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Potential utilisation of halophytes for the rehabilitation and valorisation of salt-affected areas in Tunisia

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Introduction

In arid and semi-arid regions, irrigation water contributes to salinisation of the upper layer of the soil, where most root activity takes place. Along the path of plant domestication, many crop species have lost resistance mechanisms to various stress conditions [1], including salt stress [2]. Thus, most crop plants do not fully express their original genetic potential for growth, development and yield under salt stress, and their economic value declines as salinity levels increase [3, 4]. Improving salt resistance of crop plants is, therefore, of major concern in agricultural research. A potential genetic resource for the improvement of salt resistance in crop plants resides among wild populations of halophytes [5, 6]. These can be either domesticated into new, salt-resistant crops, or used as a source of genes to be introduced into crop species by classical breeding or molecular methods.

Given the progressive scarcity of freshwater resources and soil salinisation, a major aim of investigations is to evaluate the potential of halophytic species to be widely and economically used in arid and semi-arid regions. It would encourage the sustainable use of halophytes for the creation of productive ecosystems and re-greening degraded areas, by building up a collection of halophytes with a high tolerance to salt stress and characteristics potentially exploitable from an economic point of view. Among the known 2,600 halophytic species, some present economic (human feeding, fodder, materials of high economic values) or ecological interests (soil desalinisation, dune fixation, phytoremediation, landscaping and ornamentation).

Within the framework of this approach, the Laboratory of Plant Adaptation to the Abiotic Stresses, in the National Institute of Scientific and Technical Research (INRST) of Tunisia has initiated an exploration and a physiological and biochemical characterisation of some halophyte species in order to identify the most promising ones. There are two major topics: the implication of halophyte species in the improvement of soil characteristics (desalinisation and fertilisation, heavy metal extraction), and their economical interests as oleaginous and fodder crops, for instance.

Results

Ecological interests of halophytes

Improvement of soil characteristics

Vegetation in saline habitat such as sebkha is heterogeneous. Numerous perennial tufts of strict halophytes are associated with annual species sensitive to salt and mineral deficiency stresses. *Medicago*, characterised by a high fodder value, largely contributes to the ecosystem primary production in the absence of water constraints. These annuals mainly develop within or very close to halophyte tufts. Parallel field and laboratory studies have shown that *Medicago* is sensitive to salinity [7, 8], as well as to nitrogen and phosphorus deficiencies [9]. Furthermore, the shoots of the annuals growing in association with halophyte species contains relatively low Na^+

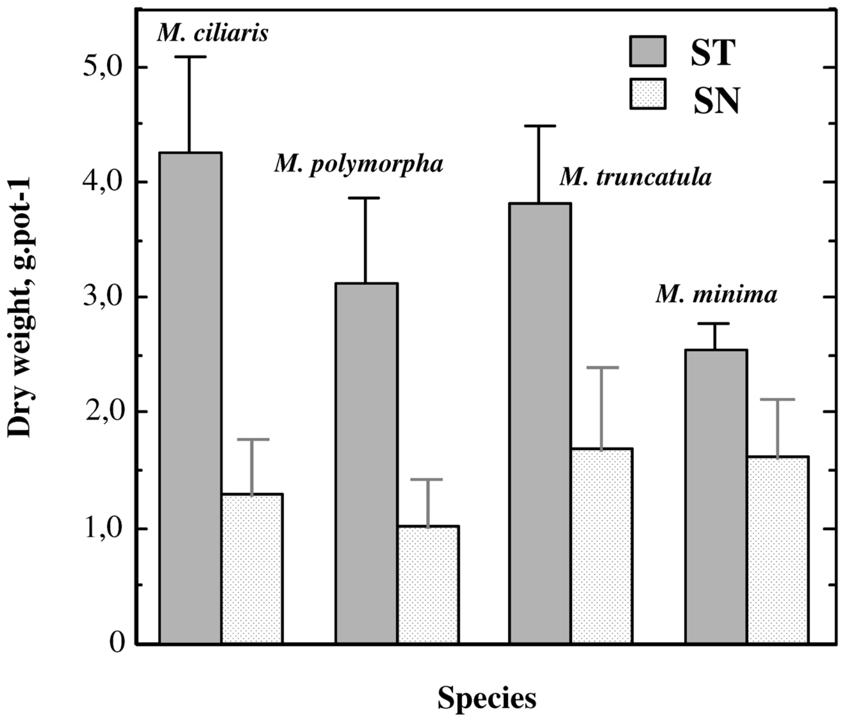

Figure 1. Changes in biomass production of *Medicago ciliaris*, *M. polymopha*, *M. truncatula* and *M. minima* (g.pot^{-1}) with soil origin. ST: culture carried out on soil sampled under halophytes tufts, SN: cultures carried out on soil sampled in outside of halophytes tufts. Means of 20 repetitions and confidence intervals at 95 %

concentrations. These data suggest that the upper horizon of spots near the halophyte tufts (where sensitive annuals grew), is fertile and contains low salt levels. Indeed, this was confirmed by the study of soil samples taken from the upper horizon in the tuft centres. Desalinisation of the upper horizon by the superficial roots of halophytes could be responsible for this microgradient of salinity. Moreover, the litter formed by halophyte fallen organs and by organic debris accumulated by the wind at the vicinity of halophyte tufts, could contribute to localised soil enrichment in N and P. This was confirmed using a biological test of soil fertility. Some tufts of *Salicornia arabica* were removed for sampling soil in the upper horizon (0–20 cm), where roots of annual plants developed. Other samples were taken between halophytes tufts, in zones devoid of vegetation or weakly populated. Four annual *Medicago* species (*M. ciliaris*, *M. polymorpha*, *M. truncatula* and *M. minima)* were grown on these soil samples, without mineral fertilisation, in a greenhouse under controlled conditions. The plants were harvested at the flowering stage. In the four species, total biomass production (dry matter per pot) was higher on soils sampled under the halophyte tufts than on soils from nude zones (Fig. 1). These studies show that perennial halophytes improve soil characteristics by lowering its salt content and by increasing nitrogen and phosphorus concentrations.

The capacity of desalination of saline soil by halophytes was also evaluated in strictly controlled conditions, using *Sesuvium portulacastrum*, an Aizoaceae. After clonal multiplication, the plants were cultivated for 2 months on saline soil, originating from the edge of a sebkha. They were irrigated with a nutrient solution deprived of $Na^+$and Cl^-, without losses by drainage. Salt export by plants was evaluated by the difference between the quantities of Na^+ and Cl^-, initially measured in the culture

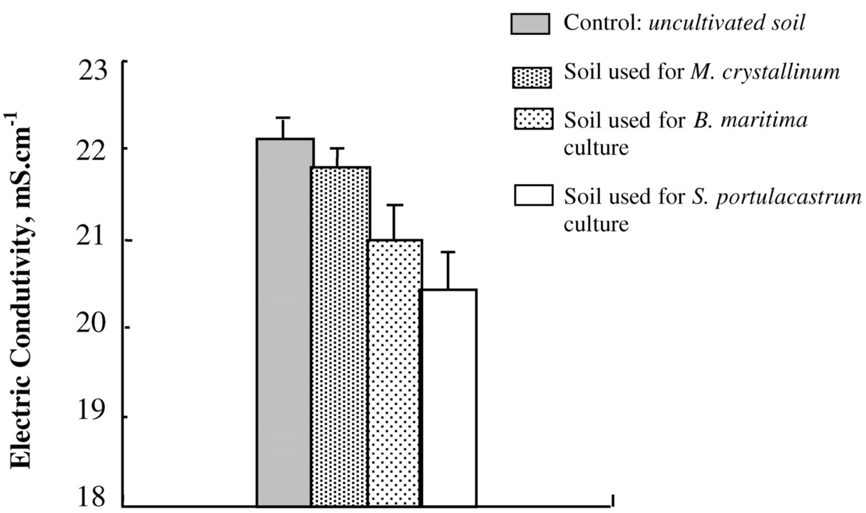

Figure 2. Electric conductivity ($mS.cm^{-1}$) of the aqueous extract (1/10) of soil used during two months for halophytes culture. Means of 12 replicates. Bars indicate ± standard errors ($p = 0.05$)

substrate, and those found at the end of the experiment in the soil. *S. portulacastrum* produced more biomass than other species used in the same experiment (*Batis maritima* and *Mesembryanthemum crystallinum*), and accumulated larger amounts of Na^{+} and Cl^{-}(about 6.5 mmol.g^{-1} DW, amounting to 30–40 % of the biomass). At the end of the cultures, the soil used for the culture of *S. portulacastrum* showed a significant (10%) decrease of its salinity estimated by electric conductivity (Fig. 2). This study demonstrates that the associated characteristics of *S. portulacastrum*, namely high growth rate and high capacity for salt accumulation, permit soil desalination, even in short-term cultures. Thus, this species would be interesting for the rehabilitation of the saline lands.

Heavy metal extraction
In Tunisia, saline depressions, colonised by halophyte species, often constitute sites of accumulation of industrial effluents contaminated by heavy metals. Indeed, preliminary studies achieved in various regions of Tunisia showed that these zones are contaminated by cadmium, nickel and lead. We studied the response to Cd of two halophyte species, *S. portulacastrum* and *M. crystallinum*. In the absence of Cd, the biomass of *M. crystallinum* plants was much larger than that of *S. portulacastrum*. However, Cd severely inhibited *M. crystallinum* growth, even at the lowest concentration (50 µM), but did not significantly modify that of *S. portulacastrum.* In the shoots, the Cd concentrations in *S. portulacastrum* shoots was half (100–350 ppm)

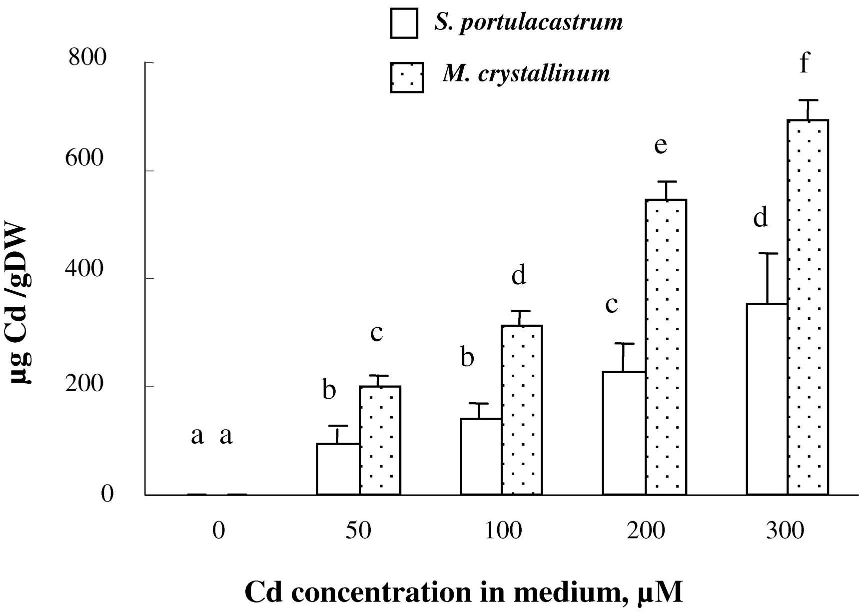

Figure 3. Changes in Cd concentration (µg.g^{-1} DW) in shoots of *S. portulacastrum* and *M. crystallinum* treated by various Cd concentrations. Means of 8 replicates. Bars marked with same letter are not significantly different at $p = 0.05$

that in *M. crystallinum* shoots (200–700 ppm) (Fig. 3). According to these data, both species would be classified among Cd hyper accumulator plants and would be of this fact interesting for the phytoremediation. The analysis of the relationship between growth and mineral status in the two halophytes suggested that the Cd-induced decrease of growth resulted not from direct effect of accumulated Cd, but rather from restriction of K^{+}, Ca^{2+}, and Fe^{2+} uptake. This hypothesis was studied using a split-root system: after a pretreatment phase, seedlings were divided into three lots. Half of the roots of the first lot were immersed in Cd free medium, while the other half were immersed in the same medium supplemented with 100 μM Cd (B/Cd). For the two other lots, the two halves of the root system were immersed either in free Cd medium (B/B) or in medium supplied with 100 μM Cd. In comparison with Cd/Cd plants, the split root Cd/B plants displayed improved growth. This effect was associated to an increase in nutrient uptake. Furthermore, the Cd/B plants accumulated Cd at a level similar to that of Cd/Cd plants. In summary, our results indicate that the Cd-induced decline growth resulted rather from an indirect Cd effect (inhibition of nutrient uptake) than from a direct Cd effect (excess of Cd accumulation) and suggest the possibility to increase the capacity of the two halophyte species to extract Cd while improving nutrient availability in the medium.

Economical interests of halophytes

Halophytes with fodder potential

In Tunisia, fodder crops occupy currently only 7 % of the cultivated zones. In addition, the surface of the uncultivated saline area is four times more important than that of the pastures. The identification of fodder halophytes would make it possible to exploit new zones of production and to reduce our deficit in fodder. Within the framework of this approach, several potentially suitable species were identified. The conditions of their multiplication and culture were established as well as their salt tolerance limits and their nutritional requirements.

Suaeda fruticosa, an indigenous Chenopodiaceae in Tunisia, is quite frequent in semi-arid, arid and desertic bioclimatic stages and well appreciated by livestock [10]. *Spartina alterniflora*, a Poaceae, is dominant in saline marsh and coastal regions in the east of USA. For *S. fruticosa*, maximal dry matter production occurred at NaCl concentrations comprised between 100–300 mM, with a 85-fold increase in dry weight following a 45 day treatment. *S. alterniflora* expressed maximal growth when irrigated with nutrient solution or containing 0–100 mM NaCl, its initial dry weight being six-fold increased after 100 days of treatment. *S. fruticosa* was more salt tolerant than S. *alterniflora* under moderate NaCl concentrations (300 mM NaCl), but more sensitive at the highest NaCl concentration (800 mM). Considering the decreasing availability of freshwater in arid regions, the utilisation of non-conventional water resources (brackish water, waste water, and seawater) constitutes a promising approach, especially as these halophytes require salt to express their maximal growth potentialities. However, the growth of both species was limited when they were irrigated with seawater, owing to the low availability of some nutrients. Indeed, they displayed higher growth rates when nitrogen and, to a lesser degree, phosphorus were added to the seawater [11].

The capacity of biomass production is an important characteristic which must be considered in the evaluation of the fodder halophytes. Using cultures carried out in pots of 0.85 m^3, we demonstrated that *S. alterniflora* could produce 7,500 kg DW per hectare in one cut. As the season of growth of this species extends from March–October, it is possible to carry out at least two cuts, which will ensure a primary production of 15 tons DW per hectare and per year. Similar estimation showed that *S. fruticosa* could produce at least 4,500 kg DW per hectare. These yields are comparable to those of the conventional fodder crops irrigated with freshwater, like alfalfa, 10 tons.ha^{-1} per year [12], and clover, 8 tons.ha^{-1} per year [13]. Analysis of published data indicates that the yield of the most productive halophytes varies from 8–17 tons DW.ha^{-1} per year. The comparison of the mineral composition of the shoots of both halophytes with that of the fodder required by livestock showed that excepting Ca^{2+} content in *S. alterniflora*, K^+, Mg^{2+} and P concentrations in tissues exceed the nutritional requirements of the cattle. The content of total nitrogen is higher in *S. fruticosa* than in *S. alterniflora*, but the digestible nitrogen fraction is similar in both halophytes, and meets perfectly the nutritional requirements of the sheep. According to Glenn and O'Leary [14] and Bayoumi et al. [15], proteins represent 15 % of DW in several halophytes. According to these data, *S. fruticosa* and *S. alterniflora* can annually produce 1.5 tons of proteins for an average biomass of 10 tons.ha^{-1}. The quantitative and qualitative yield of the two halophytes is appreciably similar to that of alfalfa irrigated with freshwater [16].

Two other halophytes from Tunisia, with fodder potential, were characterised: *Aeluropus littoralis* and *Catapodium rigidum*, respectively perennial and annual Poaceae. In the absence of salt, *Catapodium* displayed a relative growth rate (RGR) slightly higher than that of *Aeluropus*. Salt decreased this parameter in both species. On RGR basis (expressed as % of control without salt) *A. littoralis* was more tolerant than *C. rigidum*, this behaviour being accentuated with increasing salinity. In salinity range not exceeding 400 mM, RGR remained between 0.04–0.06 day^{-1}, values characteristic of spontaneous or cultivated *Medicago* species not subjected to salt, 0.08–0.09 [7, 9] or others fodder halophytes *Suaeda fruticosa*, 0.07–0.09 [11], *S. alterniflora*, 0.03 [11], *Spartina anglica*, *Puccinellia maritima*, 0.02–0.05 day^{-1} [17].

Both halophytes accumulated sodium mainly in their shoots. However, Na^+ concentrations were lower in *A. littoralis* than in *C. rigidum* (maximum values around 5 mmol. g^{-1} DW and 10 mmol. g^{-1} DW, respectively). In addition to its capacity to control Na^+ transport towards shoots, *A. littoralis* secreted more than 50% of leaf Na^+ by salt glands. Indeed, Na^+ accumulated inside tissues did not exceed 2 mmol.g^{-1} DW under the most severe salinity. This secretion seems to be selective for Na^+ and Cl^-, since K^+ was completely absent among the elements secreted on the surface of the leaves.

Oleaginous halophytes

Some Tunisian salty areas have been explored for plants which could be considered as oilseed species. Three potentially interesting species have been identified as oil producers, *Zygophyllum album* (Zygophyllaceae), *Cakile maritima* (Brassicaceae), and *Crithmum maritimum* (Apiaceae). Oil extraction was carried out on ripe seeds collected in their natural biotope. Some physiological and biochemical aspects were studied, such as individual mass of seeds, oil content, and lipid and fatty acid composition. The seeds of investigated species have a suitable size for harvesting. The dry weight of 100 seeds ranged from 133 mg in *Zygophyllum album* to 774 mg in *Cakile maritima*. The value for the latter species is nearly three times higher that for rape (*Brassica napus*) seeds, a conventional oleaginous plant. The seeds of the two other halophytes are smaller than *Cakile maritima* seeds, with mass approximately half of that of rape ones.

The seeds of *Cakile maritima* and *Crithmum maritimum* present high levels of oil, reaching respectively 42 % and 30 % of the seed DW. Oil content in *Zygophyllum album* seeds is very low (6 %). As for olive oil, fatty acid composition of *Crithmum maritimum* seeds is characterised by a high level of oleic acid (81 %), whereas that of *Zygophyllum album*, characterised by a high percentage of linoleic acid (64 %), is similar to sunflower oil composition. Therefore, these two species contain oils of good quality which can be used without any further modification. Oil of *Cakile maritima*, rich in erucic acid (25 %) may be used for industrial applications [18].

In the laboratory, growth of *Cakile maritima* was stimulated under moderate (50–100 mM) concentrations of NaCl. The response of the whole plant was essentially due to the salt effects on shoots, their growth being significantly augmented at 100 mM NaCl. In the 200–300 mM range, the whole plant biomass production was maintained at approximately 90 % of the control. At higher NaCl concentrations

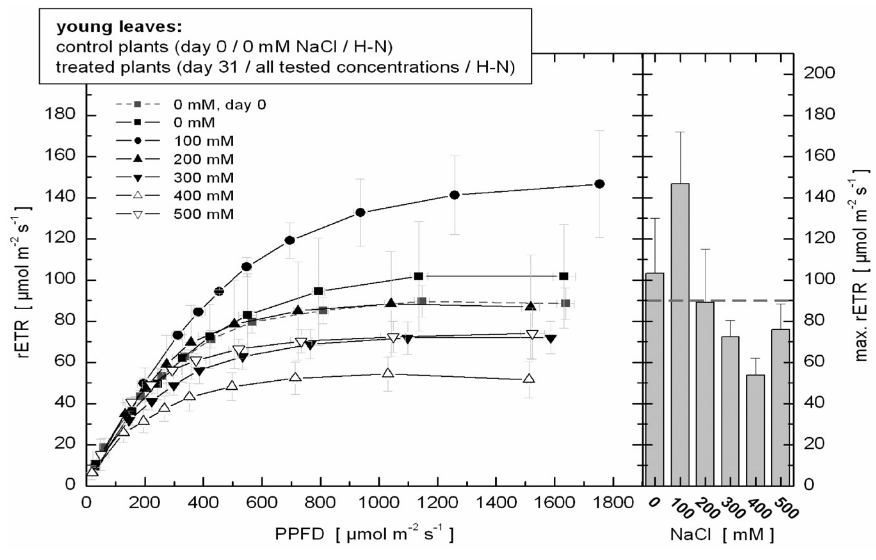

Figure 4. NaCl effect on photosynthesis. Changes in the electron transport rate (ETR) in the leaves of *Cakile maritima* subjected to increasing NaCl concentrations for 35 days. Means of 10 replicates ± standard errors (p = 0.05)

(500 mM), an important and significant reduction of growth was observed, but plants survived [19]. Concerning photosynthesis, various parameters were measured: gas exchange, net assimilation of CO_2, and electron transport rate (Fig. 4). As growth rate, these parameters indicated that the optimal physiological functioning was obtained with 50–100 mM NaCl. In these optimal conditions, the number of seeds per plant was significantly augmented by salt. At higher concentrations, a significant reduction of seed production was observed. All salt treatments resulted in seeds significantly smaller than in control. Culture of *Cakile maritima* in the presence of salt modified the biochemical composition of seeds: the oil content and the rate of erucic acid, largely used in industry, were augmented.

Conclusions

These studies were aimed at identifying among the halophytes promising species to ensure a plant productivity of economic and/or ecological interest, in the marginal zones and under conditions of irrigation with non-conventional water resources (brackish water, waste water and even seawater, more or less diluted). We identified several halophytes interesting for livestock nutrition. Monocotyledonous with a salinity avoidance strategy, enabling them to produce a biomass containing relatively little salt (*Spartina alterniflora*, *Aeluropus littoralis*, *Catapodium rigidum)* are particularly interesting. Culture conditions of these plants, their limits of salt tolerance and their nutritional requirements, are known. The data obtained so far are promising. Work is in progress to characterise their response to other constraints which could limit their yield in saline lands and particularly the low nitrogen availability [20]. Indeed, nitrogen is the most limiting nutrient for plant growth in saline ecosystems. Since a greater availability of $NH_4{}^+$ than $NO_3{}^-$ was often observed, the research of the halophytes able to use ammoniacal nitrogen would be an effective and less expensive substitute than the use of nitrogen mineral fertilisers.

Two promising oleaginous halophytes were identified: *Cakile maritima* and *Crithmum maritimum*. An important intraspecific variability was observed within these two species at the levels of i) seed biochemical characteristics (oil content, fatty acid composition), and ii) the physiological response to salt. Studies carried out on these plants from germination to the seed maturation showed that *Cakile maritima*, producing industrial oil, is relatively more tolerant to salt than *Crithmum maritimum*, producing edible oil [21]. However, recent data showed that *Crithmum maritimum*, rich in antioxidant molecules, is also interesting for medicinal purposes.

At the ecological level, particular interest was paid to the halophytes able to fix and desalinate soil. *Sesuvium portulacastrum* with its growth stimulated by high (800 mM salt) concentration under adequate mineral nutrition is a promising candidate. The analysis of its responses to heavy metals also suggests that this species is interesting for phytoremediation.

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