and cytosolic volume as high as possible and by preserving membrane integrity in desiccated organs (Darbyshire, 1974).

So, this capacity to germinate in saline conditions and this tolerance to aridity represent very important characteristics for the rehabilitation and the regeneration of our species, namely the one coming from the coastal region, which is also interesting to exploit eventually to valorize the marginalized soils and concerned by the salinization.

## CONCLUSION

The comparative physiological study realized on the steppe and coastal alpha grass allowed us to characterize the germinative behavior of the alpha caryopses of each of these two ecotypes by highlighting the effect of salinity and water stress. The study revealed that:

- Coastal caryopses are more tolerant to salinity than steppe caryopses insofar as the tolerance threshold for coastal caryopses corresponds to 136.89 mM of NaCl whereas that of steppe caryopses is around 68.44mM.
- Compared to the alpha grass caryopses of the coast, those of the steppe are much more resistant to water deficit as they are able to germinate at a low water potential of - 1.6 Mpa. The tolerance threshold for coastal caryopses is - 0.8 Mpa.

All the results obtained have made it possible to complete the corpus of data built up in relation to alpha and especially to identify some points of difference between the ecotypes studied.

On the basis of these preliminary results, it would be desirable to deepen and complete this work, using advanced techniques like molecular analysis (PCR), to identify the origin of the physiological variability recorded between these two alpha

grass provenances.

These data obtained will certainly be used in the context of the conservation of the species studied and in the ex-situ production of plants for the fixation and stabilization of steppe and coastal soils.

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