ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The effects of hydric & saline stress on the soluble sugars of *marram grass* roots (*Ammophila arenaria* L.)

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ABSTRACT

A moderate hydric stress (polyethylene glycol concentration lower or equal to 90g/l) of 5 or 12 hours duration involves an increase in the content of soluble sugars in the roots of *Ammophila arenaria* compared to the witness, whereas a severe hydric stress (concentration higher than 90g/l) involves a reduction of sugars concentration. The sodium chloride concentrations lower or equal to 12g/l cause an important increase in the soluble sugars in the roots, in particular for the treatment of 5 hours, whereas the higher concentrations involve a reduction of soluble sugars level. Soluble sugars of the root seem to increase much more in response to the saline stress than with the hydric stress.
Keywords: *Ammophila arenaria*, hydric stress, saline stress, roots, soluble sugars.

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INTRODUCTION

Ammophila arenaria (or marram grass) is a cryptophyte plant with rhizomes [38] belonging to the *Poaceae* family [9, 20].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 [31], the ears are ripe in July. Mature seeds are dispersed in September and germinate the following spring [8]. Reproduction is primarily vegetative by rhizomes [37].The fruit is an albuminous caryopsis.

The study will concern roots of *Ammophila arenaria* L. (Link), variety arundinacea (Host) [35] 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 [27], 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 [7]. The rainfall varies between 300 and 400 mm [22, 26] with an average value 330mn [41].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, 33] with a semi- arid bioclimatic weather [12].

The aim of this study is to test the effect of the intensity and the duration of the hydric and saline stress on the rate of soluble sugars of the root. This makes it possible to identify some biochemical adaptations of marram grass to these two types of stress.

MATERIALS AND METHODS

After a germination of marram grass seeds during 15 days in distilled water and in a drying oven under a 20°C of temperature, the young seedlings are cultivated under ambient temperature, in sand and peat and are daily irrigated. After five months, the roots are excised and their total soluble sugars are determined according to the method of Dubois & *al*. [11]. 100mg of fresh roots is introduced into test tubes containing various concentrations of polyethylene glycol (PEG 6000) (0g/l=Witness, 15g/l, 30g/l, 60g/l, 90g/l, 120g/l, 150g/l and 200g/l) or of sodium chloride (NaCl) (0g/l=Witness, 3g/l, 6g/l, 9g/l, 12g/l, 18g/l, 24g/l and 30g/l). Each test of concentration is done in three repetitions. After 5 hours and 12 hours of treatments (by PEG or NaCl), soluble sugars are extracted in ethanol (80%). Colorimetric analysis is done by respective addition of phenol (5%) and sulfuric acid (96%). The reading of the optical density is done at 490nm. The soluble sugar concentrations are deduced from a standard curve of glucose and expressed in µg/100mg of fresh roots.

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 hydric stress, saline stress and duration of treatment on the rate of the soluble sugars in the root.

Effect of hydric stress

For 5 hours treatments, the concentrations in PEG between 15 and 90g/l involve an increase in the rate of soluble sugars in the roots compared to the witness (figure 1). A severe hydric stress involves a great reduction in the rate of soluble sugars, and this whatever the duration of treatment. Thus for 5 hours treatments (or 12 hours), the rate of sugars for 200g/l decreases more than four times compared to the rate recorded for the concentration 90g/l. This reduction would be the result of a desiccation of the protoplasm and a structural and metabolic dysfunction at the cellular and molecular level. Indeed many studies showed that the cytoplasmic and organoids enzymes (like α amylase) can undergo important losses of activities or even be completely denatured, when they are dehydrated [36].

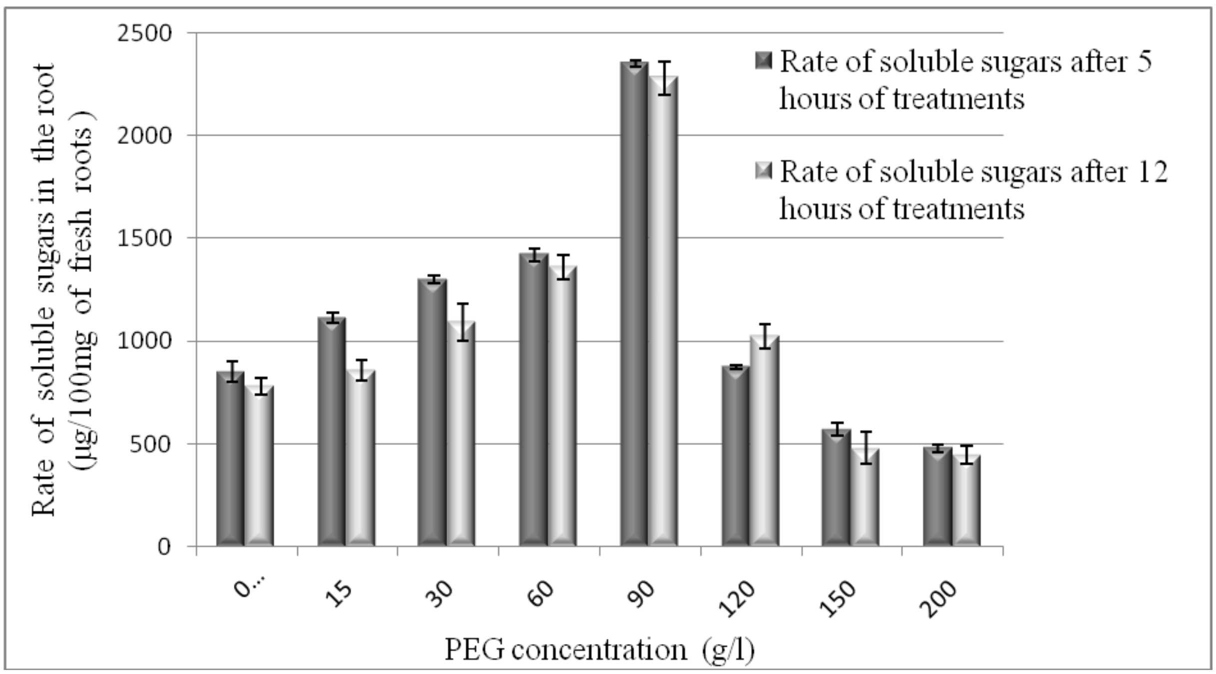

Figure 1: Effect of polyethylene glycol (PEG 6000) concentration on the rate of soluble sugars in the root of *Ammophila arenaria*

The increases in soluble sugars in the roots of marram grass are biochemical and molecular responses to the hydric stress what allows an adaptation to the environmental stresses [40]. Indeed the metabolism of sugars plays a main function in the tolerance of the plants to the drought and dehydration [32]. Soluble sugars, like saccharose, can play the role of osmoregulator during a hydric stress by maintaining the cellular osmolarity [5] and by limiting transpiration [3]. Sugars (like trehalose & saccharose) can also play a stabilizing and protective role of the phospholipids membrane systems [39, 23] and enzymatic proteins [6].

The accumulation of soluble sugars was also noted in the leaves and the rhizomes of marram grass [29] and also at other species like wheat [30, 42], soybean [28], walnut [25] and Atlas pistachio [43]. An accumulation in glucose, fructose and saccharose was also observed under hydric stress at the corn leaves [34]. Geigenberger & *al*. [14] allotted the increase in soluble sugars to a degradation of the reserve starch to saccharose, fact which could be also related to an inhibition of the starch synthesis. Du & *al.* [10] allotted the increase in glucose and the fructose to stimulation by the hydric stress of the activity of α amylase and invertase. However other authors stipulate that the accumulation of sugars is only the result of the reduction in their use following inhibition of the growth by the hydric stress [21, 2] and thus does not reflect a strategy of resistance to the drought. Moreover Ford & Wilson [13] announced that the accumulation of soluble sugars can have a weak contribution to the osmotic adjustment and that in this case, the adjustment is ensured by the accumulation of inorganic ions. The latter are especially potassium at the glycophytes [23] and sodium at the halophytes [17].

Effect of saline stress

For 5 hours treatments, the salt concentrations between 3 and 12g/l involve a great increase in the rate of soluble sugars compared to the witness (figure 2). Indeed the concentration pass from 1090µg/100mg for the witness to 3652µg/100mg for the roots incubated in 12g/l of NaCl. The salt concentrations higher than 12g/l involve a significant reduction in the sugar content. For 12 hours treatments, except for the concentration 18g/l, the salt concentrations between 3 and 24g/l seem not to involve a significant variation of the rate of soluble sugars compared to the witness. However the concentration 30g/l makes strongly decrease the rate of sugars compared to the witness.

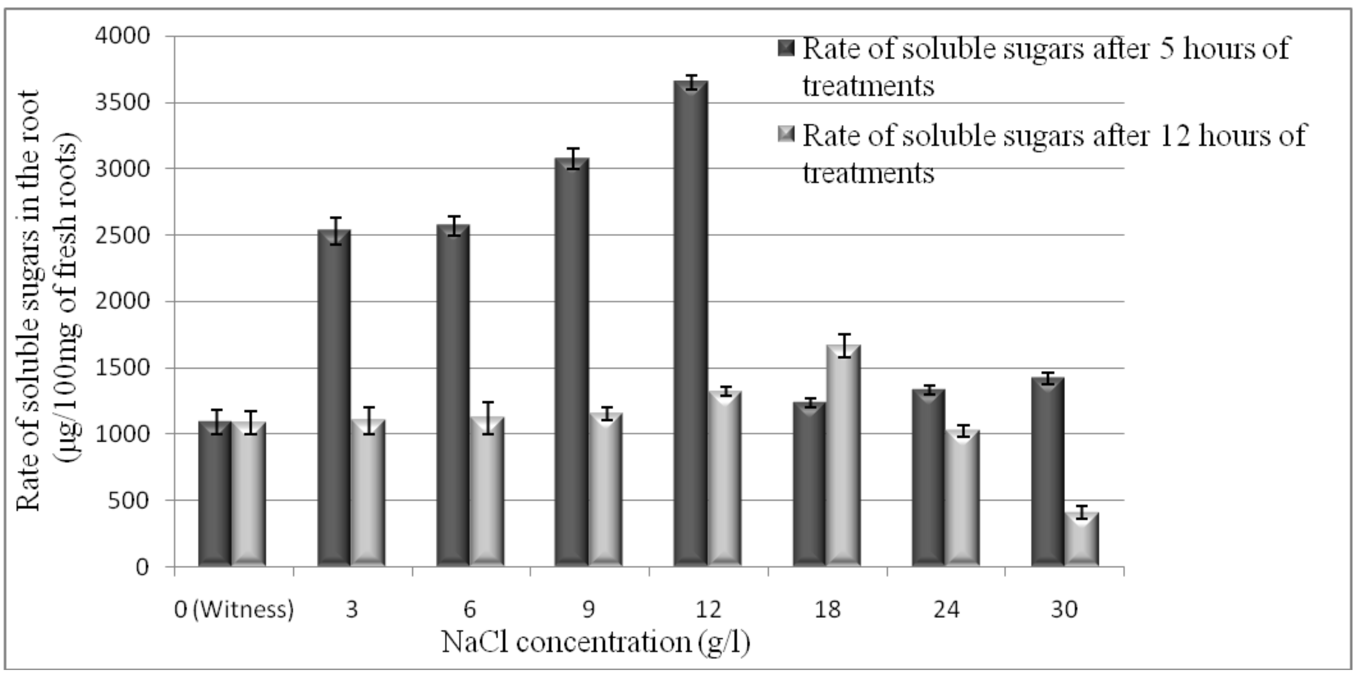

Figure 2: Effect of sodium chloride (NaCl) concentration on the rate of soluble sugars in the root of *Ammophila arenaria*

The rate of the soluble sugars accumulated in the roots in response to the saline stress (5 hours of treatments) is higher than that of the hydric stress (3652 and 2348µg/100mg respectively). The increase in soluble sugars is an adaptive biochemical response to the saline stress. Thus this rise seems to indicate that the marram grass is protected from the harmful effects of salt much more than it does it against the drought. The accumulation of soluble sugars under the saline stress was also noted on the roots of Argan [44] and Atriplex [4]. Soluble sugars, which would play the same role that those describe in the hydric stress, would be implied in the osmotic adjustment of the cytoplasm [24].

The important reduction of the rate of soluble sugars for 12 hours treatments (compared to 5 hours treatment of the saline and hydric stress) would be related either to an important conversion of sugars to other osmoregulators like the proline, and this via the glutamate [15] and ornithine [24, 16], or to an exhaustion of the starch reserves following the suppression of relationships between the roots (excised roots) and the leaves [23], or to a re-establishment of the regulation of the membrane permeability [44].

Reduction of the rate of soluble sugars for the concentrations higher or equal to 18g/l would be related on the one hand to the reduction in the availability of water [24] and on the other hand to the toxic action of salt [18]. Indeed key enzymes in the metabolism of sugars, in particular α-amylase and invertase [14, 10], would be inhibited by salt. The metabolic disturbances in the root cells in the high salt concentrations (≥18g/l), could explain why *Ammophila arenaria* under the natural conditions cannot tolerate salt concentrations higher than 20g/l [19].

CONCLUSION

The accumulation of soluble sugars in the roots of *Ammophila arenaria* depends on the type of stress of its intensity and its duration. The accumulation of sugars at the marram grass would be a biochemical adaptation to the hydric and saline stress. Thus soluble sugars would be implied in the osmotic adjustments particularly in the case of hydric stress and in the case of short-term saline stress whereas for a long-term saline stress the proline can intervene also there. Soluble sugars of the root seem to increase much more in response to the saline stress than with the hydric stress. This seems to indicate that the marram grass is protected from the harmful effects of salt much more than it does it against the drought.

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