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The effects of temperature, hydric & saline stress on the germination of marram grass seeds (*Ammophila arenaria* L.) of the SIBE of Moulouya embouchure (Mediterranean – North-eastern Morocco)

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ABSTRACT

The seeds of *Ammophila arenaria* germinate in a wide range of temperatures ranging between 15 and 30°C with a low optimal temperature (20°C). The germination rate is maximal in the distilled water recalling the germination behavior of glycophytes. At the germination stage, marram grass seems to be much more sensitive to the saline stress than to the hydric stress. Indeed if this plant can tolerate polyethylene glycol concentrations reaching 150g/l it does not support sodium chloride concentrations higher or equal to 9g/l. Thus in the littoral dunes of the SIBE of the Moulouya embouchure, the germination of seeds would be controlled by the salinity of the substrate rather than by its osmotic forces.

Keywords: *Ammophila arenaria*, germination, hydric stress, saline stress, temperature.

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INTRODUCTION

The formation of the littoral dunes is the resultant of the reciprocal action between the wind, sand and the plants. In order that the sand transported by wind, settles and accumulates it is necessary that the speed of the wind should be reduced by an obstacle like the plants [15]. The first obstacle met by the wind is marine debris, which initiates the formation of the embryonic dunes [33]. These last will be colonized by pioneer plants like *Agropyron junceum*. *Ammophila arenaria* (or marram grass) will transform these dunes into mobile dunes [19] whose substrate is not salty [11, 12]. Marram grass, which is highly adapted to the littoral dunes, will be the object of our study. *Ammophila arenaria* is a graminaceous plant with rhizome. The aerial part consists of tufts of leaves while the underground part contains a dense network of rhizomes and adventitious roots. Flowering occurs from May to August [30], the ears are ripe in July. Mature seeds are dispersed in September and germinate the following spring [18]. Reproduction is primarily vegetative by rhizomes [35]. The fruit is an albuminous caryopsis. The study will concern seeds of *Ammophila arenaria* L. (Link), variety arundinacea (Host) [34] growing on the mobile dunes of the SIBE (Site of Biological and Ecological Interest) of the Moulouya embouchure (Mediterranean – North-eastern Morocco). This SIBE classified RAMSAR site in 2005 [29], is located in the eastern region of Morocco, between the latitude 34°40'N and 35°08'N and between longitude 02°10'W and 02°50'W [16]. The rainfall varies between 300 and 400 mm [27, 28] with an average value 330mm [37]. Monthly average temperatures fluctuate between a maximum (M =31.9) during the hottest months (summer) and a minimum (m =4, 9) during the coldest months (winter). The level of vegetation is thermo Mediterranean [1, 32] with a semi- arid bioclimatic weather [21, 36].

The aim of this study is to determine the optimal temperature of germination of seeds and to test the effect of the hydric and saline stress on the rate of germination. This will make possible to compare the sensitivity of seeds with the two types of stress (hydric and saline) and to deduce which factors could control the germination of marram grass in the site under study.

MATERIALS AND METHODS

The seeds of marram grass were collected during the sampling of the dune flora in 2010 and preserved of moisture and ambient temperature. Only the intact seeds considered as being ripe and viable are retained. The seeds have undergone three treatments: initially the action of the temperature (to determine the optimal temperature of germination), then the action of the hydric stress and saline stress (in optimum condition for temperature). The seeds are initially disinfected in a bleach solution to 30% during 4 minutes, and then rinsed with distilled and sterile water during 30 minutes. The seeds are deposited in sterile petri dishes containing two layers of filter paper soaked with distilled water (effect of the temperature) or sodium chloride (NaCl) solutions (saline stress) or polyethylene glycol solutions (PEG 6000) (hydric stress). Petri dishes are incubated in a drying oven in the dark. Each test of germination is done in four repetitions of 30 seeds. After 15 days of treatment, the germinated seeds are counted. A seed is considered germinated when the radicle break through the seed coat [14].

The results of germination are expressed in rate of germination, which is the relationship between the number of germinated seeds and the full number of sown seeds. The results are analyzed statistically by the analysis of variances (ANOVA).

RESULTS AND DISCUSSION

Statistical analysis shows a highly significant effect (the threshold $\alpha = 0.05$) of the temperature, hydric stress and saline stress on the rate of seed germination.

Effect of temperature

For the temperature (figure 1), the seeds germinate (rate higher than 50%) in a wide range between 15 and 30°C (90.83 to 74.17%), thus joining the results of Bendimered & al. [5]. The optimal temperature of germination is 20°C (91.67%) what corroborates with the results of other authors: between 15 and 25°C according to Bendimered & al. [5] and lower or equal to 20°C according to Lachiheb & al. [26]. The low (5°C) or high (40°C) temperatures inhibit germination. According to the classification adopted by Nafati [31], this optimum of germination is low. This germinative behavior of marram grass is identical to that of other graminaceous plants like *Koeleria phleoïdes*, *Corynephorus articulatus* [26] and *Stipa tenacissima* [5].

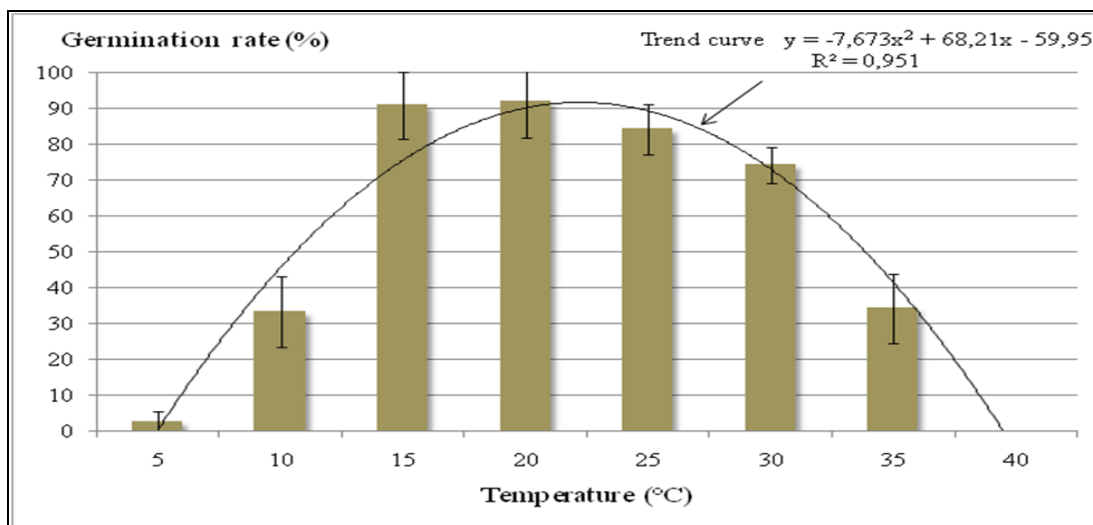


Figure 1: Effect of temperature on the germination rate of marram grass seeds

Effect of polyethylene glycol concentration

The rate of germination is maximal in distilled water (witness) and is reached 86.70% (figure 2). The concentrations in PEG between 15 and 90g/l seem to affect little or no germination and even for concentrations 120 and 150g/l the rate of germination remains higher than 50%. The germination proceeds even in the presence of high PEG concentration (200g/l) (19.10%). At 300g/l germination is completely inhibited. This shows the higher

resistance of *Ammophila arenaria* to the hydric stress at germination stage. The rates of germinations obtained are much higher than those of Talhaoui [39] (17% for 70g/l of PEG). In comparison with others halophytes like *Atriplex halimus*, *Ammophila arenaria* seems to be much more resistant to the hydric stress at the seed stage. Indeed the germination rate of seeds of *Atriplex halimus* is strongly reduced to 8.80% by 100g/l of PEG [8]. The inhibiting effect of the hydric stress at high concentration was observed at other species like *Argania spinosa* [40, 41] and *Atriplex nummularia* [25].

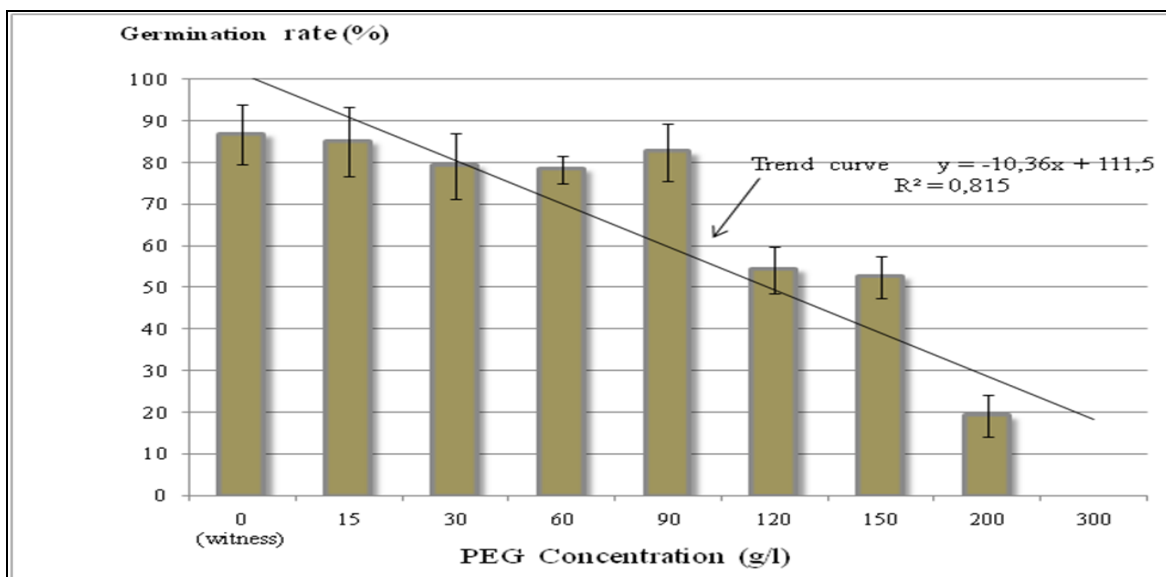


Figure 2: Effect of polyethylene glycol (PEG 6000) concentration on the germination rate of marram grass seeds

Effect of sodium chloride concentration

For salt, the germination rate decreases with the saline stress: the percentage of germination decreases from 90.83% for the witness to 0.83% for 24g/l of NaCl concentration (figure 3). The germination rate is slightly affected by concentrations 3 and 6g/l of NaCl (88, 33% and 77.50% respectively), however it is strongly reduced by the concentrations higher or equal to 9g/l. Concentrations 30 and 40g/l inhibit germination completely. The high reduction of the germination rate by the concentrations higher than 9g/l corroborates with the results of Lachiheb & al. [26] where 11,60g/l of NaCl can strongly inhibit germination. The sensitivity to salt at the seed stage was also observed at others halophyte like *Atriplex halimus* [4, 25]. The comparison of the slope of trend curves (figure 2 & figure 3) shows that *Ammophila arenaria* seems to be much more sensitive to the saline stress (toxic action of salt) than with the hydric stress at the germination stage.

Although the marram grass behaves like a halophyte at the adult stage (tolerance level of salt 20g/l, [24]), it is very sensitive to the saline stress at the germination stage. Germination is maximal in distilled water recalling the germination behavior of glycophytes [2, 6, 9 10, 13,

22]. The inhibiting effect of NaCl would be related to the toxic action of salt [4]. This toxicity would be influenced by a mineral imbalance in favor of an important load of sodium [20] and a deficiency in potassium [23] on the embryo. The hormonal intervention in this inhibition is combined with the action of salt [17]. Thus Behl and Jeschke [3] announce the important role which the abscisic acid (ABA) plays in this inhibition. The ABA has the same properties that NaCl and acts by limiting the absorption of water [38] and the synthesis of specific enzymes of germination; in particular the alpha-amylase [7].

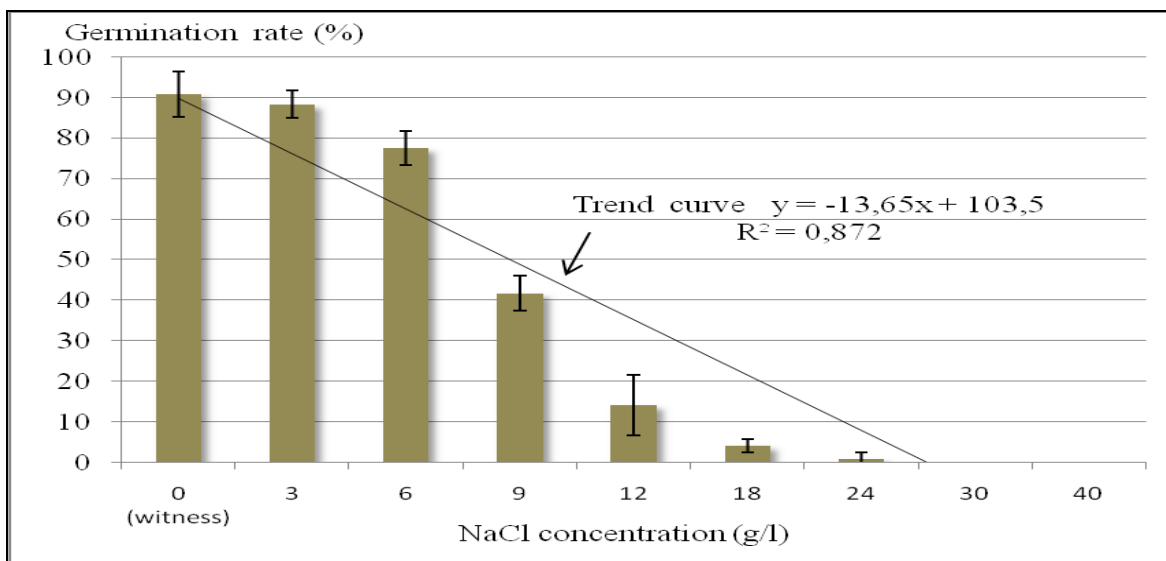


Figure 3: Effect of sodium chloride (NaCl) concentration on the germination rate of marram grass seeds

CONCLUSION

The seeds of *Ammophila arenaria* germinate in a wide range of temperature. The optimal temperature of germination is 20°C. The seeds seem to be much more sensitive to the saline stress than to the hydric stress. This germinative behavior could explain why the marram grass preferentially colonizes the unsalted dune zones like the mobile dunes.

REFERENCES

- [1] Achhal A, Akabli O, Barbero M, Benabid A, M'Hirit, O, Peyer C, Quezel P, Rivas –Martinez, S.A propos de la valeur bioclimatique et dynamique de quelques essences forestières au Maroc. *Ecologia mediterranea* 1980 ; 5 : 211- 249.
- [2] Badger K, Ungar I. The effects of salinity and temperature on the germination of the inland halophyte *Hordeum jubatum*. *Can J Bot* 1989; 67: 1420-1425.
- [3] Behl R, Jeschke W D. Influence of ABA on unidirectional fluxes and intracellular compartmentation of K⁺ and Na⁺ in excised barley roots segments. *Physiol Plant* 1981; 3: 95-100.
- [4] Belkhdja M, Bidai Y. Réponse des graines d'*Atriplex halimus* L. à la salinité au stade germination. *Sécheresse* 2004 ; 15(4) : 331-335.

- [5] Bendimered F Z, Mehdadi Z, Benhassaini H. Etude de la germination et de croissance foliaire de l'oyat (*Ammophila arenaria* (L) Link. en conditions contrôlées. *Acta Bot. Gallica* 2007; 154 (1): 129-140.
- [6] Bidai Y. Le metabolisme de la proline chez l'*Atriplex halimus* L. stressée à la salinité. Thèse de magister. Université d'Oran (Algérie), 2001, 99.
- [7] Black M. Abscisic acid in seed germination and dormancy. In Adicot FT, ed. *Abscisic acid*. New York. Praeger Publish 1983: 333-363.
- [8] Boudlal R. Etude biologique de la végétation de la rive gauche de l'embouchure de la Moulouya-Ras El Ma. Mem. Master. Université Mohammed premier, Oujda (Maroc), 2009; 73-75.
- [9] Chapman V J. The influence of salts upon the terrestrial halophytes. VIIIème Congrès Int Bot. Rapports et communications, 1954; 1994-2000.
- [10] Chatterton N J, Mckell C M. *Atriplex polycarpa* L. Germination and growth as affected by sodium chloride in water culture. *Agron J* 1969 ; 61: 448-450.
- [11] Chergui A, El Hafid L, Melhaoui M. The contribution of the study of the bordering dunes vegetation in the Moulouya embouchure: the marram grass (*Ammophila arenaria* L;) case. *J. Mater. Environ* 2011; *Sci. 2* (S1): 552-555.
- [12] Chergui A, El Hafid L, Melhaoui M. Study of some adaptive factors in coastal areas of Nador lagoon (Mediterranean – North-eastern Morocco). *Research journal of pharmaceutical, Biological and Chemical Sciences* 2012; 3(4): 740-746.
- [13] Chouki Allah R, Malcom C V, Hamdy A. *Halophytes and Biosaline Agriculture*. New York. Marcel Dekker 1997; 400.
- [14] Côme D. Les obstacles de la germination. *Masson* 1970 ; 162.
- [15] Corre J J. Structure de la végétation littorale. Les relations entre peuplements végétaux : une conséquence de leur action sur le milieu. *Acta Oecol* 1983 ; 4 : 17-27.
- [16] Daki M. Le site d'intérêt biologique et écologique de l'embouchure de la Moulouya : caractéristiques et potentialités. *Projet MedWetCoast-Maroc* 2006; 54.
- [17] Debez A, Chaibi W, Bouzid S. Effet du NaCl et de régulateurs de croissance sur la germination d'*Atriplex halimus* L. *Cah Agric* 2001 ; 10 : 135-138.
- [18] Desfossez P, Vanderbecken A. *Revue Garde : revue d'information des agents techniques des collectivités locales, chargées de l'entretien, la gestion et l'animation des sites du conservatoire de l'Espace Littoral et des Rivages Lacustres*, Paris 1988 ; 5 : 1-16.
- [19] Duvat V, Queney B.T, Auby C.C, Prat C.M. Roland Paskoff et les littoraux : regards de chercheurs. *L'Harmanttan*, Paris, 2010, 361.
- [20] El Neimi T S, William F C, Rumbaugh M D. Responses of Alfa alfa cultivar to salinity during germination growth. *Crop Sci* 1992; 32: 976-980.
- [21] Emberger L. Classification géographique des climats. *Rec, Trav, Labo, Bot, Géol, Zool, Fac, Sci, Montpellier, Série Bota* 1955 ; 3-43.
- [22] Grouzis M, Heim G, Berger A. Croissance et accumulation des sels chez deux salicornes annuelles du littoral méditerranéen. *Oecol. Plant* 1977; 12(4): 307-322.
- [23] Guerrier G. Relation entre la tolérance ou la sensibilité à la salinité lors de la germination des semences et les composantes de la nutrition en Na⁺. *Biol Planta* 1984 ; 26 : 22-28.
- [24] Huiskes A H L. Biological flora of the British Isles. *J. Ecology* 1979; 67: 363-382.

- [25] Kamari S. Contribution à l'étude écologique et écophysiological (cas de l'Atriplex halimus L.) de la flore de la plaine d'Alhoceima (Rif Central). Mem. Université Mohammed premier, Oujda (Maroc) 2005 ; 49.
- [26] Lachiheb K, Neffati M, Zid E. Aptitudes germinatives de certaines graminées halophytes spontanées de la Tunisie méridionale. CIHEAM-IAMZ. Djerba (Tunisie) 2004 ; 89-93.
- [27] Laouina A. Le Maroc nord- oriental : Reliefs modelés et dynamique du calcaire. Publ. Rectorat Université Mohammed premier, Oujda 1990 ; 605.
- [28] MATEE. Schéma de développement et d'aménagement régional (SDAR). Ministère de l'Aménagement du Territoire, de l'Eau et de l'Environnement, Maroc, 2000.
- [29] Melhaoui, M. & El Hafid, L. De l'approche GISC à la mise en place du contrat d'espace littoral : cas de la zone Moulouya- Saïdia (Méditerranée marocaine). Actes du colloque international pluridisciplinaire "Le littoral : subir, dire, agir". Lille (France) 2008 ; 7.
- [30] Munz P A, Kech D D. A California flora and supplement. Univ. California Press, 1973; Berkeley, CA.
- [31] Neffati M. Caractéristiques morphologique de certaines espèces végétales nord africaine. Implications pour l'amélioration pastorales. Thèse, université Gent 1994 ; 264.
- [32] Ozenda P. Les étages de végétation dans les montagnes du bassin méditerranéen. Doc, Cart, Ecol 1975 ; 16 :1-30.
- [33] Paskoff R. Caractéristiques et gestion d'un type de dune littorale : les avants dunes. Sécheresse 2005 ; 16: 247-253.
- [34] Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome 1, Paris (France), 1962, 565.
- [35] Ranwel D. The system and dune slack habitat. J. Ecology 1959; 47: 571-601.
- [36] Sauvage C. Etages bioclimatiques. Atlas du Maroc, Notices, applications. Physiol Météo 1963 ; 6 : 31.
- [37] Sebbar A, Badri W, Fougrach H, Hsaine M, Saloui A. Etude de la variabilité du régime pluviométrique au Maroc septentrional (1935-2004). Sécheresse 2011 ; 22 : 139-148.
- [38] Shopper P, Bajracharya D, Plachy C. Control of seed germination by abscisic acid. Time course of action in Sinapis alba L. Plant Physiol 1979; 64: 822-827.
- [39] Talhaoui A. Inventaire de la végétation de la zone côtière de Cap de l'eau et contribution à l'étude physiologique d'Ammophila arenaria. Mem. Université Mohammed premier, Oujda (Maroc) 2009 ; 46-47.
- [40] Tazi R, Berrichi A, Haloui B. Effet du polyéthylène glycol sur la germination et la croissance in vitro de l'arganier (Argania saponosa L. Skeels) des Beni-Snassen (Maroc oriental). Sécheresse 2003 ; 1 : 23-27.
- [41] Znagui F. Contribution à l'étude écophysiological de l'arganier (Argania spinosa L. Skeels) de Jbel Tikermine (Maroc oriental). Mem. Université Mohammed premier, Oujda (Maroc) 2007; 36-41.