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GENETICS AND BIOCHEMICAL VARIANTS OF SOME HALOPHYTES FROM SHALATEEN REGION, THE SOUTH-EASTERN PORTION OF EGYPT

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ABSTRACT

Seven halophytes; three excretives (***Atriplex farinosa*** Forssk., ***Rhizophora mucronata*** **Lam. and** ***Avicennia marina*** (Forssk.) Vierh) and four succulents (***Suaeda monoica*** Forssk., ***Salsola tetrandra*** Forssk., ***Halopeplis perfoliata*** (Forssk.) Bege cx Schweinf. and ***Arthrocnemum macrostchyum*** (Moric.) K.Koch) were collected from Shalateen region the south - eastern portion of Egypt to identify their biochemical fingerprint. Results revealed that the excretive halophytes attained higher content of protein than the succulent one's. Moreover, the studied species, revealed in general, a wide variation of enzyme activities. For instance, acid phosphatase (ACP) followed by alcohol dehydrogenase (ADH) enzymes exhibited the highest activities in the two halophytic groups, whereas, the Shikimate dehydrogenase (SKDH) enzyme was the lowest one. These data could be attributed to the different adaptive responses of the studied species, which are related to their genetical characteristics and/or habitat diversity. Data of the electrophoretic analysis of total protein and the different isozymes indicated that the appearance of specific band or bands inr a certain species could be expressed as specific markers for salt tolerance. Therfore, two positive markers (57.52 and 26.17 KDa) distinguished excretive halophytes, meanwhile succulents were characterized with one positive marker (34.5 KDa). On the other hand, data of isozymes showed that band (5) of ACP and ADH isozymes and bands 2 and 3 of SKDH isozymes were markedly associated with the two halophytic groups. These bands might be considered as genetic fingerprints for the studied halophytes. Moreover, some halophytic species had specific bands that may be considered as positive genetic markers for these species.

Key words: Shalateen region, Halophytes, Protein, Enzyme activity, Isozymes electrophoresis, Biochemical fingerprint

INTRODUCTION

Shalateen region is located in the south - eastern portion of Egypt, between the parallels of 220 and 240, North and between maridian of about 350 and the Red Sea coast, East. Specific salient features characterize this region, where it is bounded by Red Sea hills in the west, which are dissected by several drainage lines flowing towards the coastal sabkhas and then to the Red Sea (**Abd El Rah-**

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man, 1999). Its climate is predominantly hyper-arid; it is characterized by wide fluctuation in air temperature, both diurnally and seasonally with variable amounts of rainfall. Occasionally, heavy storms produce torrential floods through which water fill wadis for short periods. This region includes a particular phytogeographical area, where the growth of about 122 plant species is confined **(Ahmed, 1999).**

Halophytes are a group of plants that have a set of physiological adaptations that allow for the growth and completion of their cycle in media with different salinity levels **(Gallagher, 1985).** Halophytes are present in about half of the higher plant families and represent a wide diversity of plant forms **(Glenn, 1997).** They are often classified as excretives and succulents. Accordingly, **Adam (1990)** reported that excretives are widespread among many plant families, particularly those with mangarove members. Excretives have glandular cells capable of secreting excess salts from plant organs, while succulents have more common features accompanied by high osmotic concentration of the cell sap that enables the plant to absorb and retain necessary quantity of water **(Baron, 1967 and Ambasht, 1986).** Moreover, some species of halophytes can grow successfully under both saline and xeric habitats; i.e species with wide ecological amplitude **(Ismail, 1988).** Other species are known as salt regulators, although they absorb the salts, but exclude it from their roots and/or through specialized salt glands in the leaves **(Hopkins, 1999).**

Many studies have been done on the genetic make up of halophytes that can be used for gene transfer to crop species. However, nothing concrete has been achieved so far on gene transfer, mainly due that halophytes are maintained by almost 1400 genes or multigenes that is operating and control transfers. Chemical fingerprinting was developed for the identification, characterization and differentiation of several plants using electrophoretic techniques **(McKee, 1973; Abdelsalam *et al* 1998 and Vladova *et al* 2000).**

The aim of the present study is to provide a reliable identification of two halophytic groups; excretive and succulent by their biochemical fingerprints.

MATERIAL AND METHODS

Two groups of halophytic species were collected from Shalateen region (Map 1). These species are classified according to their adaptive physiological responses into two main groups,

i) excretives; ***Atriplex farinosa, Rhizophora mucronata*** **and** ***Avicennia marina,*** **and ii) succulents;** ***Suaeda monoica, Halopeplis perfoliata, Salsola tetrandra*** and ***Arthrocnemum macrostchyum.***

Leaf samples of these species were subjected to the following biochemical investigations:

(1) Total protein content and enzyme activity

Protein was extracted from each plant sample and its concentration was determined according to **(Bradford, 1976).** The enzyme activities of acid phosphatase and alcohol dehydrogenase and shikimate dehydrogenase were determined according to **Gillard and Demind, (1974); Prestamo and Manzano (1993) and Baaziz *et al* (1994),** respectively.

(2) Protein Electrophoresis (SDS – PAGE)

Extraction of total protein, gel preparations, PAGE electrophoresis runs and visualization of bands were conducted as the methods outlined by **(Stegemann, 1979 and Stegemann *et al* 1980).**

(3) Isozymes electrophoresis

Three isozymes i.e, acid phosphatase (ACP), alcohol dehydrogenase (ADH)) and shikimate dehydrogenase (SKDH), were extracted from plant samples and polyacrylamide gel electrophoresis was carried out according to the method described by **Garkova *et al* (2000).** Acid phosphatase was stained according to **Wendel and Weeden, (1989),** alcohol dehydrogenase was stained as the method given by **Soltis *et al* (1983)** and in case of shikimate dehydrogenase, the method suggested by **Vollejos (1983)** was applied.

(4) Gel analysis

All gels resulted from protion and isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. The densitometric scanning of the bands were performed on three directions. Each band is recognized by its length, with and intensity. Accordingly, relative amount of each band could be quantified and scored.

RESULTS AND DISCUSSION

Total protein content and enzyme activity

Total protein and enzyme activity levels were used as biochemical markers for the characterization of the concerned halophytic species. Data presented in Table (1) show the total protein content and the activity levels of acid phosphatase, alcohol dehydrogenase and shikimate dehydrogenase enzymes. Data revealed that the halophytic plants show highly variations in the total protein content ranging between 85±5 and 149±8 mg/g. ***Atriplex farinosa*** (excretive) attained the highest protein content, while ***Halopeplis perfoliata*** (succulent), attained the lowest content. In general, it is noticed that excretives contained higher protein contents than succulents. This may be attributed to the fact that excretive halophytes are more tolerant to salt stress than succulents, this in turn,might be due to their increased protein content. This finding is aline with those mentioned by **Strogonov, (1962)** who reported that the plants growing under salt stress show considerable difference in physiological and biochemical activities, as a result of the adaptive mechanisms during evolution. These adaptations also cause readjustment in the activities of certain key enzymes of the plant metabolism **(Ahmed, 1972 and Ahmed and Zaheer, 1974).** This indicates from the studied halophytes, which have a wide variation of enzyme activities. For instance, the highest enzyme activity in succulent halophytes was expressed acid phosphatase (ACP) which ranged from 227± 20 to 306± 10 unit g^{-1} followed by alcohol dehydrogenase (ADH) enzyme activity (156± 6 – 190± 10 unit g^{-1}). Meanwhile, the Shikimate dehydrogenase (SKDH) enzyme had the lowest activity (75± 6 - 85± 5 unit g-1). The same trend was true in case of excretives. In general ACP followed by ADH exhibited the highest activities enzymes in both excretives and succulents, whereas SKDH was the lowest one.

Table 1. Total protein content and the activity levels of the different enzymes for the studied halophytic species

Halophytic species	Total protein mg/g-1	Activity of ACP Unit g-1	Activity of ADH Unit g-1	Activity of SKDH Unit g-1
Atriplex farinosa	149±8	213±14	111±5	110±5
Rhizophora mucronata	126±5	321±10	109±4	100±4
Avicennia marina	121±5	229±12	107±20	89±3
Suaeda monoica	87±7	306±10	190±10	80±5
Halopeplis perfoliata	85±5	300±10	170±10	85±5
Salsola tetrandra	110±10	281±10	135±20	75±6
Arthrocnemum macrostchyum	108±3	277±20	156±6	77±5

Protein and isozyme electrophoresis

Electrophoretic analysis was used to identify the biolchemical genetic fingerprints of the studied halophytes using the results of total protein and three different isozymes; acid phosphatase, alcohol dehydrogenase and shikimate dehydrogenase.

Protein electrophoresis

Analysis of the total protein of the seven halophytes was carried out to identify the similarities and dissimilarities between them. The SDS-PAGE electrophoretic patterns of the seven halophytes are presented in Tables (2&3) and illustrated in Figures (1&2). Bands with different molecular weights (MW), were detected in both excretives and succulents were detected, expressed in the rate of flow (RF). Molecular weights ranged from 107.75 to 13.28 KDa, and from 120.37 to 11.91 KDa for excretives and succulents, respectively.

Regarding excretives, the total number of bands was eight. The bands, which have molecular weights of about 57.52 and 26.17 KDa, were present in all species. Consequently, these bands could be taken as positive specific markers for this group of halophytes. However, ***Atriplex farinosa*** showed a unique band of molecular weight of about 107.75 KDa. Moreover, the bands of molecular weight of about 19.97 and 14.48 KDa were detected only with ***Avicennia marina.*** Generally, the appearance of a unique band or bands for a certain species could be expressed as specific markers for salt tolerant of excretive halophytes.

On the other hand, succulents showed a maximum number of bands reaching 15 bands. The band of molecular weight of about 34.53 KDa was found in all species

Table 2. Banding patterns and molecular weight (MW) of SDS proteins for some excretive halophytes

Band No.	Mid. Mol. Marker	RF.	1*	2*	3*	Mol. Marker bands
1	97.40	0.08	+			107.75
2	66.20	0.22	+		+	64.38
3	45.0	0.30	+	+	+	57.52
4		0.40		+	+	42.62
5	31.00	0.50	+	+	+	26.17
6		0.58			+	19.97
7		0.78			+	14.48
8		0.83		+	+	13.28

(1*) *Atriplex farinosa.* (2*) *Rhizophora mucronata* (3*) *Avicennia marina.*

Table 3. Banding patterns and molecular weight (MW) of SDS proteins for some succulent halophytes

Band No.	Mid. Mol. Marker	RF.	**1	**2	**3	**4	Mol. Marker Bands
1		0.093	+				120.37
2	97.40	0.130	+				103.00
3		0.17	+	+			88.00
4	66.20	0.25		+	+	+	63.33
5		0.33	+	+	+		54.06
6	45.00	0.41		+	+	+	45.88
7	31.00	0.50	+	+	+	+	34.53
8		0.54			+		29.80
9	21.50	0.620		+			23.50
10		0.68	+	+			19.76
11		0.714				+	18.42
12		0.76		+	+	+	16.69
13	14.40	0.816	+			+	14.71
14		0.858	+				13.14
15		0.90		+			11.91

(1**) *Suaeda monoica* (2**) *Halopeplis perfoliata*
(3**) Salsola tetrandra (4**) Arthrocnemum macrostchyum

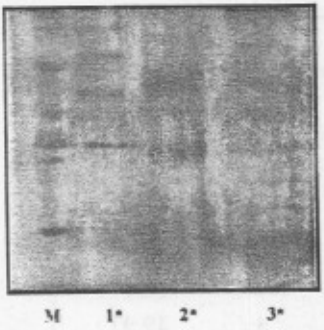

Fig. 1. Electrophoretic profiles of total protein of the studied excretive halophytes

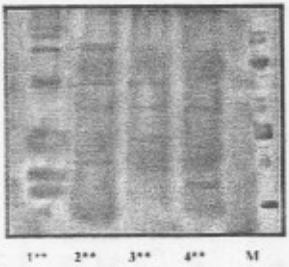

Fig. 2. Electrophoretic profiles of total protein of the studied succulent halophytes.

and hence it can be considered as a positive specific marker for succulent halophytes. However, there were different unique bands characterizing some species, which could be used in their characterization. For instances, the bands which have molecular weight of about 120.37, 103.60 and 13.14 KDa were associated with ***Suaeda monoica*** and disappeared in the other species .In addition, the bands 9 and 15 of molecular weight of about 23.50 and 11.91 KDa were accompanied with ***Halopeplis perfoliata*** and the band of molecular weight of 29.80 KDa was attached with ***Salsola tetrandra***. All these bands could be used as positive markers for the mentioned succulent halophytes.

In general, the similarity behaviour of species that have markedly high intensity of some bands was found in all excretives except ***Atriplex farinosa*** and also in all succulents except ***Suaeda monoica***. Therfore, despite the fact that all studied halophytes are salt tolerant plants, ***Atriplex farinosa*** and ***Suaeda monoica*** are considered the most tolerant species. In this regard, **Stewart *et al* (1978)** mentioned that salt tolerant plants contain high levels of amino acids. Furthermore, two positive markers can be distinguished in excretive halophytes. Meanwhile, succulents showed one positive marker. This may be attributed to their genetical variances.

Concerning the dendogram for genetic distance and similarity matrix of total protein in each of the studied halophytes, results in Figure (3) and Table (4) indicate that the highest similarity in excretive halophytes was sharing between ***Rhizophora mucronata*** and ***Avicennia marina*** (61.60 %), whereas the lowest one appeared only between ***Atriplex farinosa*** and ***Avicennia marina*** (39.0%). In case of succulent halophytes the dendogram for genetic distance and similarity matrix between their species

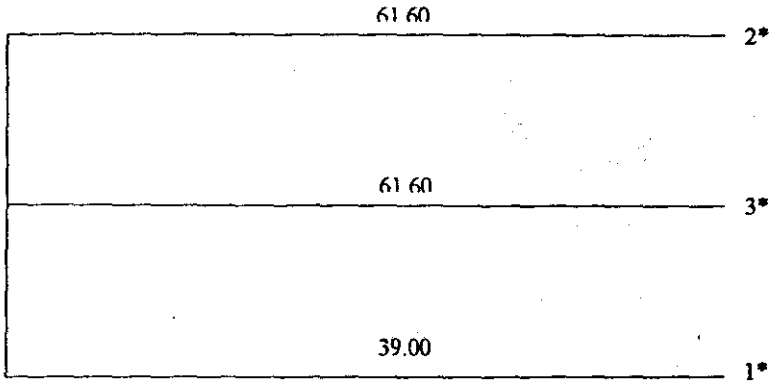

Fig. 3. The genetic distances between the three excretive *halophytes*

Table 4. Similarity matrix between the three excretive halophytes

Halophyte species	1*	2*	3*
1*	100.00	53.90	39.00
2*	53.90	100.00	61.00
3*	39.00	61.60	100.00

are shown in Figure (4) and Table (5). where the highest similarity was observed between ***Halopeplis perfoliata*** and ***Arthrocnemum macrostchyum*** (58%), while the lowest similarity was detected between ***Suaeda monoica*** and ***Arthrocnemum macrostchyum*** (10.0 %).

Isozyme electrophoresis

Three isozymes; acid phosphatase, alcohol dehydrogenase and shikimate dehydrogenase were studied to postulate the genetic fingerprint of the studied halophytic species.

Acid phosphatase isozyme (ACP)

Results of electrophoretic patterns of acid phosphatase of both excretive and succulent halophytes are shown in Table (6) and illustrated in Figure (5). Results revealed that six bands with different densities and intensities were associated with the two groups of halophytes, although their presence was not recorded in all species. For instance, band No. (5) was markedly associated with all species, though other bands were polymorphic. This indication reflects that this band might be expressed as a genetic fingerprint for the studied halophytes. Moreover, bands No. 3 and 6 were accompanied only with ***Salsola tetrandra*** (succulent). Thus, they could be clearly taken as positive markers for this species.

Alcohol dehydrogenase isozyme (ADH)

The electrophoretic patterns of alcohol dehydrogenase isozyme for the studied halophytes are presented in Table (7) and Figure (6). Accordingly, a maximum

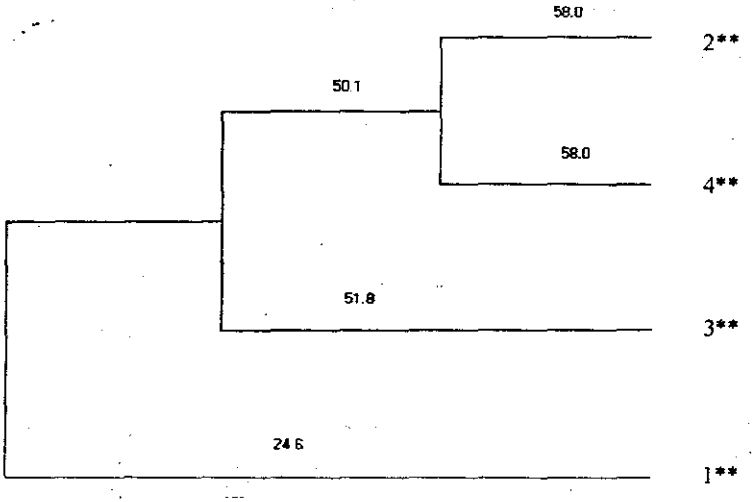

Fig. 4. The genetic distances between the four succulent halophytes

Table 5. Similarity matrix between the four succulent halophytes

Halophyte species	1**	2**	3**	4**
1**	100.00	28.9	24.6	10.00
2**	28.90	100.00	50.1	58.0
3**	24.6	50.10	100.00	51.8
4**	10.0	58.0	51.8	100.00

Table 6. Bands of acid phosphatase isozymes of the studied halophytic species

Isozyme	1*	2*	3*	1**	2**	3**	4**
ACP. 1	+		+		+	+	+
ACP. 2		+	+			+	
ACP. 3						+	
ACP. 4				+	+	+	
ACP. 5	+	+	+	+	+	+	+
ACP. 6						+	

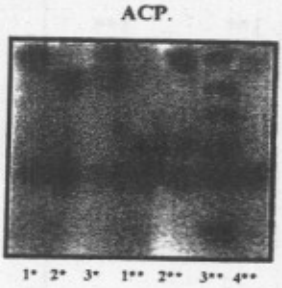

Fig. 5. Electrophoretic patterns of acid phosphatase isozyme of the studied halophytic species

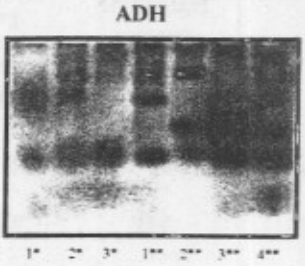

Fig. 6. Electrophoretic patterns of alcohol dehydrogenase isozyme of the studied halophytic species

Table 7. Bands of alcohol dehydrogenase isozyme of the studied halophytic species

Isozyme	1*	2*	3*	1**	2**	3**	4**
ADH.1		+	+		+	+	+
ADH.2		+	+	+	+		+
ADH.3	+	+		+		+	+
ADH.4					+	+	+
ADH.5	+	+	+	+	+	+	+
ADH.6							+

in their densities and intensities and in their polymorphic behaviour. However, a specific band (5) was noticed in all halophytes and could be expressed as a positive fingerprint for these species. However, ***Arthrocnemum macrostchyum*** (succulent) has a unique band (No.6) that was not detected in the other studied species. Accordingly, this band distinguishes this species and can be considered as a positive genetic marker.

Shikimate dehydrogenase isozyme (SKDH)

A maximum number of three bands was detected in the electrophoretic patterns of shikimate dehydrogenase isozymes of the studied halophytes as shown in Table (8) and Figure (7). These bands were present with different levels in their 3 were totally associated with all

Table 8. Bands of Shikimate Dehydrogenase isozymes of the studied halophytic species

Isozyme	1*	2*	3*	1**	2**	3**	4**
SKDH.1				+			
SKDH.2	+	+	+	+	+	+	+
SKDH.3	+	+	+	+	+	+	+

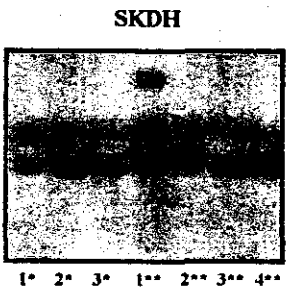

Fig. 7. Electrophoretic patterns of Shikimate dehydrogenase isozyme of the studied halophytic species
1*, 2*, 3* See table (2)
1**, 2**, 3**, 4** See table (3)

halophytes and could be considered as genetic markers for these species. However band 1 took a specific trend where it was present only in ***Suaeda monoica*** and disappeared in all the other species. This specific band may be taken as a positive genetic marker for this species.

CONCLUSIONS

From the previous discussed data, it may be concluded that excretive halophytes attained higher protein content than succulents. Meanwhile, the enzyme activities show highly variations in succulents than extretives. The ACP and ADH enzymes expressed their activities in the two halophytic groups, however shikimate enzyme was the lowest one. These findings could be attributed to the different adaptive responses of the studied halophytes, which are related to their genetical characteristics and/or habitat diversity.

The electrophoretic analyses of total protein indicated that two positive specific markers distinguished the excretive halophytes, while only one marker was associated with succulents. However, different unique band/or bands were attained by specific halophytic species; ***Atriplex farinosa*** (band No.1 of MW. 107.75KDa), ***Avicennia marina*** (bands No.6 and 7 of MW.19.97 and 14.48 KDa, respectively), ***Suaeda monoica*** (band No.1,2 and 14 of MW. 120.37,103.60 and 13.14 KDa, respectively), ***Halopeplis perfoliata*** (bands No.9 and 15 of MW.23.50 and 11.91 KDa, respectively) and ***Salsola tetrandra*** (band No.9 of MW. 29.80 KDa, respectively), and can be used as positive markers of these species.

The electrophoretic patterns of the studied isozymes revealed that band No. 5 of ACP and ADH isozyme was detected as a genetic fingerprint of the two-halophytic groups, while the bands No. 2 and 3 of SKDH isozyme were totally associated with all halophytes and could be expressed as genetic markers for the studied halophytes. Furthermore, band

No. 3 and band No. 6 of ACP isozyme, band No. 6 of ADH isozyme and band No. 1 of SKDH were accompanied with ***Salsola tetrandra***, ***Arthocnemum macrostchyum*** and ***Suaeda monoica***, respectively, and could be considered as genetic fingerprints of these halophytes.

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الاختلافات الوراثية البيوكيميائية لبعض النباتات الملحية بمنطقة الشلاتين الجزء الجنوبي الشرقي لمصر

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١- شعبة البيئة وزراعات المناطق الجافة - مركز بحوث الصحراء - المطرية - القاهرة

تم تجميع سبعة نباتات ملحية, ثلاثة منها من الأنواع المفرزة للأملاح (القطف, القندل, والشورى) واربعة منها من الأنواع العصارية (الحمد, الدمران, الهالوبلس والشينان) من منطقة الشلاتين بالجزء الجنوبي الشرقي لمصر وذلك للتعرف على بصماتها الوراثية البيوكيميائية .

أوضحت النتائج احتواء الأنواع المفرزة للأملاح على مستوى أعلى من البروتين مقارنة بتلك الأنواع العصارية. علاوة على ذلك فقد اتضح, بصورة عامة, تباين النشاط الأنزيمي في جميع النباتات موضع الدراسة فعلى سبيل المثال اظهر أنزيم (الفوسفاتيز الحمضي وأنزيم الديهيدروجينيز الكحولي) في كلا المجموعتين (المفرزة للأملاح والعصارية) أعلى درجات النشاط بينما أنزيم (الشيكيمات ديهيدروجينيز) اقلها نشاطا وقد يعزى ذلك إلى تباين ميكانيكيات التأقلم في النباتات موضع الدراسة والتي ترجع إلى خصائصها الوراثية و/أو تباين البيئات التي تنموا فيها .

أوضحت نتائج تحليل التفريد الكهربي للبروتينات الكلية وانشطة الإنزيمات التي تم تناولها أن ظهور حزمة أو حزم بروتينية لنوع نباتي معين يمكن أن يعبر أو تعبر عن دلائل وراثية خاصة لمقاومة الملوحة. بينما تميزت الأنواع المفرزة للأملاح بدلائل خاصة تتمثل في الحزم البروتينية ذات الأوزان الجزيئية (٥٧,٥٢ و ٢٦,١٧ كيلودالتون) بينما تميزت الأنواع العصارية بحزمة بروتينية ذات وزن جزيئي (٣٤,٥ كيلودالتون) ويمكن اعتبار هذه الحزم البروتينية دلائل وراثية لتحمل الملوحة, وبصمة وراثية يمكن أن تميز كل مجموعة على حدة.

على الجانب الآخر , أوضحت نتائج التفريد الكهربي لمشابهات أنزيم أن التعبير الجينى للحزمة رقم ٥ للفوسفاتيز الحمضي والديهيدروجينيز الكحولي والحزم أرقام ٢ و٣ للشيكيمات ديهيدروجينيز ارتباطا بوضوح نباتات كلا المجموعتين العصارية وتلك المفرزة للأملاح وهذه الحزم من الممكن اعتبارها كبصمات وراثية لهذه الأنواع الملحية .علاوة على ذلك أظهرت بعض الأنواع الملحية تعبير جينى خاص بها مما يمكن اعتباره دلائل وراثية ايجابية خاصة لمثل هذه الأنواع.

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