Adaptation potential of two common halophytes to salinity stress in the Salt Marshes of lake Burullus in Egypt

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Abstract
This study aims to investigate the physiology of adaptation mechanisms to increase salinity from wet to dry season when the salts are accumulated on the soil surface. Arthrocnemum macrostachyum (Moric.) K. Koch. and Halocnemum strobilaceum (Pallas) M. B are the common native plants to the salt marshes of Burullus Lake at the deltaic Mediterranean coast of Egypt. The two halophytic plants survive and grow in the prevailing high concentrations of salts. Plants and soil samples were collected from six locations depending on changes of their soil EC values. During wet season which have high rain and low temperature, the two halophytes accumulated high content of the primary metabolites carbohydrates, amino acids, proline and proteins. On the opposite at the dry season, due to increased temperature salinity increased greatly, even salt crust appears on the soil surface. The two halophytes in face to this high salinity accumulated secondary metabolites (alkaloids, phenolic compounds and polyamines) during the dry than the wet season. The high content of anthocyanins especially in A. macrostachyum showed their utilization in the two plants as a main source for pigments and antioxidants.

Key words: Alkaloids, Amino Acids, Burullus lake, Salt marshes, Halophytes, Phenolic Compounds, Proline, Protein and Polyamines.

1. INTRODUCTION
Burullus Lake is a shallow brackish lake extending for 47 km along the deltaic Mediterranean coast of Egypt and its width about (5-11 km)\(^1\). The source of lake water is drainage water through human activities, rainfall and Mediterranean Sea water through Bughaz El-Burullus. The halophytes represent the vegetation area of Burullus lake salt marshes. Halophytes are over 2000 species in the world, species of natural flora, form a group of ecologically biologically, physiologically and biochemically specialized plants capable of functioning normally and reproducing on saline soils. Halocnemum strobilaceum and Arthrocnemum macrostachyum are succulent perennial halophytes characterized by articular stems with carnose segments, reduced and stems-united leaves, and occur in highly saline environments\(^2\). Halophytes are naturally salt-tolerant plants that may be potentially useful for economical applications (oilsseed, forage, production of metabolites)\(^3\). Halophytes are plants adapted to live in saline environment, sea water, a salt-water marsh or a salt-desert\(^4\). Salt tolerance depends, to a great extent, on the cellular compartmentalization of toxic ions; tolerant plants have the ability to accumulate Na\(^+\) and Cl\(^-\) in the vacuole, so that the cytoplasm is maintained at substantially lower ion concentrations, thus avoiding the inhibition of metabolic processes\(^5\)\(^6\). The maintenance of osmotic balance requires the synthesis and accumulation in the cytoplasm of compatible solutes (osmolytes), which are not inhibitory to the metabolism even at high concentrations. These organic compounds are carbohydrates and amino acids and derivatives such as proline, glycine betaine\(^7\)^\(^8\)^\(^9\)^\(^10\). High proline content can be considered beneficial to stressed plants. Significant correlation between enhanced tolerance and proline accumulation in plants under saline condition has been reported\(^11\). Environmental stresses (salinity, drought) may trigger oxidative stress in plants, generating the formation of reactive oxygen species (ROS), leading to cellular damage, metabolic...
disorders, and senescence processes. Some antioxidant compounds are extracted from easy sources, such as agricultural and horticultural crops, or medicinal plants. Among them, halophytes are naturally salt-tolerant plants that may be potentially useful for economical applications as new sources of natural antioxidants in dietary foods. A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops, such as pea, cotton, rice. Phenolic compounds exhibit a wide spectrum of biological activities which may play numerous biological activities. Salinity stress affects the total alkaloids and phenolic compounds content in the plant. Salinity induces disturbance of the metabolic processes leading to an increase in secondary metabolites. These metabolites have an osmoregulatory role and considered as an adaptation mechanism to the imposed stress. Phenolic compounds exhibit a wide spectrum of biological activities such as anti-allergic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects. Polyamines, putrescine, spermidine, spermine, and cadaverine are low molecular weight organic polycations displaying a high biological activity. polyamines, both in the free and soluble conjugated forms, were suggested to have an important protective role against stresses such as cold, wilting, pollution, osmotic, salt, drought, heat, chilling and oxidation stresses.

2. MATERIALS AND METHODS

2.1 Plant material

Arthrocnemum macrostachyum (Moric.) K. Koch. and Halocnemum strobilaceum (pallas) M. B. represent the main vegetation of salt marshes of Burullus Lake of Egypt. The two plant species were collected during 2009 in winter (February), spring (May), summer (August) and autumn (November) from the following locations along the costal international road and they are at:

1- El hanfe village, 2- Mostroa village, 3- Godaa village, 4- El maksaba village, 5- Emad village, 6- Borg el Burullus village

The locations were arrangement in accordance with the increase in their salinity. The two plants samples were thoroughly washed with tap water followed by distilled water and then dried in an oven at 60°C for constant weight. The dried materials were powderd using an electric mill.

2.2 Soil analysis

Soil samples were taken within and away from the root systems of the two plants at depth (0-30cm) and their 1-5 soil solution electrical conductivity (EC) was measured using an electrical conductivity meter.

3. PLANT ANALYSIS

3.1 Estimation of carbohydrates

Dry shoot (0.1g) was extracted for 24 hours with a borate buffer. The borate buffer supernatants were used for estimating soluble carbohydrates. The residue was used for estimating polysaccharides quantitatively using the modified method.

3.2 Estimation of total free amino acids

Amino acid was determined from 0.1 g of the dried plant sample and the amino acid content was expressed as mg per g dry mass. Proline was determined in dry shoot samples and the proline content was expressed as mg proline per g dry mass.

3.4 Quantitative estimation of total soluble proteins

The total soluble proteins content was estimated quantitatively in the borate buffer extract. The protein content was calculated as mg/g dry mass using a prepared calibration curve by Bovine Serum Albumin protein.

3.5 Estimation of total alkaloids and phenolic compounds

Total alkaloids were measured quantitatively and expressed as mg/g dry mass of the plant samples. Total phenolic content was estimated quantitatively and expressed as mg/g dry mass of the plant sample.

3.7 Determination of polyamines

Putrescine, spermidine and spermine, were extracted and the content (ppm) of each polyamine of A. macrostachyum and H. strobilaceum was determined in four locations during summer season only. The results were statistically analyzed using the two ways analysis of variance (ANOVA) to determine the F test, LSD at 0.05 level and the degree of significance for the obtained variations by different locations, different seasons and their interactions and the analysis was carried out by SPSS statistical package.

4. RESULTS

4.1 Soil analysis

The characteristics of soil samples under and away from the two studied plant species (Arthrocnemum macrostachyum and Halocnemum strobilaceum) varied at the six locations 1, 2, 3, 4, 5 and 6 in the salt
marshes of Burullus Lake and during the four seasons (Table 1). The soil electrical conductivity (EC) show that the maximum mean (8.9 mS cm⁻¹) was in the summer season while the minimum one (3.9 mS cm⁻¹) was in the spring season. The mean values of EC in the soil away from the plants were higher than under the two studied plant species in most location and during winter and spring seasons. Also, the soil EC under Halocnemum strobilaceum was greater than under Arthrocnemum macrostachyum at all season especially at winter when soil EC under the later plant was double under the former plant. The highest EC values were recorded in location 6 during all seasons in the soil under and away from plants while the lowest one was in location 1.

4.2 Carbohydrates content
A. The direct reducing value (DRV)
Table 2 showed that the DRV of A. macrostachyum and H. strobilaceum shoot were remarkably varied in response to the effect of salinity of the different locations during all seasons. A. macrostachyum the highest value was 80 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 26 mg g⁻¹ d.m. at location 2 in summer season. H. strobilaceum acquired greater DRV sugars during most seasons compared to A. macrostachyum, the highest value was 99 mg g⁻¹ d.m. at location 5 in the spring season, while the lowest value was 25 mg g⁻¹ d.m. at location 3 in winter season.
The highest value of sucrose content in A. macrostachyum was 38.6 mg g⁻¹ d.m. at location 6 in the spring season, while the lowest value was 4.80 mg g⁻¹ d.m. at location 3 in winter season in A. macrostachyum. In H. strobilaceum the highest value was 40.0 mg g⁻¹ d.m. at location 3 in the spring season, while the lowest value was 3.6 mg g⁻¹ d.m. at location 2 in winter season.
The highest value of starch content in A. macrostachyum was 2.71 mg g⁻¹ d.m. at location 3 in the spring season, while the lowest value was 1.12 mg g⁻¹ d.m. at location 6 in winter season in A. macrostachyum. Starch content was remarkable greater in H. strobilaceum than in A. macrostachyum. The highest starch content in H. strobilaceum was 31.1 mg g⁻¹ d.m. at location 1 in the spring season, while the lowest one was 11.3 mg g⁻¹ d.m. at location 6 in summer season.
The order of variation of the three carbohydrate components was DRV > sucrose > starch showing low storage of carbohydrate in the two plants although there is a marked content of starch in H. strobilaceum (Fig. 1). The highest contents of the three sugar components and in the two halophytic plants were at the active growing spring season when edaphic factors were suitable.

4.3 Amino acids and protein content
The results represented in table 3 showed that the content of both amino acids and proteins followed the same trend of variation by seasons. Amino acids content was greater than that of protein in both plants and during all seasons of study. Proline was greatly accumulated in the two plants at the wet season (winter). It is also high in them during the dry season. The highest value of proline content was 7.10 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 0.1 mg g⁻¹ d.m. at location 3 at summer season in A. macrostachyum. In H. strobilaceum the highest value was 6.0 mg g⁻¹ d.m. at location 6 in the winter season, while the lowest value was 0.13 mg g⁻¹ d.m. at location 2 in summer season.
The highest value of amino acids content was 146 mg g⁻¹ d.m. at location 5 in the spring season, while the lowest value was 21 mg g⁻¹ d.m. at location 2 during summer season in A. macrostachyum. In H. strobilaceum the highest value was 174 mg g⁻¹ d.m. at the same location (5) and also in the spring season, while the lowest value was 50 mg g⁻¹ d.m. at location 1 during summer season.
The highest value of total protein content in A. macrostachyum was 7.2 mg g⁻¹ d.m. at location 3 in the winter season, while the lowest value was 1.45 mg g⁻¹ d.m. at location 1 during spring season. Similarly, in H. strobilaceum highest value was 6.22 mg g⁻¹ d.m. at location 6 in the spring season, while the lowest value was 1.19 mg g⁻¹ d.m. at location 2 during winter season.

4.4 Alkaloids and phenolic compounds content
The mean of alkaloids content was greater than phenolic content in the two plants during all seasons except winter (Table 4). Also the trends of variation of both alkaloids content and phenolic compounds content were similar in the different seasons and in both plants with a maximum accumulation during the hot dry season (summer). The highest value of total alkaloids was 93.0 mg g⁻¹ d.m. at location 6 during summer season, while the lowest value was 11.7 mg g⁻¹ d.m. at location 5 during winter season in A. macrostachyum. In H. strobilaceum the highest value was 99.4 mg g⁻¹ d.m. at location 6 during summer season, while the lowest value was 6.80 mg g⁻¹ d.m. at location 6 during winter season.
The highest value of total phenolic was 37.8 µg g⁻¹ d.m. during summer season at location 5, while the lowest value was 3.7 µg g⁻¹ d.m. at location 6 during spring season in A. macrostachyum. Also, in H. strobilaceum the highest value was 42.0 µg g⁻¹ d.m. during summer season at location 6, while the lowest
value was 6.40 µg g⁻¹ d.m. at location 3 during spring season.

4.5 Polyamines content
Table 5 showed that the maximum contents of putrescine, spermidine and spermine were 7.90, 10.98 and 4.24 ppm respectively in location 1 in A. macrostachyum. In H. strobilaceum the highest amounts of spermidine and spermine were 1.0 and 5.8 ppm respectively in location 6, while the highest amount of putrescine was 14.9 ppm in location 5.

5. DISCUSSION
The soil EC of the studied locations in the salt marshes of Burullus Lake was significantly increased in the summer season (dry season) due to the shortage of rain water which is required for leaching salts and decreases the soil salinity. On the opposite in winter and spring seasons (wet seasons) the rainfall led to a marked decrease of salinity. These results are in compatible with 37,38,39,40. The EC in the near soil was lower than the soil EC under A. macrostachyum. and H. strobilaceum plants because the two halophytic plants had the ability to accumulate salts in their tissues and their died parts release salts under the plants as also indicated by 41,42,43. It was observed that some halophytes act as phytoremediating plants by eliminating the heavy metals from the soil44. Also, phytoremediation could be differed by the halophytic plants as the soil EC under H. strobilaceum was greater than that under A. macrostachyum at all season especially at winter when soil EC under the later plant was double that under the former plant.

The reducing sugars of shoot were generally increased with high soil EC values in all seasons especially in plants of locations 5 and 6, and shoots accumulate reducing sugars than roots. There is a reduction in the soluble sugars during the dry season that might be a result of the general inhibition in the photosynthetic pigments (chlorophyll a and b) and due to its conversion into secondary metabolites, alkaloids and phenolic compounds.45,46 Results indicated that the starch contents of A. macrostachyum and H. strobilaceum were low during the summer and autumn seasons (dry season) especially in location-5 and 6. Statistical analysis revealed that the starch content was negatively correlated with soil-EC values, proline, soluble carbohydrates, amino acids, alkaloids and phenolic compounds for both plants during most seasons.48 This is due to the synthesis of starch was enhanced by the low salinity, but high salinity inhibited it. Also, starch can be converted to soluble sugars which play an important role in adjusting the osmotic potential of the cytoplasm; as concluded in some halophytic plants.52,53,44,55 The decrease in starch content and the increased level of sucrose of both plants with high salinity pointed a shift in the balance of sucrose–starch metabolism. Under saline conditions, the accumulation of sucrose in plants was usually considered to be the result of inhibition in sucrose oxidation in relation to shoot growth or as osmotic adjustment 47,48. On the other hand, H. strobilaceum attained high starch content compared to that of A. macrostachyum. This may be regarded as another protectant substance against dry conditions and high temperature, though H. strobilaceum withstand more sever conditions than A. macrostachyum (field observations). Hydrophilic compounds could replace water at the surface of proteins, complex and membranes, thus acting as osmo-protectants49,50. In addition to its function as an osmoprotector, proline may protect enzyme-proteins from ion inhibitory effect 51. It also stabilizes cellular structures and functions as source of carbon and nitrogen for metabolism as well as a regulator of cytosolic pH that could protect plant tissues against osmotic stress2,53.

So proline accumulation in plants was stimulated by increasing EC value at different locations particularly at location-5 and 6 of most seasons for both plants. Proline protects the plants by scavenging reactive oxygen species (hydroxyl radicals and superoxide radical), so the proline play the role of antioxidant compounds in direct quenching of free radical reactions during winter and spring seasons 54. Earlier studies revealed that the possible stimulation of total phenolics via the pentose phosphate pathway may be through steps involved in proline synthesis 55. Results indicated high values of proline and low values of phenolic compounds during winter season in A. macrostachyum and during spring season in H. strobilaceum the reverse was found in both plants during summer season. Data showed a remarkably high level of amino acids in location 5 and 6 in both plant species during most different seasons this can be attributed to the stimulation of synthesis and accumulation of amino acids with a rising in salinity for osmoregulation. These results are in agreement with Youssef (2009)56. It was observed that alkaloids usually synthesized from amino acids in particular, lysine, tyrosine, and tryptophan where there was (De Luca, 1993)57. A clear relationship was between the free amino acids content and secondary metabolites which may be increased during dry season especially in summer season instead of amino acids (primary metabolites)48. So that the results indicated that the level of free amino acids was low in both plant species during summer and autumn (dry seasons) compared to the wet seasons (winter and spring). These results are in agreement with 58. An accumulation of free amino acids with a decrease in
protein of Brassica napus was detected during water stress. This can be ascribed to enhanced protease activity, as an adaptive mechanism to stress tolerance. Plants of H. strobilaceum accumulate high amount of free amino acids than A. macrostachyum during all seasons, on the other hand A. macrostachyum accumulate high amount of soluble protein content than H. strobilaceum, which showed different mechanisms between them in osmoregulation. The reduction in the soluble protein content under elevated saline conditions may be due to:

1- Stimulating protein hydrolysis by salinity and NaCl in which Cl- inhibits NO3- uptake.
2- Shortage of nitrogen supply due to exposure to NaCl.
3- Inhibition of some regulatory enzymes for the process of protein synthesis such as nitrate reductase.

4- Reduced RNA content which is needed for protein synthesis. The decrease in protein content during summer (dry) season may be due to conversion of many primary metabolites (carbohydrates and amino acids) into secondary metabolites (alkaloids and phenolics). Gómez-Galera et al. (2007) stated that the phenylpropanoid pathway originates from phenylalanine and it is perhaps the most important biosynthetic pathway derived from any amino acid. This pathway is responsible for the synthesis of thousands secondary metabolites including lignins, salicylates, coumarins, hydroxycinnamic amides, flavonoid phytoalexins, pigments, UV light protectants, and antioxidants. These metabolites are often specific to a particular plant species. The present results showed that the total alkaloids content of both plant species was generally increased by increasing salt concentration at different locations particularly location-5 and 6 during most seasons. However the alkaloids content recorded the highest values in summer and autumn (dry) seasons in comparison with winter and spring (wet) seasons for both plants, where plants used this mechanism (secondary metabolites) at more stressed time. These results are in accordance with. The alkaloids have an osmoregulatory role and their increase was considered as an adaptation to the imposed salinity stress. reported that the increase in the alkaloids content as influenced by NaCl is a combination of an osmotic effect and a specific ion effect. The increase of alkaloids in response to salinity may be due to its role in the plant protection against the salt stress effects.

Many authors reported that accumulation of the phenolic compounds in plants by NaCl stress leading to consider that secondary metabolites may play a role in the adaptation of halophytic species to this constraint. The present results indicated that both plants possessed high level of total phenolic compounds in the summer and autumn seasons (dry season) compared to the winter and spring seasons. The total phenolic compounds remarkably increased with salinity at different locations during different seasons, where locations-5 and 6 recorded the highest value during most seasons. The total phenolic compounds increased in the summer season on account of carbohydrates content which decreased in the same season. The phenolic compounds play an important physiological and ecological role, being involved in resistance to different types of stress. Plants have different adaptive mechanisms to reduce oxidative damage resulting from salt stress through a cascade of antioxidants which stopping the propagation of oxidative chain reactions. In this case, polyphenolic compounds such as phenolic acids, flavonoids, proanthocyanidins and anthocyanins play an important role in scavenging free radicals.

The presence of large amounts of polyamines in the studied plant species was confirmed by who reported that cultivars accumulating large amounts of free polyamines, exhibit a higher tolerance to osmotic stress than other cultivars. Over expression of the arginine decarboxylase (ADC2) gene in Arabidopsis results in increased putrescine level and drought tolerance. Polyamine biosynthesis involves two alternative pathways starting from L-arginine. Putrescine was considered as the first compound produced from the two pathways, so it acts as precursors for spermidine and spermine. They observed the following interesting fact: under high salinity, A. macrostachyum accumulated spermidine in spite of low concentrations of its precursors (putrescine), the content of putrescine increased only during low salinity; thereafter, the content of polyamines dropped sharply several assumptions could explain these results. It is naturally to suppose that, during primary response to salinity, A. macrostachyum synthesized actively putrescine which was later used as substrate for spermidine and spermine synthesis or due to shortage in primary metabolites (amino acids) in summer season. In general, the polyamine contents of A. macrostachyum decreased at high levels of salinity. The total polyamines content was low at locations 5 and 6 which are distinguished by high salinity. The decrease in polyamines may be due to their conversion into secondary metabolites such as nicotine or tropane alkaloids in Solanaceae or in their conjugation with hydroxycinnamic acids or with proteins, hemicellulose or lignin.
Table 1

Electrical conductivity (EC) of soil under and away from *Arthrocnemum macrostachyum* and *Halocnemum strobilaceum* at different locations during different seasons in salt marshes of Burullus Lake.

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Table 1. continue (Statistical analysis)

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Table 2
Seasonal changes of DRV, sucrose and starch content (mg g\(^{-1}\) d.m.) of *A. macrostachyum* and *H. strobilaceum* at different locations during different seasons in salt marshes of Burullus Lake.

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Fig. 1
The content of DRV, sucrose and starch during the different seasons of study in each *H. strobilaceum* and *A. macrostachyum*.
### Table 3
Seasonal changes of proline, amino acids and protein content (mg g\(^{-1}\) d.m.) of *A. macrostachyum* and *H. strobilaceum* at different locations in salt marshes of Burullus lake.

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### Table 4
Seasonal changes of alkaloids and phenolic content (µg g\(^{-1}\) d.m.) of *A. macrostachyum* and *H. strobilaceum* at different locations in salt marshes of Burullus Lake.

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Changes of polyamines content (ppm) of A. macrostachyum and H. strobilaceum in four locations during summer season only.

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On the other hand, putrescine in H. strobilaceum shoot recorded high amount in comparison with spermidine and spermine, and this may be due to the retro conversion from spermine and spermidine to spermidine and putrescine, respectively by plant polyamine oxidases. In general the amount of polyamines of H. strobilaceum increased with salinity and recorded high levels at locations 5 and 6. These results were compatible with many researchers.

6. CONCLUSION
Our conclusion included that these halophyte plants requires more studies for the medicinal properties due to its high production of secondary metabolites (alkaloids and phenolic compounds). Use of both halophyte species for heavy metal remediation is of particular interest since these plants are naturally present in soil characterized by excess of toxic ions, mainly sodium and chloride.

7. REFERENCES


