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A comparative Study of Salt Tolerance Parameters in Three Egyptian Ecotypes of *Alhagi Maurorum* "Camel Thorn"

A. Ashgan Abou Gabal [1+], A[a]. Abed Elsalam [2], El Wakeel H. [1], Amera Zaitoun [1] and R.Nader [1]

[1] Department of Botany, Faculty of Agriculture, Alexandria University, Egypt
[2] Department of Genetics, Faculty of Agriculture, Ain Shams University, Egypt

Abstract. *Alhagi maurorum* "Camel thorn is a recognized model plant for studying its adaptation to contrasting and harsh environments. To understand the inherent molecular basis for its remarkable resistance to salinity stresses, *A. maurorum* grown in different zones (Karsheef, Khamesa and Merkeda) in Siwa Oasis of Egypt has been studied using ecological parameters, morphological, molecular and biochemical markers. The highest salinity was found in Karsheef soil (Na= 247.83 milligram equivalent per 100 gram soil and EC= 19.36 ds/m). Ecological studies revealed that *A. maurorum* showed the most homeostatic and tolerant plant in arable land and wet sabkha (Saline soil) and recorded the highest relative importance (DFD) values of all species in Karsheef saline soil (269.75). By finding the fragment of 1.2 kpb in the three *A. maurourum* ecotypes, it is clear that the gene of *P5CS* is present in the three ecotypes. However, the variation between these ecotypes may be due to gene expression. The highest proline content was founed in leaf tissues of *Alhagi maurorum* samples grown in saline soil followed by sandy sheet plants and the lowest concentration was recorded for arable soil sample. Moreover *A. maurorum* ecotypes seem to be similar in peroxidase and esterase isozymes patterns; however the intensity of esterase bands in saline soil was increased than that in sandy sheets and arable soil which means more enzymatic expression of this enzyme in saline soil. SDS-PAGE of the three *A. maurorum* ecotypes induced several low molecular weight proteins among of them the 9.5, 11.5, 16.5, 14.6 and 28.5 kDa proteins, ABA-inducible group of proteins induced by salinity and water deficit.

Keywords: *Alhagi Maurorum*, Salinity tolerance, proline, *P5CS* gene, PCR, molecular,biochemical indicators.

1. Introduction:

Salinity in soil or water is one of the most severe abiotic stress factors and, especially in arid and semi-arid regions, can severely limit crop production [1]. Today >800 million ha of land are salt affected, an area equivalent to 6% of the world's total land [2]. 2 million feddans in Egypt suffer from salinization problems [3]. Traditional breeding has few solutions for this but biotechnology has been very successful moving the genes that allow a mangrove to live in seawater, into crop plants. These salt tolerant plants will help keep the 750 million acres of salty soil in production [4].

In order to improve the performance of crops growing under salt stress, it is important to understand how plants cope under such conditions. Salt tolerance of plants is a complex phenomenon that involves physiological, biochemical, and molecular processes as well as morphological. Furthermore, salinity tolerance is unlikely to be determined by a single gene or gene product [5], but probably results from the expression of a number of genes, the importance of which is dependent upon their interaction with other salt tolerance genes and the external salt concentration. Salinity tolerance is more likely to be controlled by the complex interaction of several genes than by a single gene. The expression of these genes is influenced by

[+] Ashgan Abed El Megueed Abou Gabal Tel.: + (002 01005420541); fax: +(please specify).
E-mail address: (ashgabal@yahoo.com).

multifarious environmental factors [6]**.** Changes in their expression can be detected by studying the protein and isozyme pattern of expression [7].

Of the many biochemical indicators listed earlier, antioxidants and organic solutes such as glycinebetaine and proline have recently gained ground, plants containing high concentrations of antioxidants or either of the two organic solutes shows considerable resistance to salinity as well as other abiotic stresses. Proline accumulation is one of the most frequently reported modifications induced by water deficit and salt stresses in plants, and it is often considered to be involved in stress resistance mechanisms [8] The gene for proline synthesis, *P5CS* (Δ-1-pyrroline-5-carboxylate synthetase), is commonly detected [9]

In view of the above, this work aims to understand the genetic behavior of salt tolerance of *Alhagi maurorum*(a plant from family Fabacae) grown in different zones (Karsheef, Khamesa and Merkeda) in Siwa Oasis through test and compare three different approaches, ecological, molecular and biochemical. (i) to identify plant communities in each zone and analyze the relation between plants and soil salinity factor on *A. maurorum* of the three habitats, (ii) to apply specific primer PCR for the identification of genetic diversity among these ecological types of *A. maurorum* and (iii) investigate the total soluble protein, isozyme and proline content in the three *A. maurorum* ecotypes.

2. Material and Method

2.1. Site description and sampling:

Soil samples were collected as a profile (composite sample) at a depth of 0-50 cm below the soil surface from three different locations in Siwa Oasis within an area about one km^2, Karsheef representive (the saline soil), Khamesa (sandy sheets) and Merkeda(arable land). Soil salinity (EC) and soil reaction (pH) determined by using conductivity meter and pH meter respectively. Chlorides, bicarbonates, sulphates, calcium, magnesium, sodium and potassium were determined according to [10], **[**11].

2.2. Ecological measurements:

Fifteen quadrates were chosen randomly from the three different locations. In each quadrate the plant density and frequency were calculated according to quadrate method, whereas, the absolute density (**AD** (individual / 100 quadrates) = Total number of individuals/ Total number of quadrates), relative density (**RD** % = AD for a species/ Total AD's for all × 100), relative dominance of species (**RD** = AD of that species/ Total number of all species), absolute frequency (**AF** (Occur./ 100 quadrates= Total number of quadrate in which the species has occurred/ Total number of quadrates), and relative frequency (**RF %** = AF for a species/ Total AF's x 100).

The plant dominance of each species was calculated using the line-intercept method. Absolute dominance of species (**AD**= Total intercepts of that species/ Total length of all species) and Relative dominance of species (**RD**= AD of that species/ Total AD's of all species x 100). The three measures (relative density, relative frequency and relative dominance) were summed to give the importance value, which may lie between 0 and 300.

2.3. Molecular assays:

DNA isolation and purification was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method, according to [12] .The PCR procedures and the separation of amplified products were carried out as described by [13] **.**The Specific two primers sequences] were designed according to [14] (5 ' 3'): Forward (tac tga gac tgt gaa gtc gc) and reverse (atg gca ttg cag gct gcc g).

2.4. Biochemical assay:

Proline content was determined according to the method of [15] . About 0.5 g leaf sample was extracted with sulfosalicilic acid 3%. Coloring reaction was precede using Ninhidrin solution and glacial acetic acid and boiled for 1 hour. After addition of toluene, the chromophore was measured with a spectrophotometer at λ 520 nm. Peroxidase and Esterase iso-enzymes were extracted in an extraction buffer as describe by [16]. Bands of both enzymes were detected on the gel as described by [17].SDS Protein Electrophoresis: (SDS-PAGE) was performed according to the method of [18], employing 10% resolving gel and 4% stacking gel

2.5. Statistical analysis:

The statistical analysis was carried out using JMP IN5 software [19]. The analysis of variance (ANOVA) was calculated and the means were compared using LSD values according to [20] (0.01 probability level).

3. Result and Discussion

Soil salinity is the most important ecological factor affecting *A. maurorum* biology in Siwa oasis. The highest salinity was found in Karsheef soil (Na= 247.83 milligram equivalent / 100 gram soil and EC= 19.36 ds/m). This type of soil is localized around the lakes in the depression and flooded by their water [21]. In contrast, Khmaisa soil (sandy sheets) exhibited the lowest concentration of salt (Na= 13.04 Meg/ 100 gm and EC= 1.76 ds/m). Merkeda (arable soil) its EC values were (Na= 78.26 Meg/ 100 gm and EC= 9.77 ds/m). While sandy sheets lacking necessary elements and exposed to drought, plants in Karsheef have enough soil elements but exposed to high level of Na ions and EC value which limit the absorption of other ions and nutrients required for growth, this investigation supported by the results of [22] who showed that Na^+ competes with K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}, Cl^- restricts the absorption of NO^{3-}, PO_4^{2-} and SO_4^{2-}

The ecological studies revealed that there were three species in Merkeda (the arable land); *A. maurorum*, *Imprata cylindrical* and *Cynodon dactylon*. In El-Khameesa, sandy sheets, *Cornulaca monacantha* was more abundant but associated with *A. maurorum* which recorded the lowest DFD value for its appearance(148.92) , while recorded the highest DFD value (269.57) in the saline soil(El-Karsheef), associated with *I. cylindrical* species. Moreover, it was clear that *A. maurorum* represents the most adapted and tolerant plant under different environmental conditions varying from sand sheets to wet sabkha, these results were in agreement with the results of [23] who mentioned that *A. maurorum* has wide ecological amplitude, since it grows in different salinities habitats in the salt marsh plant of Siwa oasis. Moreover, [21] indicated that the natural vegetation in Siwa depression depending directly on the environmental factors. However, *A. maurorum* had the highest homeostasis ability to habitat changes than other species, tends to dominate the area and replace other species such as *C. monacantha* which originally inhabited the area of sandy sheets.

By finding the fragment of 1.2 kpb in the three *A. maurourum* ecotypes, it is clear that the gene of *P5CS* which is conserved region presented in the three ecotypes and is no obvious variation in-between them. However, the variation between these ecotypes may be due to of gene expression, (Figure, 1). [24] found by amplification of sugarcane DNA template and using primers within the conserved region of *P5CS* gene a fragment with molecular weight 1.2 kpb. The gene for proline synthesis, *P5CS* (Δ-1-pyrroline-5- carboxylate synthetase), is commonly detected [9]**.** *P5CS* involved in proline biosynthesis from glutamate has been reported to accumulate in leaves and roots in response to salt-stress in Pisum sativum [25] while in *Oryza sativa* and *A. thaliana*, by salt dehydration and ABA [26]

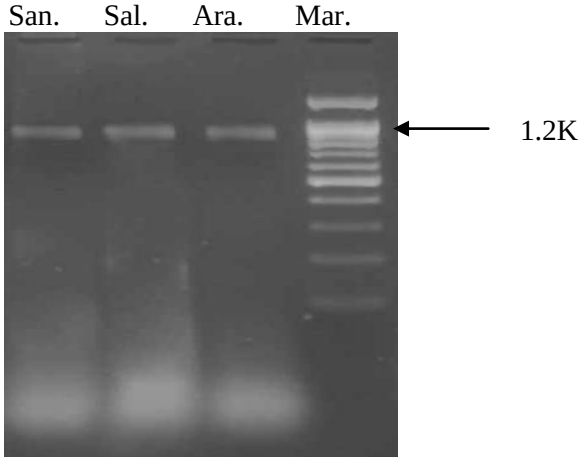


Fig. 1: Screening of the *P5CS* gene amplified from the *Alhagi maurorum* ecotypes

Proline accumulation is one of the most frequently reported modifications induced by water deficit and salt stresses in plants, and it is often considered to be involved in stress resistance mechanisms [8] The present results indicated that there were significant differences in leaves proline content of the three studied camel thorn ecotypes. Plants grown in saline soil showed the highest proline content (41.730 mg/g fresh weight). The lowest concentration of proline (11.734 mg/g fr wt) was recorded for arable soil sample. The sandy sheets sample recorded a concentration of (11.760 mg/g fr. wt.) of proline. These results were in acceptance with many other studies on legumes [27] who found that accumulation of proline in the leaves of

different population of *Alhagi maurorum* in Siwa oasis is associated with salt stress. The results of [28] was also in agreement with this study as they reported that exposure of chickpea to salt stress led to increase in proline concentration [29] also reported that salt treatment in *C. arietinum* cultivars provoked an accumulation of proline in the radicals of salt-tolerant cultivar ILC1919.

SDS-PAGE was performed to study variation in soluble proteins of the three *A. maurorum* ecotypes. The total proteins bands exhibited in all *Alhagi* ecotypes 57 (19 for each ecotype). The polymorphic bands were ten and the percentage of polymorphism was 17.5. The protein fractions from all *A. maurorum* ecotypes distributed along a wide range of molecular weights in seven zones, it was obvious that *A. maurorum* ecotypes induced several low molecular weight proteins out of which 9.5, 11.5, 16.5, 14.6 and 28.5 kDa proteins, (Figure, 2), [30] indicated that proteins may be synthesized de novo in response to salt stress or may be present constitutively at low concentration and increased when plants are exposed to salt stress**.** It was found that many salt-induced bands appeared during characterization of salt-induced proteins, in tobacco, a 26 kDa protein named as osmotin was detected [31]. In barley, two 26 kDa polypeptides, not immunologically related to osmotin, identified as germin, were increased in response to salt stress [32] and [33] found a 22 kDa protein in response to salt stress in radish. In finger millet (*Eleusine coracana*), [34] found 54 kDa and 23–24 kDa proteins responsible for salt or drought tolerance. Moreover, the study of [35] on five mangroves showed extra numbers of protein bands expressed with relatively low molecular weight in saline habitat and in all salinity imposed plants.

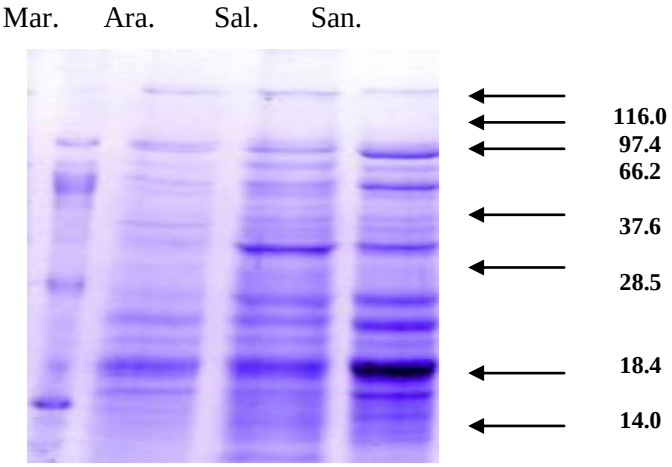
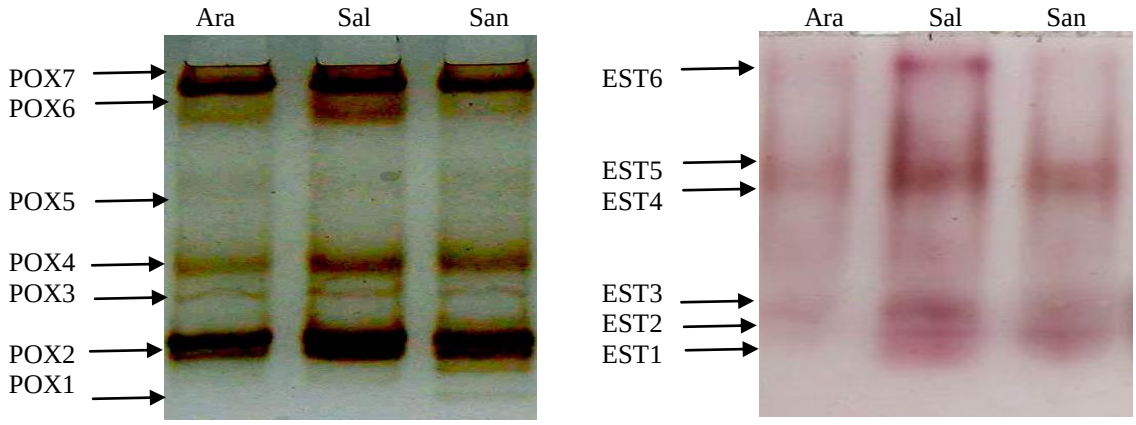


Fig. 2: SDS-Polyacrylamide gel electrophoresis of protein banding patterns of *A. maurorum* ecotypes.

Esterase isozyme pattern revealed three polymorphic bands (16.7%), these bands were 1, 4 and 6. Plants grown in arable soil showed the absence of these bands, while sandy sheets plants recorded the absence of band number 1.On the hand the three polymorphic bands appeared in saline soil plants pattern indicated that esterase isozymes were more stable under salt stress. Moreover the intensity of bands in saline soil was increased than that in sandy sheets and arable soil which means more enzymatic activity in this sample. Bands number 2, 3 and 5 were found as common bands in all *Alhagi* ecotypes, but with different enzymatic activity, they were strong in plants which grown in saline soil. Band number 1 was found only in saline soil plants with intermediate activity so it can be considered as a potential marker associated with salinity stress, (Figure, 3a). From this data it is clear that salinity stress led to increase both number and intensity of bands of esterase isozyme. [36] showed that the increase in lipids hydrolysis due to the increase in esterase activity under salinity was detected in peanut, and he reported that treatment the peanut plants with 150 mM NaCl lowered the lipids content and this decrease in lipids content was associateed with increased number and/ or activity esterase isozymes. Also [27] examined the isozyme pattern of ten different *A. maurorum* populations at different habits of Siwa depression and he found that the banding pattern exhibited a wide range of esterase variability.

Peroxidases are important components in the detoxification process, reactive oxygen species under stress conditions result in a significant damage of the cellular constituents and even cell death if the protective mechanisms fail to detoxify them [37]. A total number of seven isoperoxidases were detected in the three *A. maurorum* ecotypes, except band number 7 which was found as a weak band only in sandy sheets plants. The intensity of all bands in saline soil was strong in comparison to arable soil and sandy ecotypes. Moreover the intensity of all peroxidase isozyme bands increased with salinity increases which means that salinity increase the expression dose and not isoenzymes forms, (Figure, 3b)



(b) (a)

Fig. 3: Polyacrylamide gel (7.5%) electrophoresis of (a) esterase isozymes banding patterns and (b) peroxidase isozymes banding patterns of *Alhagi* ecotypes. .(Ara= Arable soil, Sal= Saline soil, San.= Sandy sheets)

[38] concluded that the increase in peroxidase activity may be due to the rise in gene expression of some peroxidases which was represented by the increase of staining intensity of the bands of *Alhagi garecorum* exposed to high concentrations of sodium chloride **[39]** reported similar results of peroxidase isozymes activity under salt stress in *Solanum tuberosum* L. Also, [40] reported that isozyme pattern of peroxidase showed over expression of all the five constitutive isozymes of peroxidase in French bean (*Phaseolus vulgaris*) under salt stress. Correlation between the increase in peroxidase expression and the resistance to stress condition has been established in many plant species [38]-[41] Peroxidase activity may be due to the increase in the number of peroxidase isoenzyme forms, or the rise in expression of some of them which was represented by the increase of staining intensity of these bands [42]. With these results it was clear that the polymorphism was increased in total proteins than that in esterase or peroxidase isozymes. This may be due to the rise in expression of proteins under saline conditions.

In conclusion, results revealed that *Alhagi* ecotype from highly saline habitat was relatively less affected by salt stress in terms of various ecological parameters studied than their counterparts from moderately saline, sandy soil and low saline arable land. At the biochemical levels plants grown in saline soil show increase in proline content, number of bands, increased activity of esterase & peroxidase isozymes and, protein banding pattern. By finding such specific gene (*P5CS*), Camel thorn can be used as a donor to transfer this gene to other economical plants salinity sensitive especially related to family Fabacae, i.e. faba bean (*Vicia faba* L.).

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