

The effects of temperature, hydric and saline stress on the seedling growth of marram grass (*Ammophila arenaria* L.)

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Abstract

The maximum growth of marram grass seedling is carried out at 25°C. The tests of stress (under the optimum temperature of germination; 20°C) showed on the one hand the great resistance of the radicle to the hydric stress and, on the other hand the great sensitivity of the coleoptile to the saline stress. The growth of the radicle and the coleoptile is affected much more by the saline stress than by the hydric stress. In the coastal dunes, the growth of embryonic axes would be controlled by the salinity of the substrate rather than by its water potential. The culture of marram grass seeds in distilled water, successively at 20°C (until germination) and 25°C makes it possible to obtain many seedlings with a good development of the embryonic axes. These seedlings could be used for the restoration and the rehabilitation of the degraded coastal dunes.

Keywords: Coastal dunes, marram grass, radicle, coleoptile, growth, stress.

INTRODUCTION

The most typical plant of coastal dunes (especially mobile dunes) in the Eastern Mediterranean region of Morocco is marram grass (*Ammophila arenaria* (L.), sub-species *arundinacea*) [1,2]. *Ammophila arenaria* is a herbaceous perennial cryptophyte [3]. The aerial part consists of tufts of leaves while the underground part contains a dense network of rhizomes and adventitious roots [4]. Flowering occurs

from may to August [5], the ears are ripe in July. Mature seeds are dispersed in September and germinate the following spring [6]. Reproduction is primarily vegetative by rhizomes [7].

These dunes in semi-arid bioclimate [8,9], are located in two SIBEs (Site of Biological and Ecological Interest) : Moulouya embouchure and Marchica lagoon, which are classified as RAMSAR sites [10]. However, these coastal ecosystems are currently under intense anthropic pressure especially because of urbanization and tourism, which threaten their biodiversity and their ecological balance [11,12].

The aim of this study is to test the effects of temperature (to determinate the optimum temperature of seedling growth), the hydric and saline stress on the seedling growth of marram grass in vitro culture. This make it possible to deduce which soil factor (salinity and water potential) could control much more the seedling growth in the coastal dunes. Thus, if we want to use direct seeding for restoration of the degraded coastal dunes, the operation must take into account this factor. Moreover the control of the optimal conditions for early seedling growth (in vitro culture) makes it possible to obtain many seedlings with a good development of the radicle and the coleoptile. These seedlings could be used for the restoration and the rehabilitation of the degraded coastal dunes.

MATERIALS AND METHODS

The seeds were collected from the SIBE of the Moulouya embouchure and preserved of moisture and ambient temperature. Only the intact seeds considered as being ripe and viable are retained. The seeds are initially disinfected in a bleach solution at 30% during 4 minutes, and then rinsed with distilled and sterile water during 30 minutes [13]. The seeds are deposited in sterile Petri dishes containing two layers of filter paper [14]. The seeds have undergone three treatments: the action of the temperature (in distilled water), the action of the hydric stress (polyethylene glycol solutions, PEG 6000) and the saline stress (sodium chloride solutions, NaCl). Petri dishes are incubated in a drying oven in the dark.

After 15 days of treatment, the length of the root and the coleoptile (with the sheets) is measured using a graduated ruler. The study of hydric and saline stress is carried out under the optimum temperature of germination (20 °C) [2].

Each test is done in four repetitions of 30 seeds. The results are analyzed statistically by the analysis of variances (One- Factor ANOVA, SPSS software 11.5).

RESULTS AND DISCUSSION

Effect of temperature

Statistical analysis shows a significant effect of the temperature on the length of the radicle and the coleoptile (Probability <0. 05 : significant differences a threshold of 5%).

The length of the radicle and the coleoptile increases with the temperature until reaching the maximum size at 25°C (figure 1). Beyond this thermal optimum, their growth decreases.

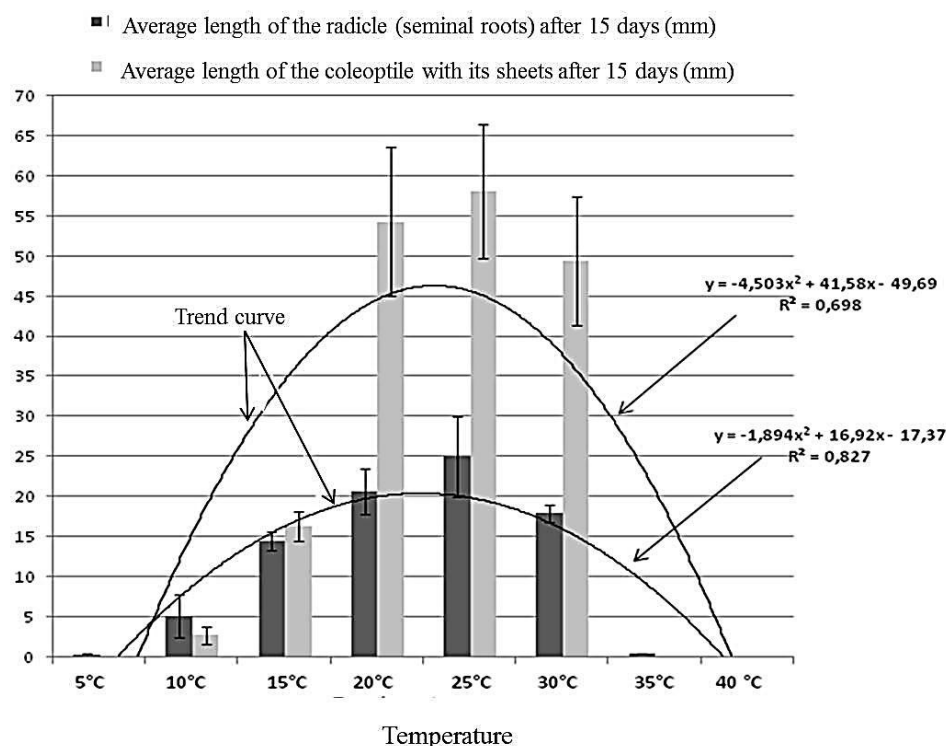


Figure 1: Effect of temperature on the growth of the radicle and the coleoptile of marram grass

The effect of the temperature on germination and seedling growth was quoted by several authors [15-19]. The extreme temperatures (5°C and 40°C) can deteriorate the plasmic and mitochondrial membrane [20] which inhibits the processes of germination [17,21] and consequently the growth of the embryonic axes. Thus the low temperatures modify membrane lipids which disturbs the cell membrane permeability. In the same way, the low temperatures involve a disturbance and a delay of coordination during the mobilization of the reserves [22] and a reduction of the speed of water absorption [23]. The high temperature causes a denaturation of the membrane proteins [24], an inhibition of synthesis and/or activity of the enzymes implied in the mobilization of the reserves [17] and a reduction in the quantity of oxygen which arrives at the embryo [25].

The culture of marram grass seeds in distilled water, successively at 20°C (until germination) and 25°C makes it possible to obtain many seedlings with a good development of the embryonic axes. These seedlings could be used for the restoration and the rehabilitation of the degraded coastal dunes.

Effect of hydric stress

Statistical analysis shows a significant effect of the hydric stress on the length of the radicle and the coleoptile (Probability <0. 05 : significant differences a threshold of 5%).

The maximum development of the embryonic axes is carried out in distilled water (figure 2).

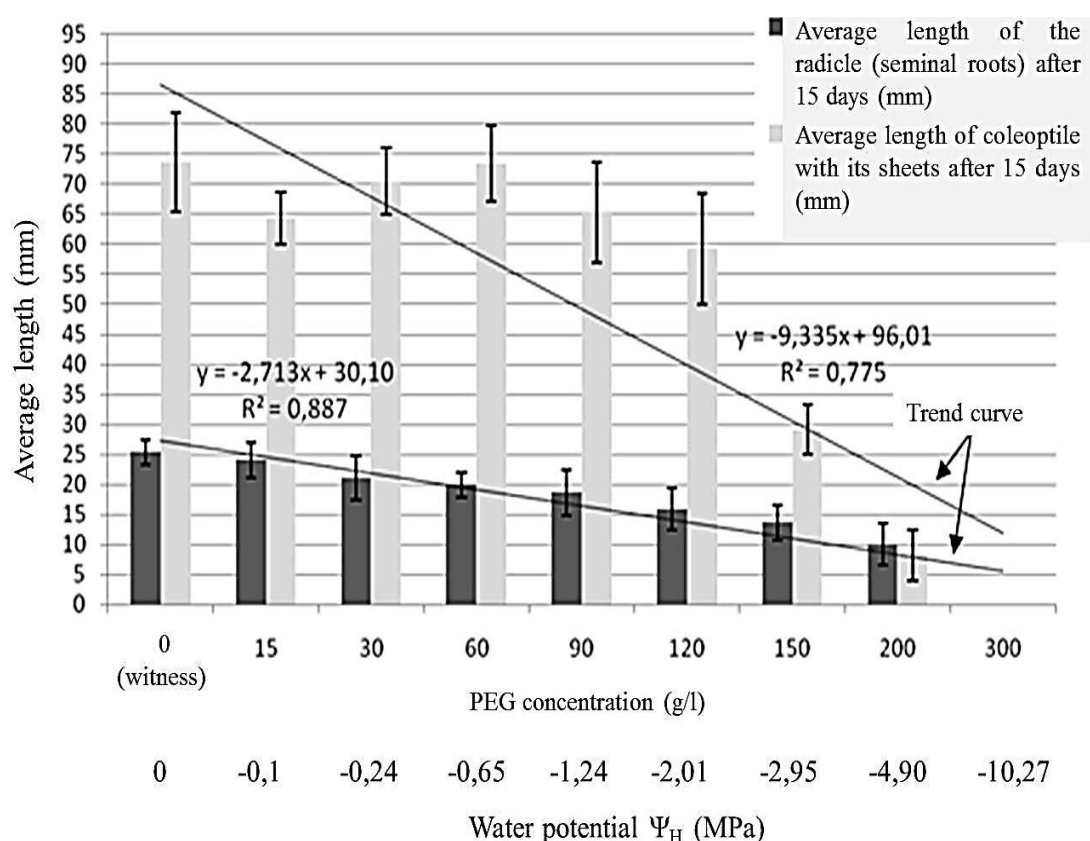


Figure 2: Effect of PEG 6000 concentration on the growth of the radicle and the coleoptile of marram grass

In general, the length of the radicle and the coleoptile decreases with the intensity of the hydric stress. The reduction of the growth of the seminal roots of marram grass is related to the inhibition of the multiplication and the elongation of root cells [26, 27]. This reduction could be also related to a reduction in dimensions of the epiblema cells and a reduction in the speed division of root apical meristem [28]. The inhibiting effect of hydric stress on the growth of the seminal roots was also reported at other graminaceous plants like Barley [29], Oats [30], Rice [31] and Wheat [28].

The concentrations between 15 (-0.1MPa) and 120g/l (-2.01MPa) may decrease a little or not at all the length of the radicle and the coleoptile. However, the

concentrations higher than 120g/l seem to affect much more the growth of the coleoptile than of the radicle. Thus, the radicle is more resistant than the coleoptile; the maintenance of the growth of the seminal roots (in comparison with the coleoptile), under hydric stress, plays a great role in the water and mineral nutrition of the plant at early stages of development [28].

In comparison with the seminal roots, the hydric stress involves a stimulation of the growth of the adventitious roots as at Durum wheat [32], [33] and Corn [26].

Effect of saline stress

Statistical analysis shows a significant effect of the saline stress on the length of the radicle and the coleoptile (Probability <0. 05 : significant differences a threshold of 5%).

The maximum development of the embryonic axes is carried out in distilled water (figure 3).

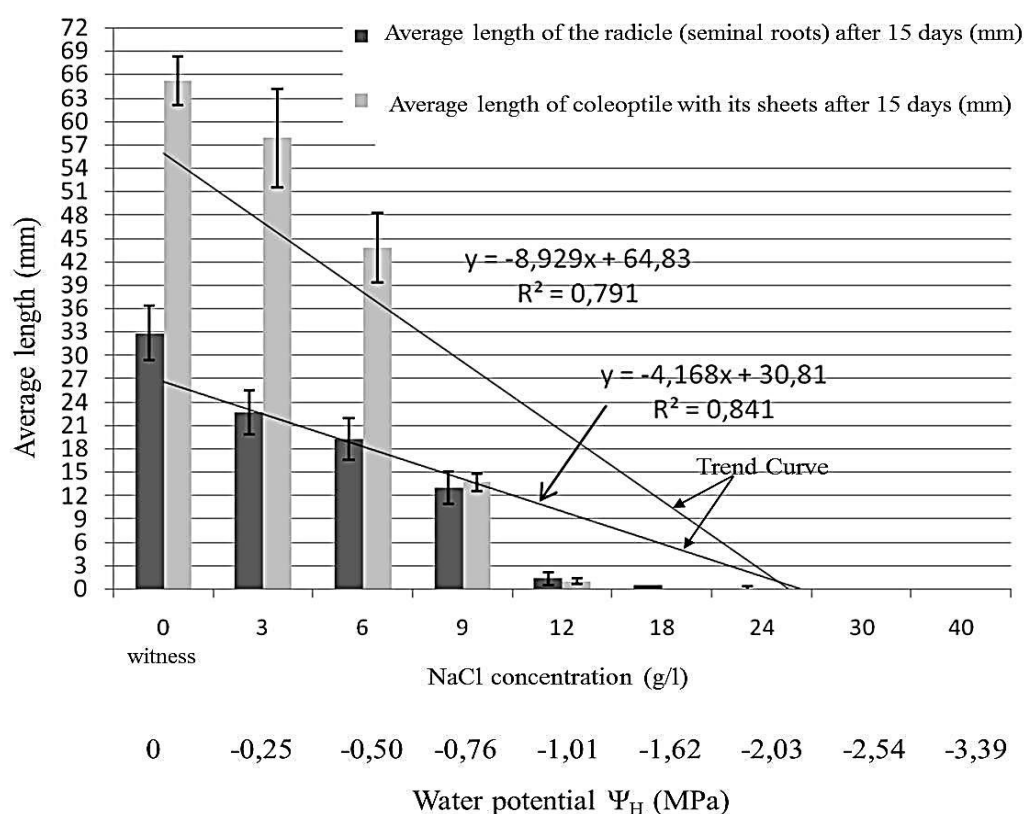


Figure 3: Effect of NaCl concentration on the growth of the radicle and the coleoptile of marram grass

The growth of the radicle and the coleoptile decreases with the increase in the NaCl concentration. The concentrations higher or equal to 9g/l decrease strongly the growth of the radicle and the coleoptile. This behavior was also observed at the other graminaceous plants like Wheat [34].

The inhibiting effect of NaCl on the growth of the seminal roots is related to the reduction of the absorption of water by the embryo and the cotyledon [35], on to the reduction of the water flow towards the radicle [36] and to the toxic action of salt [37]. This toxicity would be influenced by a mineral imbalance in favor of an important load of sodium [38] and a deficiency in potassium [39] on the embryo.

Salt can involve an inhibition of the activity of the α amylase and a reduction of hexoses (glucose and fructose) on the embryo [40, 41]. The hormonal intervention in this inhibition is combined with the action of salt [42]. Thus, Behl and Jeschke [43] 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 [44] and the synthesis of specific enzymes of germination; in particular the alpha-amylase [45].

The proline can be also implied in the reduction of the growth of the plants under saline stress. Indeed under salines constraints, a negative correlation was observed between the growth of the roots and the air parts of the Sea daffodil and the content of their tissus in proline [46].

The radicle is much more resistant to the saline stress than the coleoptile. This tow organ seems to be much more resistant to the hydric stress than the saline stress (figure 2 and figure 3). Thus, in the coastal dunes, the seedling growth of marram grass would be controlled by the salinity of the substrate rather than by its water potential.

CONCLUSION

The maximum development (in distilled water) of the embryonic axes of maram grass is carried out at 25°C. The results of stress tests showed the great resistance of the radicle to the hydric stress and the great sensitivity of the coleoptile to the saline stress. The growth of the radicle and the coleoptile is affected much more by the saline stress than by the hydric stress. Thus in the coastal dunes, the growth of embryonic axes would be controlled by the salinity of the substrate rather than by its water potential.

The culture of marram grass seeds in distilled water, successively at 20°C (until germination) and 25°C makes it possible to obtain many seedlings with a good development of the embryonic axes. These seedlings could be used for the restoration and the rehabilitation of the degraded coastal dunes. If we want to use direct seeding for restoration of the degraded coastal dunes, the operation must take into account the salinity of soil witch must be low ($<0.9\text{g/l}$), the ambient temperature (20°C–25°C) and the precipitation ($\Psi_H > -2.01\text{MPa}$).

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REFERNCE

- [1]. Quezel, P., and Santa, S., 1962, Nouvelle flore de l'Algérie et des régions désertiques méridionales, CNRS, Paris, 565p.
- [2]. Chergui, A., El Hafid, L., and Melhaoui, M., 2013, The effects of temperature, hydric and saline stress on the germination of marram grass seeds (*Ammophila arenaria* L.) of the SIBE of Moulouya embouchure (Mediterranean – North-eastern Morocco), R.J.P.B.C.S., 4 (1), 1333–1339.
- [3]. Raunkiaer, C., 1934, The life of plants and statistical geography, Claredon, Oxford, 632p
- [4]. Chergui, A., El Hafid, L., and Melhaoui, M., 2017, Characteristics of Marram Grass (*Ammophila arenaria* L.), Plant of The Coastal Dunes of The Mediterranean Eastern Morocco : Ecological, Morpho-anatomical and Physiological Aspects, J.M.E.S., 8 (10), 3759–3765.
- [5]. Munz, P.A., and Kech, D.D., 1973, A California flora and supplement, University California Press, Berkeley, 1905p.
- [6]. Desfossez, P., and Vanderbecken, A., 1988, Revue d'information des agents techniques des collectivités locales, chargés de l'entretien, la gestion et l'animation, des sites du conservatoire de l'Espace Littoral et des Rivages Lacustres, Revue Garde (n°5), Paris, 16p
- [7]. Ranwel, D., 1959, The system and dune slack habitat, J.Ecolo., 47, 571–601.
- [8]. Emberger, L., 1955, Une classification biogéographique des climats, Rec. Trav. Lab.Bot. Géol. Fac. Sc., 7 (11), 3–43.
- [9]. Sauvage, C., 1963, Étages bioclimatiques. Atlas du Maroc, Notices explicatives, Comité national de géographie du Maroc, Rabat, 31p.
- [10]. Melhaoui, M., and El Hafid, L., 2008, De l'approche GIZC à la mise en place du contrat d'espace littoral : cas de la zone littorale Moulouya-Saïdia (Méditerranée marocaine), Actes du colloque international pluridisciplinaire, eds., Lille, France, 7p.
- [11]. Ley de la Vega, C., Favennec, J., Gallego-Fernández, J., and Pascual Vidal, C., 2012, Conservation des dunes côtières : restauration et gestion durables en Méditerranée occidentale, eds., Union Internationale pour la Conservation de la Nature et de ses ressources, Gland, Suisse et Malaga, Espagne, 124p.

- [12]. Moulis, D., 2004, Compte rendu de la mission d'expertise sur le littoral Nord-Est Marocain, Projet MedWetCoast, Maroc, 11p.
- [13]. Tomaszewska, S.M., and Figas, A., 2011, Optimization of the processes of sterilization and micropropagation of cup plant (*Silphium perfoliatum* L.) from apical explants of seedlings in vitro cultures, *Acta Agrobot.*, 64 (4), 3–10.
- [14]. Lachiheb, K., Neffati, M., and Zid, E., 2004, Aptitudes germinatives de certaines graminées halophytes spontanées de la Tunisie méridionale in Ferchichi A. (comp.), Ferchichi A, eds., *Réhabilitation des pâturages et des parcours en milieux méditerranéens*, Cahiers Options Méditerranéennes, Djerba, pp.89–93.
- [15]. Heller, R., Esnault, R., and Lance, C., 2000, *Physiologie végétale. 2-Développement*, Dunod, Paris, 366p.
- [16]. Hopkins, W.G., 2003, *Physiologie végétale*, De Boeck université, Bruxelles, 514p.
- [17]. Bendkhil, B., and Denden M., 2014, Effet de la température sur la germination, la dégradation des réserves protéiques et minérales des graines du gombo (*Abelmoschus esculentus* L.), *J. New Sci.*, 5 (4), 25–33.
- [18]. Hawker, J.S., and Jenner, C. F., 1993, High Temperature Affects the Activity of Enzymes in the Committed Pathway of Starch Synthesis in Developing Wheat Endosperm, *Aust. J.Plant. Physiol.*, 20(2), 197–209.
- [19]. Guan, B., Zhou, D., Zhang, H., Tian, Y., Japhet, W., and Wangc, P., 2009, Germination responses of *Medicago ruthenica* seeds to salinity, alkalinity, and temperature, *J. Arid Environ.*, 73, 135–138.
- [20]. Bouzoubaâ K., and El Mousadik, A., 2003, Effet de la température, du déficit hydrique et de la salinité sur la germination de l'Arganier -*Argania spinosa* (L.) Skeels, *Acta. Bot. Gallica.*, 150 (3), 321–330.
- [21]. Ibanez, A. N., and Passera, C.B. ,1997, Factors affecting the germination of albaida (*Anthyllis cytisoides* L.), a forage legume of the Mediterranean coast, *J. Arid Environ.*, 35, 225–231.
- [22]. Nykiforuk, C. L., and Johnson-Flanagan, A. M., 1994, Germination and early seedling development under low temperature in Canola, *Crop Sci.*, 34, 1047–1054.
- [23]. Nykiforuk, C. L., and Johnson-Flanagan, A.M., 1999, Storage reserve mobilization during low temperature germination and early seedling growth in *Brassica napus*, *Plant Physiol.Biochem.*, 37, 939–947.
- [24]. Mayer, A.M., and Poljakoff-Mayber, A., 1989, *The germination of seed*, Fourth ed, Pergamon Press, Oxford, 270p.
- [25]. Moore, R.P., 1985, *Tetrazolium testing manual*, International seed testing association, Zurich, 99p.

- [26]. Sharp, R., Poroyko, V., Hejlek, L.G., Spollen, W.G., Springer, G.K., Bohnert, H.J. , and Nguyen, H.T., 2004, Root growth maintenance during water deficit: physiology to functional genomics, *J.Exp.Bot.*, 55, 2243–2351.
- [27]. Malamy, J.E., 2005, Intrinsic and environmental response pathway that regulate root system architecture, *Plant. Cell.Environ.*, 28: 67–77.
- [28]. Adda, H., Sahnoune, M., Kaid-Harch, M., and Merah, O., 2005, Impact of water intensity on durum wheat seminal roots, *C. R. Biologies.*, 328, 919–927.
- [29]. Grando, S., and Ceccarelli, S., 1995, Seminal root morphology and coleoptiles length in wild (*Hordeum vulgare* ssp. spontaneum) and cultivated (*Hordeum vulgare* ssp. vulgare) barley, *Euphytica*, 86, 73–80.
- [30]. Murphy, C.F., Long, R.C., and Nelson, L.A., 1982, Variability of seedling growth characteristics among oat genotypes, *Crop Sci.*, 22,1005–1009.
- [31]. Boonjung, H.,and Fukai, S., 1996, Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions.1. Growth during drought, *Field Crop Res.*, 48 , 37–45.
- [32]. Benlaribi, M., Monneveux, P., and Grignac, P., 1990, Étude des caractères d'enracinement et de leur rôle dans l'adaptation au déficit hydrique chez le blé dur (*Triticum durum* Desf.), *Agronomie.*, 10, 305–322.
- [33]. Ali Dib, T., and Monneveux, P., 1992, Adaptation à la sécheresse et notion d'idéotype chez le blé dur. I. Caractères morphologiques d'enracinement, *Agronomie.*, 12, 371–379.
- [34]. Ben Naceur, M., Rahmoune, C., Sdin, H., Meddahi, M.L., and Selmi, M., 2001,Effet du stress salin sur la germination, la croissance et la production en grains de quelques variétés maghrébines de blé. *Sciences et changements planétaires, Sécheresse.*, 12 (3) ,167–174.
- [35]. Prado, F.E., Gonzalez, J.A., Gallardo, M., Moris, M., Boero, C.,and Kortsarz, A., 1995, Changes in soluble carbohydrates and invertase activity in *Chenopodium quinoa* developed for saline stress during germination, *Phytochem.*, 14, 1–5.
- [36]. Waisel, Y., 1972 , *Biology of halophytes*, Academic Press, New York and London,410p.
- [37]. Belkhodja, M., and Bidai, Y., 2004, Réponse des graines d'*Atriplex halimus* L. à la salinité au stade germination, *Sécheresse*, 15 (4), 331–335.
- [38]. El Neimi, T.S., William, F. C., and Rumbaugh, M.D., 1992, Responses of Alfa alfa cultivar to salinity during germination growth, *Crop Sci.*, 32 , 976–980.
- [39]. Guerrier, G., 1984, 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.*, 26 , 22–28.
- [40]. Lin, C.C., and Kao, C.H., 1995, NaCl stress in rice seedling : starch

- mobilization and the influence of gibberellic acid on seedling growth, Bot. Bull. Acad. Sin., 36 ,169–173.
- [41]. Prado, F.E., Boero, C., Gallardo, M., and Gonzalez, J.A., 2000, Effects of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. Seeds, Bot. Bull. Acad. Sin., 41 , 27–34.
- [42]. Debez, A., Chaibi, W., and Bouzid, S., 2001, Effet du NaCl et de régulateurs de croissance sur la germination d'*Atriplex halimus* L, Cah. Agric., 10 ,135–138.
- [43]. Behl, R., and Jeschke , W. D.,1981, Influence of ABA on unidirectional fluxes and intracellular compartmentation of K^+ and Na^+ in excised barley roots segments, Physiol. Plant., 3, 95–100
- [44]. Shopper, P., Bajracharya, D.,and Plachy, C., 1979, Control of seed germination by abscisic acid. Time course of action in *Sinapis alba* L, Plant Physiol., 64 , 822–827.
- [45]. Black, M., 1983, Absciscic acid in seed germination and dormancy. in Adicot FT, eds., Absciscic acid, Praeger Publish, New York, 1983, pp. 333–363
- [46]. Khedr, A.H.A., Abbas, M.A., Abdel Wahid, A.A., Paul Quick, W., and Abogadallah, G.M., 2003, Proline induces the expression of salt -stress-responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt-stress, J. Exp. Bot., 54 (392) , 2553–2562.