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 determine the optimum temperature of seedling growth), the hydric and saline stress on the seedling growth of marram grass in vitro culture. This makes 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 at 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 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 marram 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 (<0.9g/l), the ambient temperature (20°C–25°C) and the precipitation ($\Psi_H > -2.01\text{MPa}$).
ACKNOWLEDGMENT

This work is completed within the framework of the P3 program of cooperation CUD/UMP. Let us thank the CUD from Belgium for the means provided. I would like to express my very sincere thanks to all the partners who are working with us on this project. I take this opportunity to thank all the scholars who kindly undertook the task of revising and correcting this manuscript.

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