

Analysis of the state of floristic diversity by the comparison of germination between two native species *Atriplex halimus* and *Lygeum spartum*; sustainability of the steppe landscape of western Tlemcen Algeria

Análise do estado da diversidade florística por meio da comparação da germinação entre duas espécies nativas *Atriplex halimus* et *Lygeum spartum*; sustentabilidade da paisagem de estepe do ocidental de Tlemcen Argélia

Análisis del estado de la diversidad florística mediante la comparación de la germinación entre dos especies nativas *Atriplex halimus* y *Lygeum spartum*; sostenibilidad del paisaje estepario del Tlemcen occidental de Argelia

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ABSTRACT

This article proposes a comparison of the germination of two halophytes. *Atriplex halimus* and *Lygeum spartum* subjected to different concentrations of Na + Cl⁻ (2g / l, 4g / l, 5g / l, 6g / l, 7g / l, 9g / l) and in different culture media (Nutrient agar, Mac Conkey, Mannitol Mobility, Chapman, PDA).

To remove the dormancy of the seeds, four pretreatments were carried out: scarification with razor blade, distilled water, boiling water and sulfuric acid.

The seeds exhibit various behaviors with respect to the concentration of Na⁺ Cl⁻ and the chemical composition of culture medium. It suggests that *Lygeum spartum* supports high salinity compared to the *Atriplex halimus*. Thus, the PDA culture medium accelerates the germination of the seeds because of its richness in phosphorus.

Keywords: germination, stress, salinity, poaceae, halophytes.

RESUMO

Este artigo propõe uma comparação da germinação de duas halófitas. *Atriplex halimus* e *Lygeum spartum* submetidos a diferentes concentrações de Na + Cl⁻ (2g/l, 4g/l, 5g/l, 6g/l, 7g/l, 9g/l) e em diferentes meios de cultura (Ágar Nutriente, Mac Conkey, Mobilidade de Manitol, Chapman, PDA).

Para retirada da dormência das sementes foram realizados quatro pré-tratamentos: escarificação com lâmina de barbear, água destilada, água fervente e ácido sulfúrico.

As sementes apresentam comportamentos variados em relação à concentração de Na⁺ Cl⁻ e à composição química do meio de cultivo. Isso sugere que *Lygeum spartum* suporta alta salinidade em comparação com *Atriplex halimus*. Assim, o meio de cultura BDA acelera a germinação das sementes devido à sua riqueza em fósforo.

Palavras-chave: germinação, estresse, salinidade, poaceae, halófitas.

RESUMEN

Este artículo propone una comparación de la germinación de dos halófitas. *Atriplex halimus* y *Lygeum spartum* sometidos a diferentes concentraciones de Na+Cl⁻ (2g/l, 4g/l, 5g/l, 6g/l, 7g/l, 9g/l) y en diferentes medios de cultivo (Agar nutritivo, Mac Conkey, Manitol Mobility, Chapman, PDA). Para eliminar la latencia de las semillas se realizaron cuatro pretratamientos: escarificación con navaja, agua destilada, agua hirviendo y ácido sulfúrico.

Las semillas exhiben diversos comportamientos con respecto a la concentración de Na⁺ Cl⁻ y la composición química del medio de cultivo. Sugiere que *Lygeum spartum* soporta una alta salinidad en comparación con *Atriplex halimus*. Así, el medio de cultivo PDA acelera la germinación de las semillas debido a su riqueza en fósforo.

Palabras clave: germinación, estrés, salinidad, poaceae, halófitas.

1 INTRODUCTION

Salinity plays a predominant role. The abundance and richness of species are negatively correlated with the electrical conductivity of the soil (Canadas et al., 2010). However, the existence of temporal spatial salinity gradients has traditionally been considered one of the most important factors in these physical environments to influence the distribution of plants (Chapman, 1974).

Subsequent work (Marshner, 1986) shows that inhibition of the growth of salt-sensitive species is mainly due to the toxicity of ions even at low concentrations of salts. In fact, the term salt stress applies mainly to an excess of ions, in particular, but not exclusively, to Na⁺ and Cl⁻ ions.

It is often difficult to separate the effects of high salinity from those generated by low water potential or complex interactions with other ions (De Boeck et al., 2003). Therefore, understanding the functioning of the ecosystem requires knowledge of the modes of growth and production that are fundamental aspects of the response of individuals or plant communities to the conditions of the environment (Cornet, 1981).

The aim of this work is to compare the seed germination of two halophytic species *Atriplex halimus* L. (Amaranthaceae family) and *Lygeum spartum* L. (Poaceae family) to salt stress. The germination of the seeds was carried out in vitro, following their kinetics of germination and the effects of the culture media. Very little work of this kind (germinative comparison of seeds subjected to different treatments) was carried out. Can salt concentrations and synthetic culture media promote or inhibit this phenological stage of the plant?

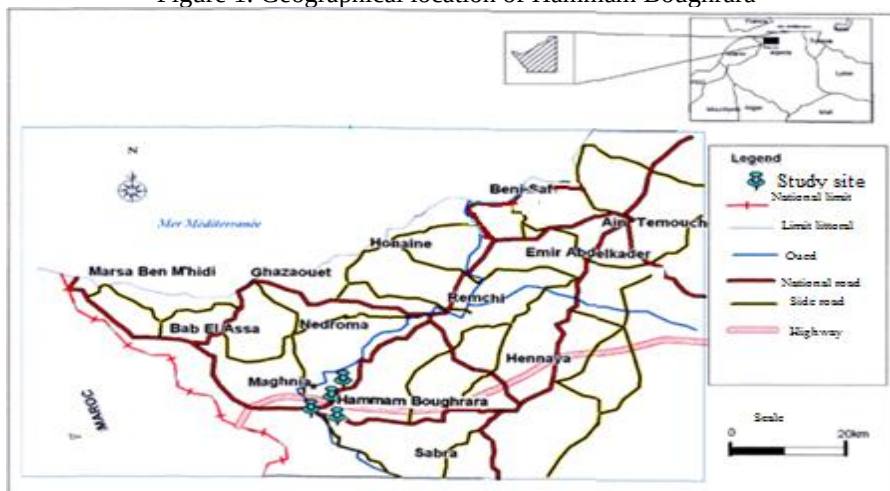
2 MATERIALS AND METHODS

2.1 ENVIRONMENT AND LOCATION

The area of Hammam Boughrara is situated in the North West of Western Algeria latitude 34 ° 54' et 37'de 1 ° longitude west. It rises at an altitude of about 280 m. It belongs to the wilaya of Tlemcen. This zone is characterized by low slopes (<10%), a rather sparse herbaceous vegetation and a friable substrate of sandy loam texture (72% sand), a large amount of CaCO₃ (higher than 25%), a low alkaline pH 23.57%), a high salinity of 1.5 Ms / CM, a low organic matter content of 0.5% -0.81% and a soil color accorded by the Munsell code: (5/3 5YR), (4/3 5YR).

The study area is characterized by summer drought and high temperatures also abundant autumn rainfall. The minimum is recorded in July and August, thus constituting a summer low characteristic of the Mediterranean climate.

Figure 1. Geographical location of Hammam Bouhrara



Source: Prepared by the authors themselves.

2.2 PRE-TREATMENT OF SEEDS

To remove seed dormancy from the seeds, four pretreatments were performed as follows:

- The first is manual scarification (cracking seeds) using a razor blade,
- The second pretreatment consists in imbibing the seeds with distilled water,
- The third is the soaking of the seeds in boiling water for 3 mn,
- The fourth is the immersion of the seeds in dilute sulfuric acid for 3 mn.

2.3 METHODOLOGY

2.3.1 Experience 1

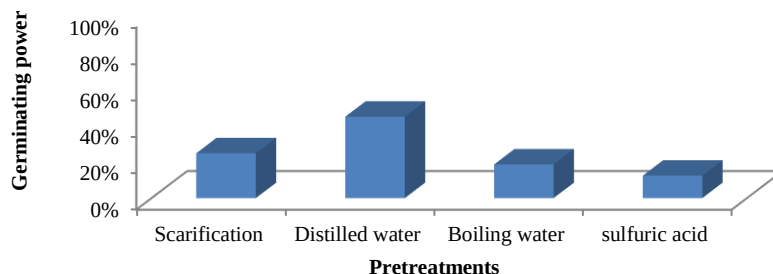
Seeds of *Atriplex halimus* and *Lygeum spartum* are placed in petri dishes like 10 seeds per box, repeated 3 times and then subcultured in the following culture media: Nutritive Agar, Mac Conkey, Mannitol Mobility, Chapman, and Potato Dextrose (PDA).

2.3.2 Experience 2

The seeds of *Atriplex halimus* and *Lygeum spartum* are put in Petri dishes like 10 seeds per box, repeated 3 times and treated with NaCl (sodium chloride) at various concentrations: 2 g / l, 4 g / l, 5 g / l, 6 g / l, 7 g / l, 9g / l, in order to determine the threshold likely to impede germination. These seeds from the two experiments subjected to pretreatment (Scarification, distilled water, boiling water, sulfuric acid) were placed in an oven at 25 ° C. $\pm$ 2 ° C. and then germinated. Every two days, the germinated seeds are counted (corresponding to the piercing of the teguments by the radical) for a period of 30 days. Recalling that seed germination is considered positive when the radicals reach 5 mm in length (Kaur et Duffus, 1989).

3 RESULTS

Figure 2: Power germination of the seeds of *Atriplex halimus* and *Lygeum spartum* for each pretreatment



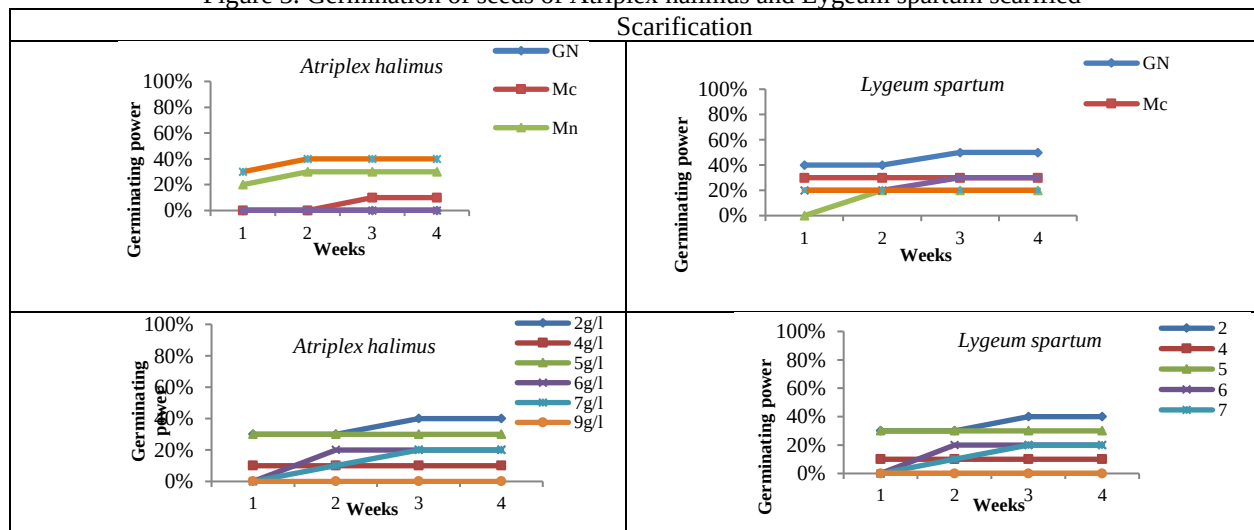
Source: Prepared by the authors themselves.

The results of these pre-treatments (presented below) show a difference in germination (Figure 2) of the seeds having undergone the various treatments:

- Distilled water was the most efficient with 44.62%,
- In second position we have the scarification with 24.61%, the latter facilitates the imbibitions of the seeds and the fast water intake by the embryo which induces the germination and the growth.
- The treatment with boiling water occupies the third position with 18.46%, this technique softens the integuments and favors the triggering of the germination.
- The germination rate treated with dilute sulfuric acid occupies the last position with 12.31%.

3.1 SCARIFICATION

Figure 3. Germination of seeds of *Atriplex halimus* and *Lygeum spartum* scarified



Source: Prepared by the authors themselves.

Placed in different culture media and treated by increasing saline concentrations

➤ ***Atriplex halimus***

- Nutrient agar (GN): For this culture medium no germination is recorded.
- Mac Conkey (Mc): The culture medium Mc triggers germination from the 2nd week with 10% and stabilizes until the 4th week 10%.
- Mannitol Mobility (Mn): This culture medium Mn increases the germinate power from the 2nd week and stabilizes it until the 4th week at 30%.
- Chapman (CH): No germination is recorded for this culture medium.
- Potato Dextrose (PDA): This shows that the germinating power increases from the 1st to the 2nd week from 30% to 40%.

This last percentage does not change until the fourth week.

The best result is obtained with the PDA for the species *Atriplex halimus* (40%), in other media germination is difficult (10%) or sometimes does not occur.

➤ ***Lygeum spartum***

- Nutrient agar (NG): 40% of the seeds germinated during the first week. This rate increases and stabilizes during the 3rd and 4th week at 50%.
- Mac Conkey (Mc): The germination is 30% in the first week and will not increase in the other weeks.
- Mannitol Mobility (Mn): The seeds sown in the Mn culture medium begin to germinate after the second week (20%). This percentage will not change during the remaining weeks (3rd and 4th).
- Chapman (CH): The germination in the CH culture medium starts from the 2nd week with a percentage of 20%. This rate increases the 3rd week to stop at 30% in the 4th.
- Potato dextrose (PDA): 20% of germination obtained at the first week and stabilizes during the successive weeks (2nd, 3rd and 4th).

The best result is obtained with nutrient agar for *Lygeum spartum* (40%). In the other media, the germination decreases substantially to 30% for the medium Mc and 20% for the Mn.

➤ ***Atriplex halimus***

The germination fluctuates from one treatment to another (increasing concentrations of NaCl) during the different weeks as follows:

- 2 g / l: The germination rate obtained from the 2nd week is 30%, this one does not undergo any change until the fourth week.
- 4 g / l: 10% germination is recorded from the first week, which is not changed until the fourth week.
- 5g / l: 30% germination obtained from the first week, this rate does not undergo any change until the fourth week.
- 6g / l: From the 2nd week onwards, 20% germination is obtained, which is not changed until the fourth week.
- 7g / l: 20% germination is registered from the 3rd week, this percentage does not change.
- 9g / l: Germination did not occur at this high concentration.

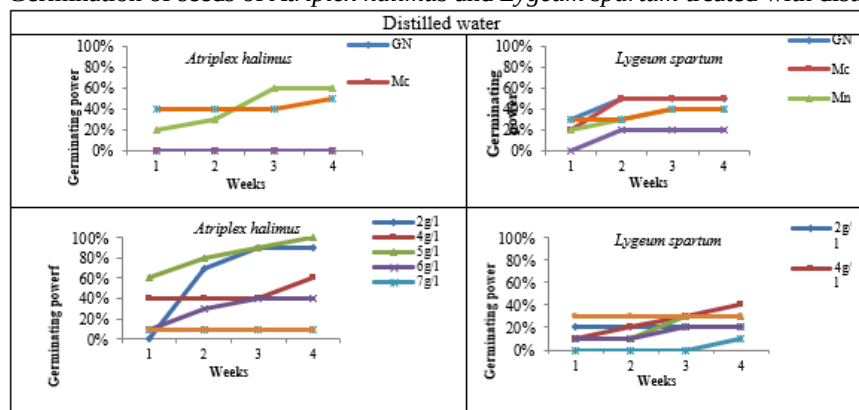
For all these treatments on the *Atriplex halimus*, the germination initiated in front of the concentrations with 10 to 30% in the first weeks will not undergo any variation during the rest of the time. The NaCl concentration at 9 g / l completely inhibits germination.

➤ *Lygeum spartum*

- 2g / l: Germination did not occur at this concentration.
- 4g / l: The germination did not occur at this concentration either.
- 5 g / l: 10% germination obtained from the first week, then it rose to 30% during the third week, and stabilized at the fourth week.
- 6 g / l: 10% germination is obtained from the first week; this percentage does not change until the fourth week.
- 7g / l: From the first week 20% of germination is obtained, this one does not undergo any change until the fourth week.
- 9g / l: Germination did not occur at this high concentration.

3.2 DISTILLED WATER

Figure 4. Germination of seeds of *Atriplex halimus* and *Lygeum spartum* treated with distilled water.



Source: Prepared by the authors themselves.

Distilled water placed in different culture media and treated by increasing saline concentrations

The best germination is obtained with the concentration 5 g / l for the species *Lygeum spartum* (30%), in the other concentrations the germination is hardly done (10%) or sometimes does not take place at all.

➤ ***Atriplex halimus***

- Nutrient agar (GN): For this culture medium no germination is recorded.
- Mac Conkey (Mc): there is no germination for this culture medium.
- Mannitol mobility (Mn) : This culture medium shows that the germinative power increases from the 1st to the 2nd week from 20% to 30%, this rate increases the 3rd week to stop at 60% in the 4th week.
- Chapman (CH): No germination is recorded for this culture medium.
- Potato Dextrose (PDA): For the PDA culture medium the seeds germinated from the 3rd week (40%) to stabilize at the 4th week at 50%.

The best germination for *Atriplex halimus* is obtained with Mn culture media (60% at the 4th week) and PDA (50% at the 4th week), in other media there is little (10%) or no germination.

➤ ***Lygeum spartum***

- Nutrient agar (GN): 30% of the seeds germinated during the first week and 50% during the second week, this rate stabilizes at the 4th week.
- Mac Conkey (Mc): This culture medium shows that the germinating power increases from the first to the second week, from 20% to 50%, and stabilizes at the 4th week.
- Mannitol mobility (Mn): 20% of germination is obtained from the first week, then 30% during the second week and 40% during the third week; it will remain until the fourth week.
- Chapman (CH): From the first week 20% of seeds germinate, this rate will not change until the fourth week.
- Potato dextrose (PDA): 30% of germination is obtained from the first and the second week; this rate successively rises and stabilizes at 40% at the 3rd and 4th week.

The best germination for *Lygeum spartum* is obtained with GN culture media (50% at the 4th week), Mc (50% at the 4th week) and 40% (3rd and 4th week) for the PDA, at the other media showed little germination (20%).

➤ ***Atriplex halimus***

- 2 g / l: Germination begins in the 2nd week with 70% germination, and then 90% in the third week, this percentage remains unchanged until the fourth week.
- 4g / l: 40% of seeds germinates from the first week and stay until the 3rd, then this rate is 60% at the 4th week.
- 5 g / l: 60% germination is obtained from the first week, then it reaches 80% during the 2nd week, and 90% during the 3rd week and reaches 100% during the 4th week.
- 6 g / l: From the first week, 10% germination is obtained, then 30% during the 2nd week, and 40% at the 3rd week, there is no change until the fourth week.
- 7g / l: We have 10% germination from the first week; this one does not undergo any change until the fourth week.
- 9g / l: 10% germination from the first week, this one will remain at a constant level until the fourth week.

The best germination for the species *Atriplex halimus* is obtained with the concentration 5g / l (100%) and 2g / l (80%), in the other concentrations there is between 10% and 60% germination.

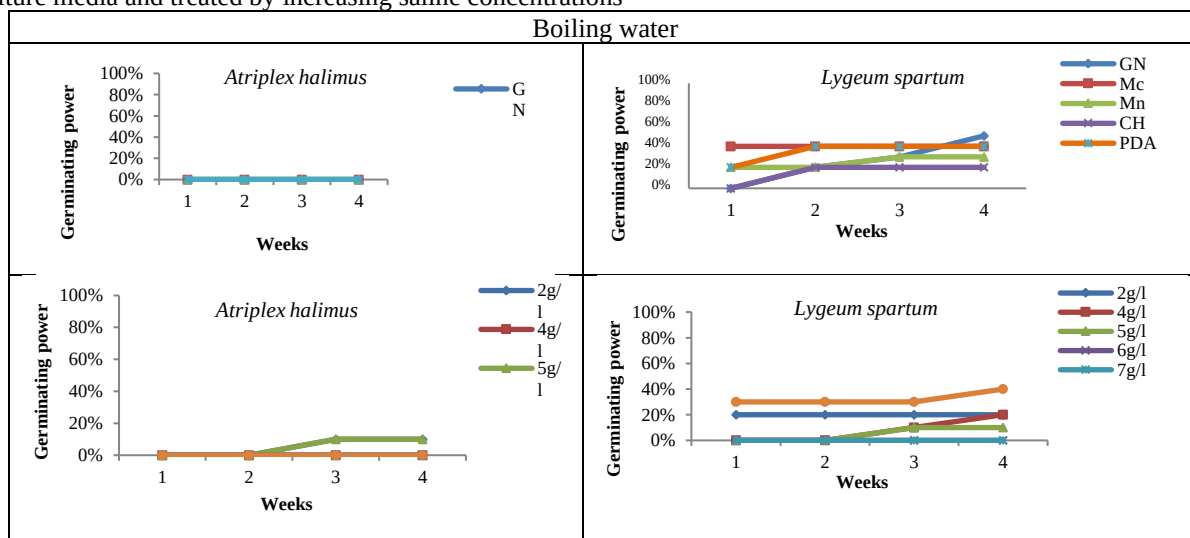
➤ ***Lygeum spartum***

- 2g / l: 20% germination is obtained from the first week, this figure remains constant until the fourth week.
- 4g / l: 10% germination obtained from the first week, then it amounts to 20% during the 2nd week to reach respectively 30% and 40% during the 3rd and 4th week.
- 5g / l: 10% germinate from the 1st week, this rate remains stable until 2nd week, then it rises to 30% during the 3rd week and will not undergo any change until the fourth week.
- 6 g / l: 10% germination is obtained from the first week, this rate remains stable until the second week, amounts to 20% at the 3rd week to remain constant at the fourth week.
- 7g / l: No germination 0% is obtained during the first 3 weeks; it is only at the 4th week that germination starts (10%).
- 9g / l: 30% seeds germinate at week 1, this percentage does not change until the fourth week.

The best germination for the species *Lygeum spartum* is obtained with the concentration 4 g / l (40% obtained at the 4th week), in the other concentrations there is between 10% and 30% germination.

3.3 BOILING WATER

Figure 5. Germination of seeds of *Atriplex halimus* and *Lygeum spartum* treated with boiling water placed in different culture media and treated by increasing saline concentrations



Source: Prepared by the authors themselves.

➤ *Atriplex halimus*

For all of these culture media Nutrient agar (GN) Mac Conkey (Mc) Mannitol mobility (Mn) Chapman (CH) Dextrose of potato (PDA) no germination is recorded.

➤ *Lygeum spartum*

- Nutrient agar (GN): The germination starts from the 2nd week 20%, then it rises to 30% during the 3rd week and reaches 50% during the 4th week.
- Mac Conkey (Mc): 40% germination is obtained from the first week; this rate will remain stable until the fourth week.
- Mannitol mobility (Mn): 20% germination obtained from the first week, this rate remains stable until 2nd week, then it is 30% and remains unchanged at the fourth week.
- Chapman (CH): The germination starts from the 2nd week (20%), this one will not undergo any change during the other weeks.
- Potato Dextrose (PDA): 20% of seeds germinate at the first week, at the 2nd week and then it rises to 40% and stabilizes during the other weeks.

The best germination for *Lygeum spartum* is obtained with GN media (50% at the 4th week), Mc and PDA (40% at the 4th week), in other media the percentage of germination varies between 20 % - thirty).

➤ ***Atriplex halimus***

- 2g / l: The germination takes place from the 3rd week (10%) and will remain stable at the fourth week.
- 5g / l: From the 3rd week the seeds germinate (10%) this rate will remain stable at the fourth week.
- Germination did not occur at concentrations: 4g / l, 6g / l 7g / l and 9g / l.

Germination for the species *Atriplex halimus* is obtained only at 2g / l and 5g / l (10% at 4th week), in the other concentrations, no germination is recorded.

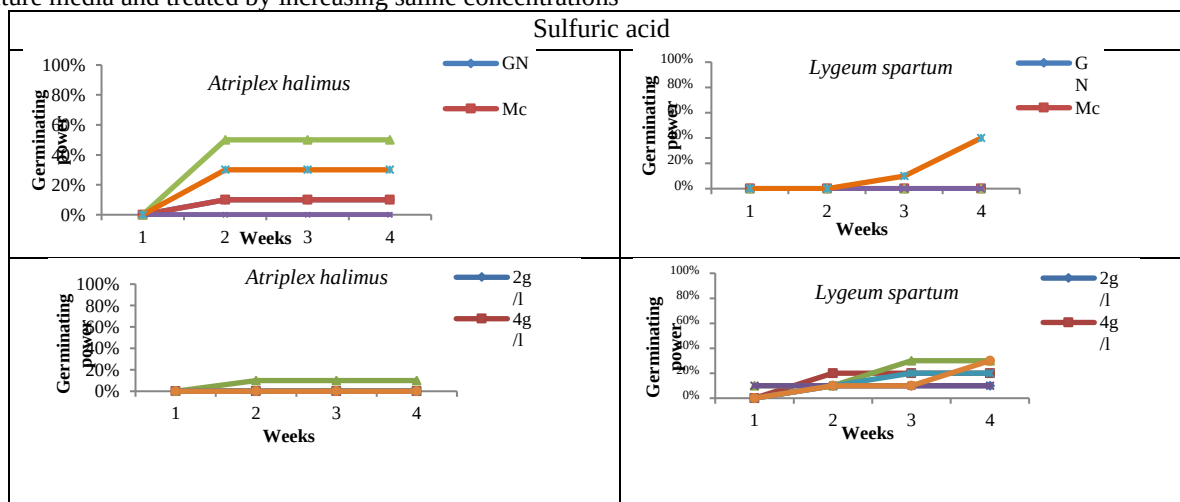
➤ ***Lygeum spartum***

- 2g / l: 20% germinate from the first week, this rate remains stable until 4th week.
- 4g / l: The germination takes place from the 3rd week (10%), this one will not undergo any change during the other weeks.
- 5g / l: The germination takes place from the 3rd week (10%), this rate will not change during the other weeks.
- 6g / l: No germination is recorded.
- 7g / l: There is also no germination.
- 9g / l: 30% germinates from the first week, this rate remains stable until 3rd week, and then it is 40% during the 4th week.

The best germination for the species *Lygeum spartum* is obtained with concentrations 9g / l (40% at the 4th week), in the other concentrations it varies between 10% and 20%.

3.4 SULFURIC ACID

Figure 6. Germination of seeds of *Atriplex halimus* and *Lygeum spartum* treated with sulfuric acid placed in different culture media and treated by increasing saline concentrations



Source: Prepared by the authors themselves.

➤ ***Atriplex halimus***

- Nutrient agar (GN): 10% germinate at the 2nd week; this rate will remain stable until the fourth week.
- Mac Conkey (Mc): 10% germinate from the 2nd week; this percentage will remain unchanged until the fourth week.
- Mannitol mobility (Mn): 50% germinate at the 2nd week, this rate will remain stable until the fourth week.
- Chapman (CH): No germination is recorded for this culture medium.
- Dextrose potato (PDA): 30% germinate at the 2nd week; this percentage will remain stable until the fourth week.

For *Atriplex halimus*, germination is obtained with Mn culture media (50% at the 4th week), PDA (30% at the 4th week), in the other concentrations it is low and varies between 0% and 10 %.

➤ ***Lygeum spartum***

No germination is obtained for these culture media: Nutrient agar (GN), Mac Conkey (Mc), Mannitol mobility (Mn), Chapman (CH).

- Potato Dextrose (PDA): For this medium germination occurs during the 3rd week (10%), this figure will remain stable during the other weeks.

For the *Lygeum spartum* species germination is low and obtained with the PDA culture medium (10% at the 4th week), in the other culture media, no germination is observed.

➤ ***Atriplex halimus***

- No germination occurs for 2g / l, 4g / l, 6g / l, 7g / l and 9g / l.
- 5g / l: Germination begins on the 3rd week (10%), this percentage will remain stable until the end.

The percentage of germination is low (10%) and occurs only at the 4th week with the concentration at 5 g / l.

➤ ***Lygeum spartum***

- 2g / l: Germination starts at the 2nd week (10%), this figure will remain stable until the end.
- 4g / l: The germination is obtained from the first week (0%), and then it rises to 20% during the 2nd week so as not to undergo any change during the other weeks.
- 5 g / l: 10% germination obtained from the first week, this rate remains stable until 2nd week, and then it rose to 30% during the 3rd week, at the fourth week.

- 6g / l: 10% seeds germinate from the first week, which will not change until the fourth week.
- 7g / l: The germination is obtained from the 2nd week 10%, and then it rises to 30% during the third week and will not change during the fourth week.
- 9g / l: The germination is obtained from the 2nd week 10%, and then it rises to 30% during the 4th week.

The best germination for the species *Lygeum spartum* is obtained with concentrations 5g / l and 9g / l (30% at the 4th week), in the other concentrations it varies between 10% and 20%

4 DISCUSSION AND CONCLUSION

The mechanical scarification carried out manually on the seeds allows the germination of nearly 30% of the seeds to be germinated. So, it seems that this will allow better access to water for the embryo; although, hydration is important for humidification of the embryo. Thus, the absorbed water must allow a homogeneous modification of the tissues while minimizing the growth and respiration of the embryo (Chapon, 1960).

Seeds with better germination were those treated with distilled water (Rémi, 2003). It is said that imbibing the seeds in distilled water for 24 hours is an effective way to achieve good germination. These results confirm the results obtained by several other authors concerning seed germination (Grousis, 1973; Platt- aloia et Thomson, 1986; Dorgham, 1989; Ismail, 1990; Neffati, 1994 ...) and have indicated that the seeds of most halophytes reach their maximum germination in distilled water.

In the warm water the germination treatment reached 20%. This treatment makes it possible to remove the waxy cuticle from the seeds, it is an effective way to soften the seed coats and to reduce the impermeability to water (Li et Foley, 1997; Aduradola et Badru, 2004; Roos, 1980; Tran et Cavanagh, 1984).

As the concentration of NaCl increases in the solution, the rate and germination capacity of the *Atriplex halimus* seeds decreases. Reduction of germination rates under saline conditions could be attributed to the increase in the osmotic pressure of the surrounding environment which decreases the rate of water absorption by the seed (Prisco et Oleary, 1970). Causing water stress and an inhibition of the mobilization of seed reserves for the growth of the embryo. Subsequent work, (Prisco *et al.*, 1981), (Prodo *et al.*, 2000) showed that this decrease in the germination rate of the seeds subjected to a salt stress is due to a process of osmotic dormancy developed under these conditions of stress, and thus representing a strategy of adaptation with respect to environmental constraints.

Inhibition of the growth of salt-sensitive species is mainly due to the toxicity of ions even at low salt concentrations (Marshner, 1986). In *Lygeum spartum*, however, this increase in NaCl concentration stimulates germination and seed development, particularly in poaceae. These results are confirmed in similar works (Sibi et Fakiri, 2000).

Moreover, chemical scarification by sulfuric acid is less effective compared to other treatments, of which we have recorded a low germinate power. This explains why this substance would destroy the embryo of the seeds and thus prevent their germination (Joshi et Pant, 2010). It has also been shown that $\text{H}_2^+\text{SO}_4^-$, while improving germination of seeds (experiment on *Canna indica* L.). Their viability when the immersion time becomes long. Thus, germination of barley can occur in the presence of high concentrations of 10 g / l of NaCl, which can confirm the high salt resistance in poaceae.

It is recognized that seed germination decreases with time, even under ideal and constant environmental conditions (Bewley et Black, 1994) (Hilli *et al.*, 2003). This explains our seed germination result of *Atriplex halimus* and *Lygeum spartum* in Nutrient agar media as well as Mannitol mobility.

It appears that the PDA culture medium accelerates seed germination because of its high phosphorus content. This is an indispensable element for the development of crops in the early stages of growth (Gervy, 1970). This PDA medium also promotes the multiplication of fungi that attack seeds. This may explain their inability to germinate. The germination in Mac Conkey culture was too low, this is due to the acidity of the medium by fermentation of lactose. This acidity causes the lesion of the tissues with formation of necroses and losses of mineral substances (Rémi, 2003).

In a medium rich in NaCl, Chapman (75g) germination in the first and second week was low, while during the third and fourth week the germination was important (Benmiled *et al.*, 1986). The study of the kinetics of germination shows that the salinity delays the germination and slows down its speed. This delay corresponds to the time required for the seed to set up mechanisms allowing it to adjust its internal osmotic pressure. Stress tolerance requires that the organism be in thermodynamic equilibrium with stress, which means that conditions in the plant are in balance with the conditions of the external environment (Hopkins, 2003).

The comparison of seed germination and the emergence of the vegetative apparatus of the species of *Atriplex halimus* and *Lygeum spartum* in the presence of NaCl show very highly significant differences with respect to salinity tolerance. The germinations of seeds of *Lygeum spartum* in 9 g / l of NaCl and in a nutrient medium Chapman at 75 g / l of NaCl follow a kinetics characterized initially by a remarkable increase in the percentage of germination. On the other hand,

the germination kinetics of *Atriplex halimus* showed the opposite, where it tolerates low concentrations of NaCl (0 and 5 g / l).

Thus, it seems that the germination is stimulated by the presence of a medium rich in nutrients. This is the case of the PDA environment. This ability to germinate is interesting in saline conditions and may be an important feature for the rehabilitation and reforestation of space. But it is also interesting to exploit to valorize and protect the marginalized soil affected by the salinity in steppe environment.

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