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Characteristics of Marram Grass (*Ammophila arenaria* L.), Plant of The Coastal Dunes of The Mediterranean Eastern Morocco :Ecological, Morpho-anatomical and Physiological Aspects

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Abstract

The Coastal dunes of the Mediterranean Eastern Morocco show a large floristic and faunistic biodiversity and represent a national heritage of a great value at the ecological, socio-economic and eco-touristic levels. On the morpho-anatomical level, marram grass (*Ammophila arenaria* L.), a typical granimeous plant of the coastal dunes, is well adapted to its biotope. Thanks to its high adaptations, this xerophyte and halophyte plays several ecological roles the most important of which is the fixing of sand. On the physiological level, the optimum germination temperature of seeds in distilled water is 20°C. This culture in vitro makes it possible to obtain a large number of marram grass seedlings which could be used for the restoration, and the rehabilitation of the degraded dunes. The coastal dunes of the Mediterranean Eastern Morocco are currently under an intense anthropic pressure which threatens their ecological balance and their biodiversity. In absence of the littoral rights and a plan of integrated management, which ensures a sustainable development in these coastal areas, the anthropic pressures can only increase and the evolution of the dune ecosystem can only regress.

1. Introduction

The coastal dunes are naturally issued from a fixation and stabilization by sandy vegetation. Marram grass (*Ammophila arenaria* L. (Link)), subspecies *arundinacea* (Host) [1], is the most typical plant of coastal dunes (especially mobile dunes) in the Eastern Mediterranean region of Morocco. These dunes in semi-arid bioclimate [2, 3], are preserved in two SIBEs (Site of Biological and Ecological Interest) : Moulouya embouchure and Marchica lagoon, which are classified as RAMSAR sites [4]. Mobile dunes play several ecological roles : They fix the sand and protect the hinterland [5, 6]. They constitute physical barriers against the sea spray [6], the wind [7], the waves and the tides [8]. They prevent the contamination of the fresh groundwater by the seawater. They constitute a sand reverse [9, 10], show a large floristic [11-15] and faunistic [16-18] biodiversity and represent a national heritage of a great value at ecological, socio-economic and eco-touristic levels. These dunes are currently under intense anthropic pressure especially urbanization and tourism [19] which threaten their biodiversity and their ecological balance [8, 20].

The study will focus on temperature germination of seeds and the morpho-anatomical organization of marram grass.

The aim of this study is to determine the optimal temperature of germination of marram grass seeds and to put under focus the relation between morphological and anatomical organization of this species and its adaptation to its habitat.

2. Materials and methods

2.1 *Morphological and histological study*

The study is carried out on fresh samples of marram grass resulting from the mobile dunes of Moulouya embouchure. The morphological study (vegetative and floral organs) is made visually and by observation under the binocular magnifying glass. The histological study (vegetative organs) is made by photonic microscopy observation after treatment according to the double staining technique (Laboratory of Water Sciences, the Environment & Ecology, Mohammed Ist University, Faculty of Sciences, Oujda – Morocco) : histological sections of the vegetative organs are put in bleach solution during 2 minutes. After a rinsing in distilled water during 3 minutes, the sections are put in the acetic acid at 20% during 5 minutes. The sections are rinsed again during 3 minutes, then are stained by carmine and iodine green solution during 10mn. After rinsing, the sections are held between the cover slip and a microscope slide and are observed under the optical microscope. The cells with cellulose walls are coloured pink, those with lignified walls are coloured green, whereas those with suberized walls are coloured yellow.

2.2 *Effect of temperature*

The seeds 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 are initially disinfected in a bleach solution at 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. Petri dishes are incubated at different temperatures 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 breaks through the seed coat [21].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 (One-Factor ANOVA, SPSS software 11.5).

3. Results and discussion

3.1 *Morphological study*

Marram grass is an herbaceous perennial cryptophyte [22, 23]. The air part consists of tufts of leaves in the rosette form (figure 1). The external face of the leaves is smooth, whereas the internal part contains parallel veins characteristic of the monocot plant [24] (figure 1).

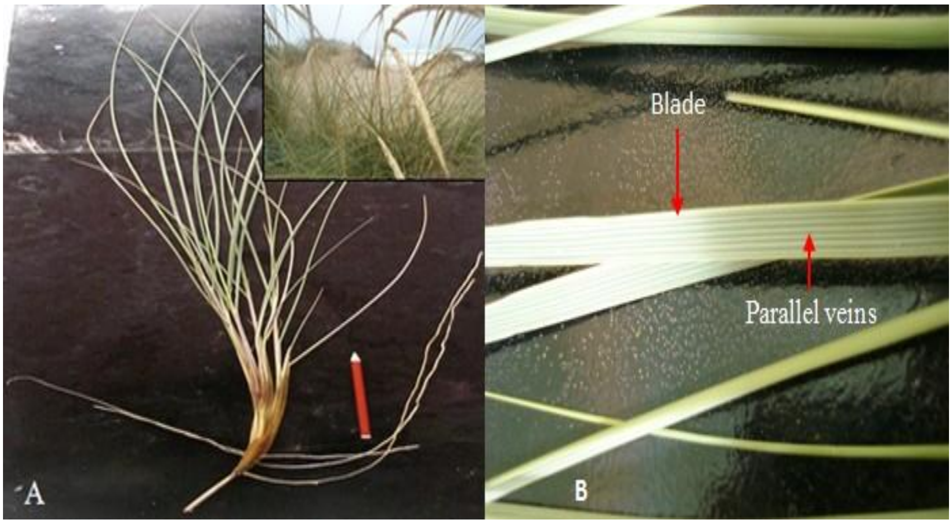

Figure 1: *Ammophila arenaria:* general form (A), leaf form (B)

The fibrous root and the rhizome (figure 2) are well developed allowing a good fixing of sand and plant, while ensuring a good water provision. The reduction of the leaf area decreases transpiration and offers a protection against the abrasive action of the grains of sand and the harmful effect of salt [25].

The buds (figure 2) are fragile, their sand builds up with approximately 10cm of depth is an adaptation to the salted, dry and windy habitat. The buds of the species of the fixed dunes, which venture on the mobile dunes, do not support the spray charged with salt and the impacts of sand, whereas those of *Ammophila arenaria* are in the shelter under surface. Marram grass grows and develops to the rhythm of the sand deposition ; the link between these two phenomena is very strong: the sand “stimulates” the development of marram grass [26-30] which by retaining sand, supports the sand deposition and thus the formation of the dunes. This adaptation to the sand gives it a great advantage compared to other species [23].

Marram grass is a monoicous plant with hermaphrodite flowers (figure 3). The inflorescence is a panicle [24] formed by condensed spikelet simulating a spike. The spikelet carried by course branches, has only one fertile flower with two acute glumes, lemma and palea (figure 3).

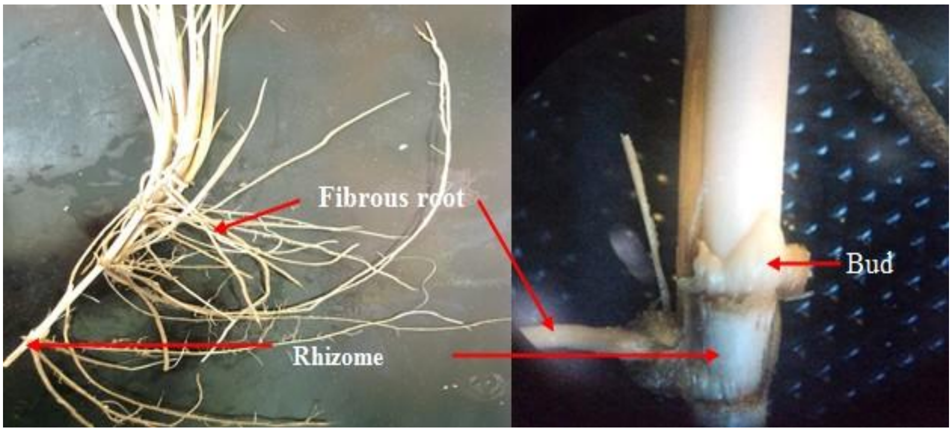

Figure 2: Roots, stem (rhizome) and buds of *Ammophila arenaria*

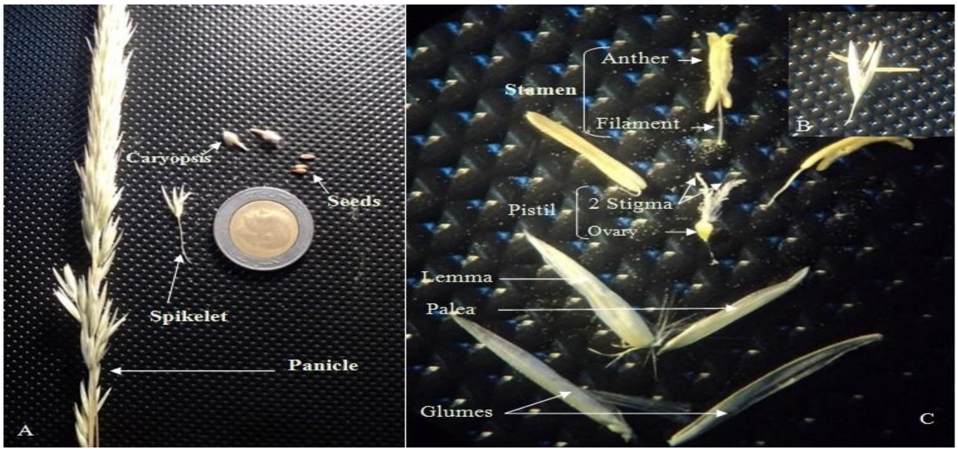

Figure 3: Inflorescence, caryopsis and seeds of *Ammophila arenaria* (A), general form of flower (B), detail of the floral parts (C)

Lemma, the larger, is indented at the top, whereas palea, the smaller, is included in the lemma [31]. The fertile flower contains three stamens with short filament. The pistil is formed by two welded carpels forming a unilocular superior ovary. This latter is surmounted by two feathery stigma carried by two styles (figure 3). Ovary contains only one ovule with basal placentation .Anemophily pollination and the lightweight of albuminous seeds (the fruit is a caryopsis [30]) support dissemination of marram grass in its windy environment. The weight of seeds can control their burial depth [6]. Thus the weight of seeds can support a seed bank accumulation in the shallowest sand layers making the dune ecosystem capable to a dynamic re-colonization after natural or anthropic ecological disturbance [6].

3.2 *Histological study*

3.2.1 *The root (figure 4)*

The anatomy is typical of monocot root [24, 32]. The bark contains an epiblema (Rhizodermis), suberized tissue with several assizes, a parenchyma which is well developed, formed by large cells, and a lignified endodermis (the pericycle appears absent). The central cylinder contains phloem and xylem (large metaxylem, xylem and phloem alternate with each other) with exarch differentiation, and a lignified medullary parenchyma.The root is rich in lignified and suberized tissues which offer rigidity, a protection (corrosion, dehydration) and a flexibility in the sandy soil. The absence of wood (secondary xylem) is compensated by the presence of large vessels of metaxylem which ensures an effective conduction of the crude sap [33, 34]. The important network of root hair increases the surface exchange with the soil solution, which makes it possible for the plant to resist to the hydric stress. That has a great advantage in the sand which retains water slightly [34].

3.2.2 *The Stem (figure 5)*

The anatomy is also typical of monocot stem [24, 32].The bark contains an epidermis covered by cuticle, a cortical parenchyma and an endodermis (difficult to visualize). The central cylinder, more developed than the bark, contains conducting vessels superimposed and disposed according to several circles (at least 3 circles), a sheath of sclerenchyma which surrounds the vascular bundles, and a marrow.

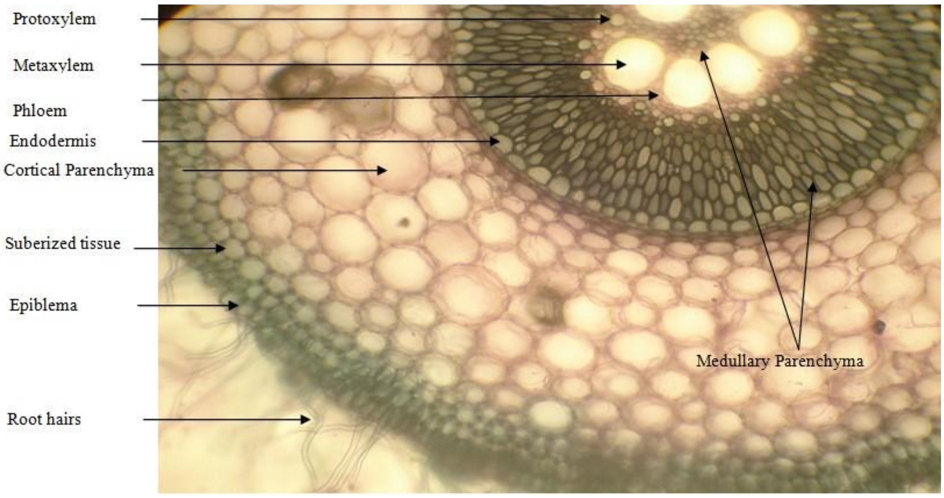

Figure 4: Transverse section of *Ammophila arenaria* root (Enlargement x 100)

The xylem well developed, is in the shape of "V" with end arch differentiation (the protoxylem and the metaxylem are very visible). The phloem has an exarch differentiation.The sclerenchyma, which is well developed, ensures at the same time a resistance and flexibility to the rhizome in the sandy substrate [33]. In the same way, this tissue surrounds vascular bundles and limits the water loss. The parenchyma is reduced; that cortical is thin, whereas that medullary is mainly resorbed (except on the level of the node). The large vessels of the metaxylem ensure, as at the root, a fast and efficient conduction of the crude sap [34, 35].

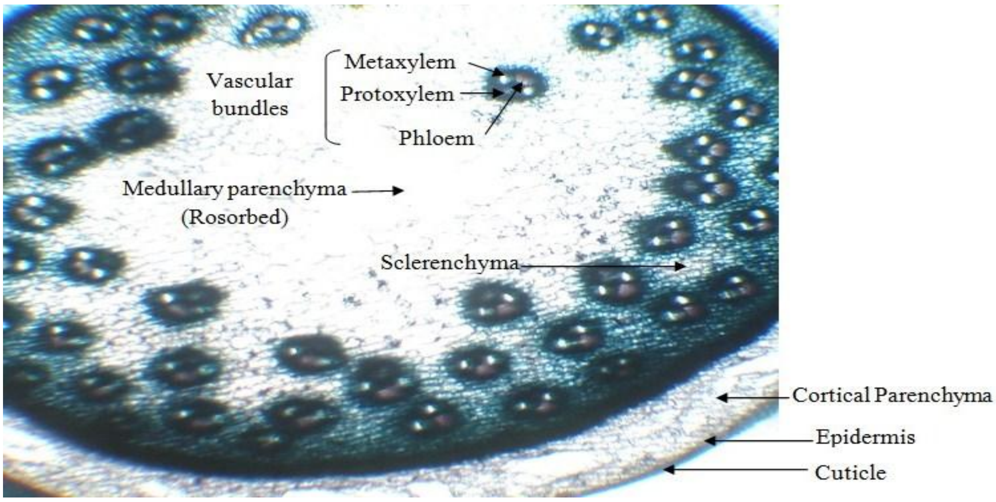

Figure 5: Transverse section of *Ammophila arenaria* stem (Enlargement x40)

3.2.3 The leaf (figure 6)

At the anatomical level, the adaptation of the leaves to the hydric and saline stress is more sophisticated [34]. The functional organization of the leaf reflects such adaptations perfectly. The external cuticle, thick and clear, plays several roles: the clear cuticle increases the albedo of the plant protecting it from the radiation effects (resulting from the albedo of the sandy substrate) which could deteriorate the photosynthetic pigments of the parenchyma [34, 36]. It also forms a physical barrier against the harmful effect of salt. The hydrophobic character of the cuticle (waxy coating) prevents the too important losses of water by evapotranspiration [34, 37]. The lower epidermis is deprived of stomata. The reduction of the number of stomata and the thickening of the cuticle decrease the transpiration [38].The abundant sclerenchyma ensures a mechanical protection of the plant against the aggressions by the grains of sand [28]. It allows especially a support particularly adapted to the windy environment since it is at the same time flexible and resistant. The upper epidermis contains stomata, which are inserted in the stomatal crypts: sunken stomata in the crypts and the hairs reduce movement of air, which decreases the transpiration [32, 34, 39]. These crypts also exist at the Oleander and the Esparto [40] and are very characteristic of the plants which live in the dry environment [41]. This differential organization between the two leaf faces joined that of other species like Oleander and Carob [40].

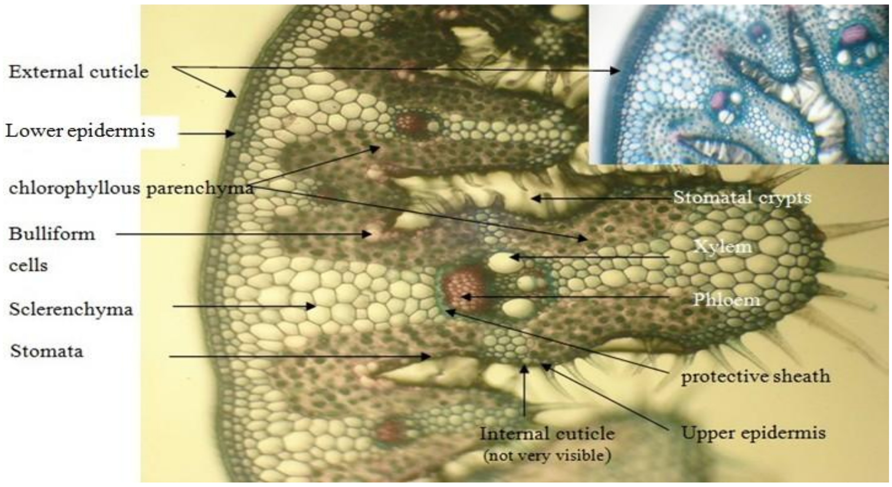

Figure 6: Transverse section of *Ammophila arenaria* leaf (Enlargement x100)

The bulliform cells, located at the base of the crypts, ensure an osmoregulation which controls the movement of the leaf blade [42]. These specialized motor cells facilitate the leaf rolling: in dry condition the bulliform cells become plasmolysed; consequently the leaf is rolled along its principal vein [39]. In wet conditions the bulliform cells are turgid which causes unrolling of the leaf [39]. This leaf rolling is an adaptation to the drought [32, 34, 39]. Thus this mechanism of water conservation makes it possible to the plant to resist to the hydric stress [43]; the surface exchange which decreases considerably, limit water loss by evapotranspiration [28]. In the same way, the leaf rolling reduces the abrasive impacts of the grains of sand on leaf tissues.
The chlorophyllous parenchyma is well protected and reduced to thin bands, whereas the vascular bundles are surrounded by a protective sheath formed by lignified fibers, which limits the water loss [32].

3.3 *Effect of temperature (Figure 7)*
Statistical analysis shows a significant effect of the temperature on the rate of seed germination (Probability <0, 05: significant differences a threshold of 5%). The effect of the temperature on germination was quoted by several authors [44-47].The seeds germinate (rate higher than 50%) in a wide range between 15 and 30°C (90.83 to 74.17%).

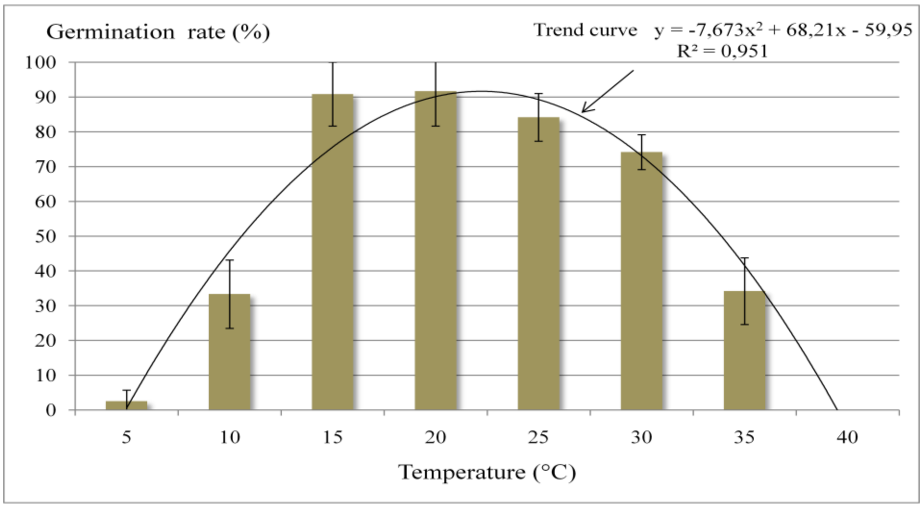

Figure 7: Effect of temperature on the germination rate of *Ammophila arenaria* seeds

The optimum germination temperature is 20°C (91.67%) which corroborates with the results of other authors: between 15 and 25°C according to Bendimered & *al*. [48] and lower or equal to 20°C according to Lachiheb & *al*. [49]. According to the classification adopted by Neffati [50], the optimum of germination is low. This germinative behavior of marram grass is identical to that of other graminaceous plants like *Koeleria phleoïdes*, *Corynephorus articulatus* [49] and *Stipa tenacissima* [48]. This optimum temperature is close to the average temperatures of spring which supports germination especially with abundant precipitations during this season [35]. The low (5°C) or high (40°C) temperatures inhibit germination. Indeed extreme temperatures can deteriorate the plasmic and mitochondrial membrane [51] which inhibits the processes of germination [47, 52]. Thus the low temperatures modify membrane lipids which disturbes 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 [53] and a reduction of the speed of water absorption [54]. The high temperature causes a denaturation of the membrane proteins [55], an inhibition of synthesis and/or activity of the enzymes implied in the mobilization of the reserves [47] and a reduction in the quantity of oxygen which arrives at the embryo [56]. This thermal inhibition was also reported for seeds of Celery [57] and Spinach [58].The culture of the seeds at 20°C temperature allows the production of a large number of seedlings of marram grass which will be used for the restoration and the rehabilitations of the degraded dunes. If we want to use direct seeding, the operation must take into account the ambient temperature, precipitation and the salinity [6, 59] of the soil which must be low [60,61]: the operation must be realized during the beginning of autumn or during spring [8].

Conclusion

All the parts of marram grass are well adapted to the life in the mobile dunes. The adaptation of this xerophyte and halophyte to the hydric stress, saline stress and sand build up is visible as well on the morphological, anatomical and physiological levels. In vitro germination at 20°C temperature makes it possible to obtain a large number of seedlings of marram grass which could be used for the restoration and the rehabilitation of the degraded dunes. Due to the great germination capacity of seeds of a subspecies *arundinacea* and its weak cover in the Mediterranean coasts, it is recommended to proceed with the plantations starting from seeds instead of the cuttings and of the rhizomes.

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