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Infra-Specific Pollen Diversity of Atriplex halimus L. in Egyptian Flora

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Abstract: *The taxonomic revision of Atriplex halimus L. (Chenopodiaceae) in Egypt revealed the presence of two subspecies namely: subsp. halimus L. and subsp. schweinfurthii Boiss. Microscopic examination of A. halimus populations revealed that each of the studied subspecies (subsp. halimus "AHH" and subsp. schweinfurthii "AHS") was traced in high variability within and between the studied populations, this study grouped all these variables under two forms one form has all male flowers with five tepals (T_5) and five stamens named typical form and the other form has some male flowers with four tepals and four stamens and other male flowers with five tepals and five stamens (T_4+T_5) named non typical form. Soil analysis and calculation the percentage of pollen grain sterility of the studied 52 populations were carried out. The correlation between the studied items revealed that in case of AHH the pollen sterility increase with the increase of soil salinity and aridity. While, AHS showed more adaptive character to arid and saline soil, in addition, the pollen fertility increased under these harsh environmental conditions. Scanning electron microscope (SEM) of the two subspecies pollen revealed that both are characterized by spheroidal to subspheroidal shape with pantoporate spiny exine with 60-95 pores, and presence of subspecies variability.*

Keywords: *Chenopodiaceae, Atriplex, A. halimus subsp. halimus, A. halimus subsp. schweinfurthii, pollen grain, SEM, Egypt.*

1. Introduction

Chenopodiaceae (Goosefoot family) includes about 105 genera and 1500 species worldwide, especially in desert and semi-desert regions, often in alkaline and saline habitats. It is represented in Egypt with 20 genera and 76 species (Boulos 2000). Genus *Atriplex* L. includes about 250 species worldwide and represented in Egypt by only 18 species. This genus attains its potential after citation of *Atriplex halimus* L. in FAO annex 1, which list it among the potential forage crops (Annex1: International treaty on Plant Genetic Resources for Food and Agriculture, FAO 2009). *Atriplex* species possesses tolerant traits so can be planted in soils having saline water or having low water requirement and can play an important role in semiarid environments due to its tolerance to salinity, drought and high temperature.

Several authors have suggested the potential taxonomic value of pollen in Chenopodiaceae. For example, Tsukada (1967) hinted at the importance of spinule and puncta density, and spinule: puncta ratios in the identification of Chenopodiaceae at generic and at species level. Pollen grains of the Chenopodiaceae resemble golfballs, with a spheroidal shape and pantoporate surface, pores are distributed evenly across the pollen grain. They are characterized by a spinulose and punctate tectum, with tectum perforations often being minute (Nowicke, 1975; Skvarla & Nowicke, 1976). The pantoporate pollen grains of *Atripliceae* are characterized by their spheroidal or subspheroidal shape, flat or moderately vaulted mesoporia with 21–120 pores (Olvera, Soriano & Hernandez, 2006).

Frankton and Bassett (1970) suggested that pore size, pore number, and spinule shape are useful characters in *Atriplex* L., however pollen morphology of members of Chenopodiaceae especially species of *Atriplex* L., is poorly documented. Nevertheless, a few data about pollen diameter, pore number, spinule and puncta densities are scattered in the literature (e.g. Wodehouse, 1965; Nair & Rastogi, 1966-67; Tsukada, 1967; Nowicke, 1975; Frankton & Bassett, 1970; Bassett *et al*., 1983; Chu, 1987; Hao *et al*., 1989 and Flores Olvera, 1992).

The need for assessing viability of pollen is important in the understanding of sterility problems and hybridization programs (Gupta and Murty, 1985), fruit breeding programs (Oberle and Watson, 1953), and evolutionary ecology (Thomson *et al.* 1994). However the reasons for pollen grain abortion in natural populations are varied, with factors intrinsic to the individual in addition to environmental factors (Illescas *et al*., 2010).

2. THE AIM OF THIS STUDY IS TO

1. Describe, document the pollen diversity within *Atriplex halimus* L. species.
2. Explore the potential taxonomic utility of pollen character to identify the subspecific taxa of *Atriplex halimus* L. within the Egyptian geographic borders.
3. Study the relation between pollen fertility in different *A. halimus* populations and different environmental factors especially the salinity and aridity.

3. MATERIALS AND METHODS

Atriplex halimus L. was subjected to taxonomic revision and the species was found to be represented in Egypt by two subspecies (Sallam Master thesis under work). Both of the two studied subspecies have two forms typical form (T_5) specimens in which all male flowers have five perianth segments and five stamens, and nontypical form (T_4+T_5) specimens in which some male flowers have five perianth segments and five stamens and other male flowers have four perianth segments and four stamens.

Flowers of 52 populations were selected to represent the two *Atriplex halimus* L. subspecies (subsp. *halimus* L. and subsp. *schweinfurthii* Boiss.), were collected from their natural habitats (coastal salt affected land and semidesert areas) for the study of pollen grains sterility, and flowers of 5 representative samples were used for SEM study.

Table 1. *Locality of the studied Atriplex halimus L. populations*

		Population type	Lat.	Long.
subsp. *halimus* populations				
Coastal salt affected land	Sallum	Typical	31°34'	25°09'
	Rosetta	Typical	31°24'	30°25'
	El-Mahmoudia	Typical	31°08'	30°12'
	Burg El-Arab site 2	Typical	30°55'	29°32'
	221.5 km Alex- Matruh	Typical	31°04'	29°34'
	Burg El-Arab site1	Typical	30°54'	29°28'
	227 km Alex- Matruh	Typical	31°05'	29°38'
	237 km Alex- Matruh	Typical	31°07'	29°45'
	Abu Qir	Typical	31°19'	30°04'
	Gamasa	Non typical	31°26'	31°32'
	140 km,Alex- Matruh	Non typical	30°50'	28°12'
	10km from El-Alamein	Non typical	30°49'	28°59'
	El-Busseili	Non typical	31°20'	30°25'
Semi-arid desert	Maadi desert plain	Non typical	29°58'	31°15'
	Wadi Digla site6	Non typical	29°57'12''	31°20'25''
	Wadi Digla site5	Non typical	29°57'11''	31°20'25''
	Wadi Digla site 4	Non typical	29°57'7''	31°21'23''

	Wadi Digla site3	Non typical	29°57'7"	31°20'22"
	Wadi Digla site2	Typical	29°57'1"	31°20'45"
	Wadi Digla site1	Typical	29°56'45"	31°29'3"
	Wadi Hof site1	Non typical	29°52'45"	31°25'20"
	Wadi Hof site2	Non typical	29°52'32"	31°25'54"
	Wadi Hof site3	Non typical	29°52'11"	31°25'08"
	Wadi Haggag,Sinai	Typical	28°52'	34°25'
subsp. *schweinforthii* Boiss. populations				
Coastal salt affected land	Ain Sokhna S. of Suez	Non typical	29°37'57"	32°19'48"
	Ain Sokhna S.of Suez site 2	Non typical	29°37'55"	32°19'43"
	206 km Alex- Matruh	Non typical	30°53'	29°24'
	26 km east of Matruh	Typical	31°14'	27°32'
	Abu Matamir (Western Delta)	Typical	30°54'	30°10'
	Amria	Typical	31°01'	29°48'
	Sidi Krer	Typical	31° 1'0.3"	29°38'44"
	11 km east of Sallum	Typical	31°32'	25°20'
	Idko	Typical	31°18'	30°18'
	Ras El Hekma	Typical	31°15'	27°52'
	Burg El-Arab site 2	Typical	30°56'	29°39'
	Burg El-Arab site1	Typical	30°54'	29°33'
	Gamasa	Typical	31°26'	31°32'
Semi-arid desert	Wadi Digla site10	Non typical	29°57'58"	31°20'22"
	Wadi Digla site 9	Non typical	29°57'56"	31°20'35"
	Wadi Digla site 8	non typical	29°57'53"	31°20'48"
	Wadi Digla site 7	Non typical	29°57'48"	31°21'28"
	Wadi Digla site 6	Non typical	29°57'43"	31°21'13"
	Wadi Digla site 5	Non typical	29°57'39"	31°21'15"
	Wadi Digla site 4	Non typical	29°57'34"	31°20'28"
	Wadi Digla site 3	Non typical	29°57'22"	31°20'35"
	Wadi Digla site2	Typical	29°57'15"	31°20'22"
	Wadi Digla site1	Typical	29°56'40"	31°29'15"
	Wadi Hof site 4	Non typical	29°52'55"	31°23'8"
	Wadi Hof site 3	Non typical	29°52'54"	31°23'09"
	Wadi Hof site 2	Non typical	29°52'53"	31°23'9"
	Wadi Hof site1	Typical	29°52'49"	31°23'10"
	Sinai	Typical	28°45'	34°12'

3.1. Soil Samples

Soil samples were collected by cylindrical vial from the stands in which the studied *A. halimus* L. populations were collected. Two soil samples were collected from each population stand at depth 20-40 cm. Each sample was air dried, sieved by 2 mm sieve to remove gravel and plant debris, and then packed in plastic bags to be ready for physical and chemical analysis.

3.2. Malachite Green-Acid Fuchsin

The stain solution was prepared by adding the following constituents: 10 ml 95% alcohol, 1 ml Malachite green (1% solution in 95% alcohol), 50 ml Distilled water, 25 ml Glycerol, 5 ml Acid fuchsin (1% solution in water), 0.5 ml Orange G (1% solution in water), 4 ml Glacial acetic acid, 4.5 ml distilled water to a total volume of 100 ml, and then the final stain solution is stored in dark until use.

3.3. 2- Methods

Flowers of these samples are fixed in FAA (50 ml ethyl alcohol, 10 ml formaldehyde, 5 ml glacial acetic acid and 35 ml distilled water). Atleast five closed anthers from five flowers were randomly selected from the studied population samples for light microscope study, opened onto a drop of lactophenol cotton blue stain on a glass slide, stained for two hours, and examined under LM.

Two hundred grains from each plant were observed under light microscope, as fertile pollen are stained dark blue, and aborted (sterile) pollen appear pale blue and the aborted pollen percentage was calculated. Verification was carried out by Malachite green-acid fuchsin dye which is a simplified staining method that rapidly produces a clear differentiation between sterile and fertile pollen, non-aborted pollen grains stained magenta-red and aborted pollen grains stained pale green.

The anthers from the samples used for Scanning Electron Microscope study are removed from FAA and then placed through an ETOH dehydration series, dried with a Denton DCP-1 apparatus, mounted and coated with 600 Å of Gold-Paladium mixture with a Technics Hummer V Sputter Coater. Samples are scanned on a Joel 1200 EX II SEM at 20 kv. Size measurements were obtained from the average of 10 randomly selected grains when possible. The used pollen terminology in this study followed Erdtman (1952) and Walker & Doyle (1975).

3.4. Soil Analysis

Soil texture was determined by using the sieve method according to Allen *et al.* (1974). In applying this method, a known weight 100 g of air dried soil samples were passed through a series of sieves to separate soil fractions according to Wentworth scale (American System) Ryan *et al.* (1996).

3.5. Chemical Analysis

Soil–water extract (1:1) was prepared by adding 100 ml of distilled water to 100 g of air-dried soil. This filtrate was used in the determination of electrical conductivity (EC), soil reaction (pH) and soluble anions: chlorides (Cl^-), sulphates (SO_4^{2-}) and bicarbonates (HCO_3^-) and soluble cations (Na^+, K^+, Ca^{2+} and Mg^{2+}). Calcium carbonate was estimated using the air-dried soil as powder.

- Hydrogen ion concentration was measured in the soil extract by using Electric pH meter with a glass and calomel electrodes (Ryan *et al*., 1996).
- Electric conductivity was measured in the soil extract by using electrical conductivity meter with conductivity cell (Ryan *et al*., 1996). The accuracy of EC meter was adjusted using 0.01 N KCL.
- Soluble chloride (meq L^{-1}) was determined in the soil extract by silver nitrate titration methods (Ryan *et al*., 1996).
- Sulphate (meq L^{-1}) was determined in the soil extract by the turbidity method using spectrophotometer (Model, Spectronic 20 D) on transmittance at wavelength of 420 nm according to Standard methods (1989).
- Bicarbonates (meq L^{-1}) were estimated by using acid neutralization method against 0.1 N sulphoric acid, where methyl orange acts as an indicator (Ryan *et al*., 1996).
- Calcium ions were determined by versenate titrations method (Ryan *et al*., 1996).
- Sodium (Na^+) and potassium (K^+) concentrations (meq L^{-1}) in the soil extract were determined by using flame photometer technique according to (Ryan *et al*., 1996). Calcium carbonate was determined volumetrically using Collin's Calcimeter (Piper, 1950).

3.6. Statistical Analysis

Statistical analysis was performed using the correlation analyses to compute the correlation coefficients (r) between soil parameters (physical & chemical), along with the fertility, regression analyses also performed to computed the determination factor (r^2) for electrical conductivity and chloride as independent factors with the fertility as a dependent factor. All statistics were carried out using Statistical Analysis Systems (SAS) program Ver. 9.2 (SAS, 2009).

4. RESULTS

The taxonomic revision of *Atriplex halimus* L. in Egypt revealed the presence of two subspecies (subsp. *halimus* L. and subsp. *schweinfurthii* Boiss). Microscopic examination of *A. halimus* populations revealed that each of the two subspecies (subsp. *halimus* "AHH" and subsp. *schweinfurthii* "AHS") was traced in two forms one form has all male flowers with five tepals (T_5) and five stamens named typical form and the other form has some male flowers with four tepals and four stamens and other male flowers with five tepals and five stamens (T_4+T_5) named non typical form.

4.1. Pollen Sterility

By examining under LM using lactophenol cotton blue stain, fertile pollen appear dark blue, while sterile pollen appear pale blue. But by using Malachite green-acid fuchsin dye, fertile pollen stained magenta-red and sterile pollen stained pale green (Figs. 3 & 4).

Then the percentage of pollen grain sterility was calculated after observing at least two hundred grains from each plant and the results appeared as in Tables (2 & 3). Pollen sterility of the studied 14 AHH populations from coastal salt affected land were examined and outlined in Table (2), the pollen fertility showed the highest value (99%) at Rosetta, this fertility decreases southward to reach its lowest value at El-Busseili area (68%). Populations of the same subspecies in semi-arid non-saline desert showed maximum fertility (73%; Table 2) in northern desert wadi (W. Digla, downstream site with less arid soil) and lowest fertility to the south (8%; Table 2), at the upstream sites of this wadi (more arid). But for AHS the populations in semi-arid non-saline desert showed maximum fertility in upstream sites (less arid soil) of both Wadi Hof (98%; Table 3) and Wadi Digla (93%; Table 3), and lowest fertility in the downstream sites (more arid soil of Wadi Digla (29%; Table 3) and Wadi Hof (33%; Table 3).

The bio-statistical relation between the pollen grains fertility with both of electrical conductivity and the chloride ion concentration in soil as outlined in Figures 91 & 2), showed that increasing both EC and Cl^- conc. causing decrease in pollen fertility (negative relationship) in *A. halimus* subsp. *halimus* (AHH), as when EC was 1.5 ds/m, and Cl^- conc. was 11 meq/L, the fertility was 70%, and when EC was 12.2 ds/m, and Cl^- conc. was 109.5 meq/L, the fertility was 20%. In the opposite side, the *A. halimus* subsp. *schweinfurthii* (AHS) the increase in both EC and Cl^- conc. causing the increase in pollen fertility (positive relationship) as when EC was 2.2 ds/m, and Cl^- conc. was 16 meq/L, the fertility was 29%, and when EC was 10.5 ds/m, and Cl^- conc. was 91 meq/L the fertility was 83%, as shown in Figures (1 & 2).

Table 2. *Pollen fertility in AHH populations in different habitats in Egypt*

	Locality	Perianth segments	Pollen fertility	Pollen sterility
Coastal salt affected land	Rosetta (Markaz Rashid)	Typical	99%	1%
	El-Mahmoudia	Typical	98%	2%
	Sallum	Typical	95%	5%
	Sinai(Wadi Haggag)	Typical	94%	6%
	Burg El-Arab site2	Typical	87%	13%
	221.5 km Alex- Matruh	Typical	84%	16%
	Burg El-Arab site1	Typical	75%	25%
	227 km Alex-Mersa Matruh	Typical	70%	30%
	237 km Alex-Mersa Matruh	Typical	87%	13%
	Abu Qir	Typical	79%	21%
	140 km Alex-Mersa Matruh	Non typical	82%	18%
	10km from El-Alamein	Non typical	70%	30%
	Gamasa	Non typical	92%	8%
	El-Busseili	Non typical	68%	32%
Semi-arid non saline desert	Wadi Digla site2	Typical	73%	27%
	Wadi Digla site1	Typical	57%	43%
	Wadi Hof site1	Non typical	56%	44%
	Wadi Hof site2	Non typical	50%	50%
	Wadi Hof site3	Non typical	47%	53%
	Maadi desert	Non typical	46%	54%
	Wadi Digla site6	Non typical	44%	56%
	Wadi Digla site3	Non typical	35%	65%
	Wadi Digla site5	Non typical	20%	80%
	Wadi Digla site4	Non typical	8%	92%

Table 3. *Pollen fertility in AHS populations in different habitats in Egypt*

	Locality	Perianth segments	Pollen fertility	Pollen sterility
Coastal salt affected land	Ain Sokhna S. of Suez	Non typical	95%	5%
	26 km east of Matruh	Typical	95%	5%
	Abu Matamir	Typical	95%	5%
	Amria	Typical	95%	5%
	Sidi Krer	Typical	83%	17%
	11 km east of Sollum	Typical	80%	20%
	Ain Sokhna S. of Suez site2	Non typical	77%	23%
	Idko	Typical	70%	30%
	Ras El Hekma	Typical	62%	38%
	Burg El-Arab site2	Typical	61%	39%
	Burg El-Arab site1	Typical	56%	44%
	206 km Alex- Matruh	Non typical	45%	55%
	Gamasa	Typical	40%	60%
Semi-arid non salinedesert	Wadi Hof site3	Non typical	98%	2%
	Wadi Digla site9	Non typical	93%	7%
	Wadi Digla site8	Non typical	86%	14%
	Wadi Digla site7	Non typical	81%	19%
	Wadi Digla site4	Non typical	77%	23%
	Wadi Digla site10	Non typical	75%	25%
	Wadi Hof site2	Non typical	73%	27%
	Wadi Hof site4	Non typical	72%	28%
	Wadi Digla site5	Non typical	54%	46%
	Wadi Digla site3	Non typical	46%	54%
	Wadi Digla site1	Typical	43%	57%
	Sinai	Typical	41%	59%
	Wadi Digla site6	Non typical	38%	62%
	Wadi Hof site1	Typical	33%	67%
	Wadi Digla site2	Typical	29%	71%

Table 4. *Relation of Atriplex halimus L. fertility with chemical and physical character of soil Typical = T5 and non typical = (T4 &T5) W=Wadi*

	Locality	Fertility %	EC ds/m	Ca^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	PH	% sand
AHH												
Typical	237 k Alex-Matruh	87	1.9	10.5	1.9	2.5	24	2.5	14	0.9	8.2	69.1
	227 k Alex-Matruh	80	1.4	6	2.1	1.2	12	2.5	9	1.3	8.5	50
	221.5 k Alex-Matruh	84	1.5	8.5	2.4	1.3	15	2.6	10.4	1.5	8.3	51.3
	Abu Qir	79	6.2	20.5	25.6	1.4	29.8	5	51.8	2.7	8.1	70.2
	W Digla K1	57	4.2	21	3.2	1.6	18.9	3	31	7.3	7.7	76.3
	W Digla K3	49	4.7	22.3	4.8	0.9	29.7	4.2	33.6	7.4	8.1	38.7
Non typical	Gamasa	92	2.4	3.5	15.6	0.8	6.5	2.5	18.5	1.4	8.2	93.5
	140 k to Matruh	82	6.9	21.5	36.8	1.8	16	3.5	58.5	5.1	8	53.3
	10 k from Alamein	70	1.5	9	1.8	0.8	23.5	2.5	11	1.1	8.5	76
	W Digla K3	44	4.4	26	4.2	0.7	30.5	4	32.5	7.4	8.1	38.4
	W Digla K1	20	12.2	61.5	40.2	2.6	28	5	109.5	4.8	8.2	48.1
	W Digla K2	8	2.2	10.5	2.4	0.7	16.3	2.5	16	1.6	7.8	73
AHS												

Typical	Amria	95	6.8	22.3	26	1.1	27.4	4.8	53.5	2.8	8.8	69.8
	Sidi Krer	83	10.5	30	62.4	2.7	15.5	3.5	91	6.6	8	78.4
	155 k to Matruh	60	6.5	21	36.4	1.7	20.3	3.3	57.6	4.9	8.2	73
	W Digla K1	43	13.7	61	46.8	1.9	26.9	6	117.5	5.8	8.1	64
	W Hof K1	33	3.9	23	4.5	0.8	27.8	5.2	30.4	8.5	8.1	65
	W Digla K2	29	2.2	10.5	2.4	0.7	16.3	2.5	16	1.6	7.8	73
Non typical	W Digla K3	81	12.6	63	25.4	2.5	18	6	108	6.9	7.9	83.3
	W Digla K3	77	11.6	29	65.7	2.9	22.4	4	101	7.2	7.7	70
	W Digla K2	75	7.1	43	11.7	1.4	20	5	52.5	13.1	8.1	80.1
	Nubaria canal	72	7.2	31	22.5	1.2	8.2	5	60.5	3.7	7.9	90.8
	Alamein	52	7.5	28.5	14.8	1.9	21.7	4	80.6	3.4	7.9	75.1
	206 k Alex-Matruh	45	9.3	27	42.6	6.2	9.2	4	82.5	3.3	7.9	91.8

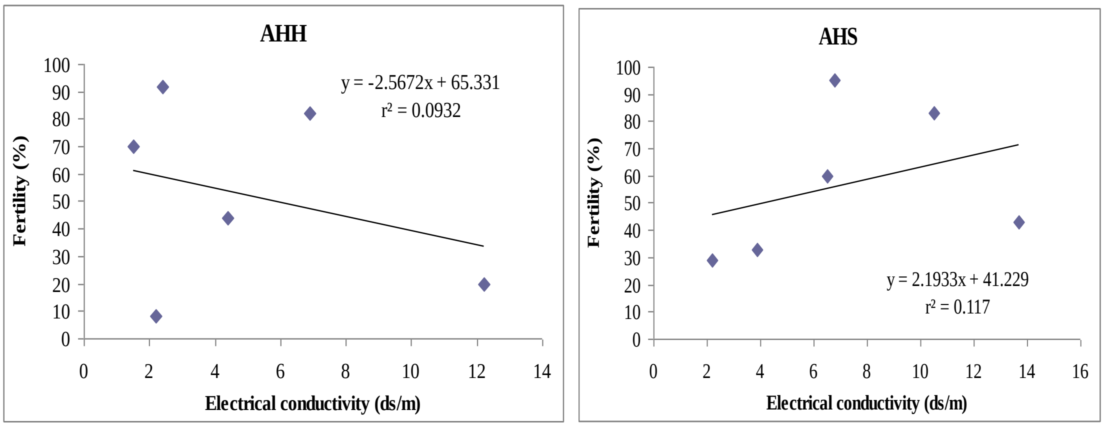


Figure1. *The relation between fertility of both subspecies of A. halimus and electrical conductivity*

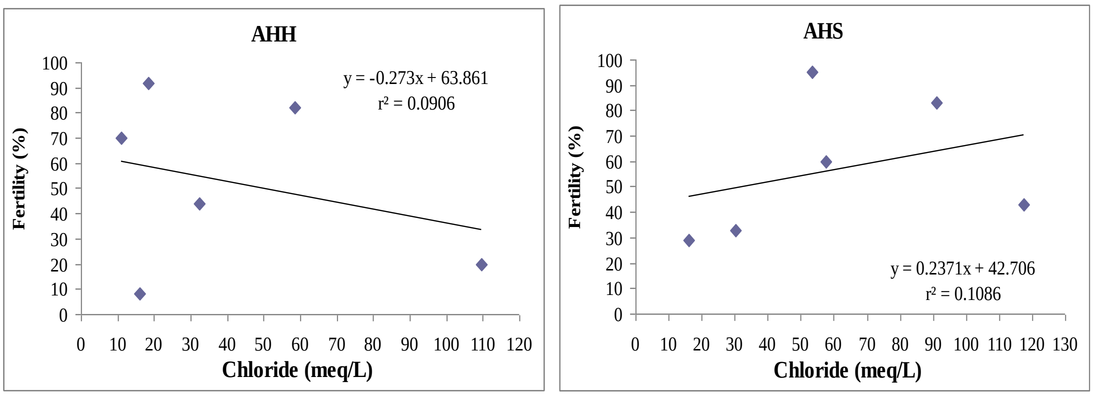
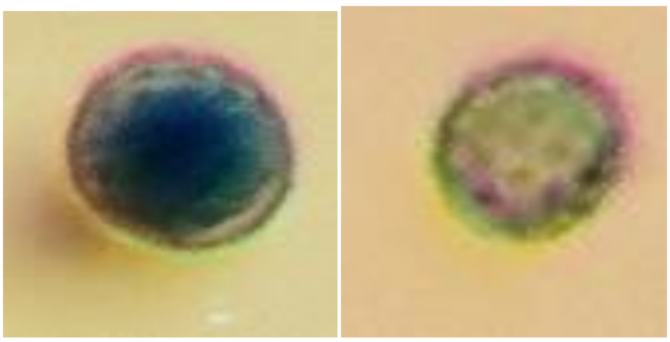
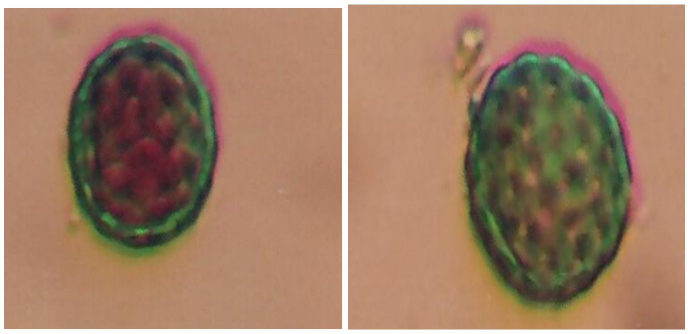


Figure 2. *The relation between fertility of both subspecies of A. halimus and concentration of chloride*



A **B**

Figure3. *Pollen in both subspecies of Atriplex halimus under LM using lactophenol cotton blue stain (x 500). A: Dark blue: fertile pollen & B: Pale blue: sterile pollen*



A B

Figure 4. *Pollen in both subspecies of Atriplex halimus under LM using Malachite green-acid fuchsin dye (x 500). A: Red: fertile pollen & B: Pale green: sterile pollen*

4.2. SEM:

By examining using SEM pollen grains of the two subspecies of *Atriplex halimus* L. (Table 5, Fig 5) showed that the pollen are spheroidal or subsdpheroidal, pantoporate with numerous pores, rangs from 60 to 95, having numerous evenly distributed spinules.

The pollen diameter of the two subspecies of *A. halimus* L. (subsp. *halimus* and subsp. *schweinfurthii* Boiss.) as measured by SEM cleared that the smallest pollen grain diameter among the two studied subspecies was found in non typical form specimens of AHH with diameter ranging between 15.6-16.4 µm (Table 5 & Fig 5) and non typical form specimens of AHS with diameter 15.6-18.7 µm (Table 5 & Fig 5),While the largest pollen grain diameter was found in typical form of AHH specimens, the diameter ranging between 18.5-21.3 µm as shown in Table 5 & Fig 5.

Exine feature: The exine has numerous evenly distributed spinules in both non typical form of AHH, and typical form of (AHS) as shown in Fig (6), in addition to these spinules, the exine is wavy, and have punctate tectum in both typical form of AHH, and non typical form of (AHS) as shown in Fig (6).

Number of pores: Pollen grains of the two subspecies of *Atriplex halimus* L. are pantoporate with numerous pores, the number of pores ranges from 60 to 90 pores, the highest number of pores is found in typical form of AHH (Fig 5) as the number of pores is 90, while the lowest number of pores is found in non typical form of AHH as the number of pores is 60 (Fig 5).The number of pores in AHS in both typical and non typical forms ranges from 62 in typical form to 75 in non typical form as shown in Fig (5).

Pore diameter: The diameter of pore (Table 5) is non distinctive character in both of the studied subspecies.

Pore shape: The shape of pore is irregular in typical form of AHH (Fig 6), and is spherical in both typical and non typical forms of AHS, and non typical form of AHH (Fig 6).

Pore margin: The pore margin is non distinct in both typical form of AHH, and non typical form of AHS as shown in Fig (6), and is distinct in both typical form of AHS, and non typical form of AHH as shown in Fig (6).

Number of worts on pore membrane: The smallest number of worts is found in non typical form of AHH (Table 5 & Fig 6), as the number of worts are 7-9, the highest number of worts is found in typical form of AHH and AHS (Table 5 & Fig 6), as the number of worts are 12-23.

Interporal distance: Which is the space of the exine between pores and has a range between 1.5 and 2.4 µm (Table 5), the longest interporal distance (2.3-2.4 µm) was found in typical form of AHS, while the shortest interporal distance (1.5-2.12 µm) is found in typical form of AHH (Table 5, Fig 6).

Spinule density: Spinules are evenly distributed over the entire pollen surface. Spinule density ranges from 2-8/μm^2 (Table 5). Spinule density is high in both non typical form of AHH, and ranges from 5-8 spinules/ μm^2, and in typical form of AHS, and ranges from 6-8 spinules/μm^2

(Fig 6). While the density is low (2-5 spinules/ μm^2) in typical form of AHH (Fig 6). In non typical form of AHS, the density has a wide range 2-8 spinules/μm^2 (Fig 6).

Spinule shape: Spinule has rounded tip in both non typical form of AHH, and in typical form of AHS (Fig 6), but the spinule has acute or rounded tip in both typical form of AHH, and non typical form of AHS (Fig 6).

Spinule height: Typical form of AHS exhibits spinules with extreme height values 0.15-0.67 μm high (table 5 & Fig 6), while the other three forms have spinules with height ranges from 0.12 to 0.39 μm.

Table 5. *Pollen morphology of A. halimus L. (subsp. halimus & subsp. schweinfurthii)*

Character	*A. halimus* subsp. *halimus*		*A. halimus* subsp. *schweinfurthii*	
	Typical	**Non typical**	**Typical**	**Non typical**
Pollen spheroidal	+	+	+	+
Pollen diameter (μm)	18.5-21.3	15.6-16.4	17.2-18.7	15.6-18.7
Exine feature	spiny, wavy and porolate	spiny	spiny	spiny, wavy and porolate
Number of pores	90	60	62	75
Number of worts on pore membrane	>12	7-9	up to 23	up to 12
Number of pores	0.9-1.2	0.8-1.0	0.85-1.5	0.76-1.4
Pore diameter (μm)	irregular	Circular	Circular	Circular
Pore shape	Non distinct	Distinct	distinct	non distinct
Pore margin	23-26	17-18	16-17	16-19
Number of pores/mm	1.5-2.12	1.97-2.4	2.3-2.4	1.9-2.3
Interporal distance (μm)	2-5	5-8	6-8	2-8
Number of spinulae in 1 μm^2 (spinulae density)	acute or rounded	rounded	rounded	acute or rounded
Spinule shape	0.15-0.39	0.15-0.2	0.15-0.67	0.12-0.24

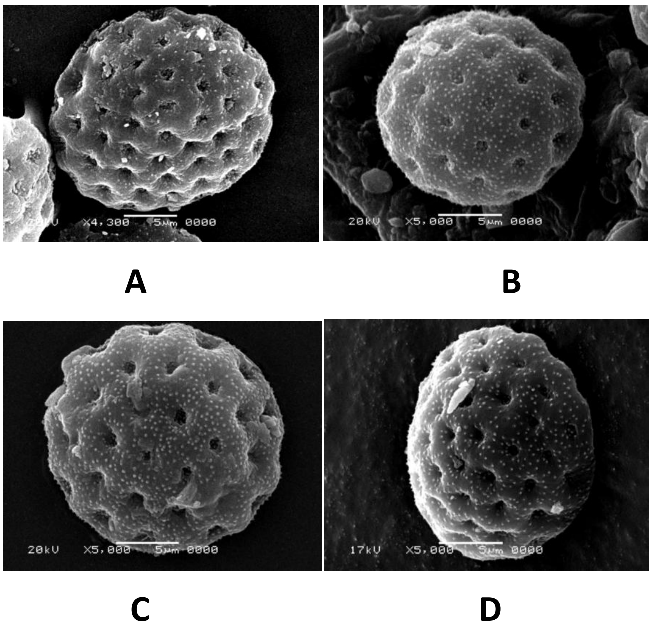


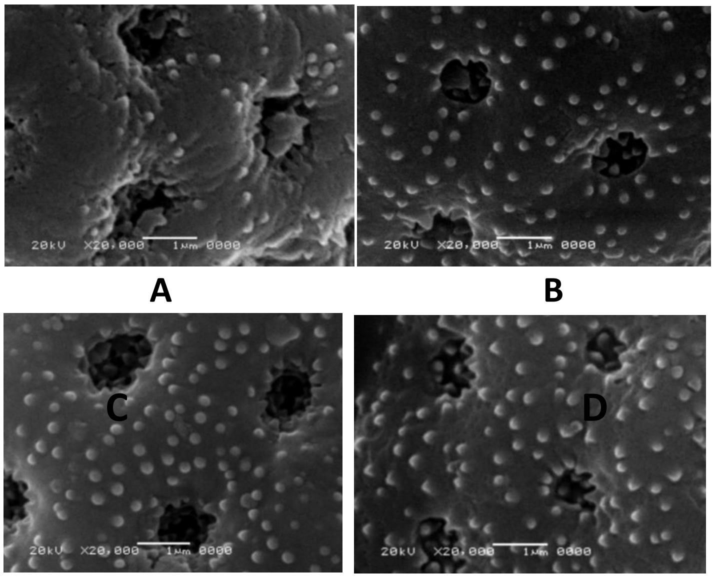
Figure5. *Pollen grains of the two Atriplex halimus subspecies under SEM.*

A: Typical form of A. halimus subsp. halimus.

B: Non typical form of A. halimus subsp. halimus.

C: Typical form of A. halimus subsp. schweinfurthii.

D: Non typical form of A. halimus subsp. schweinfurthii.



C D

Figure 6. *SEM micrographs showed magnified exine of the studied A. halimus subspecies*

A: Typical form of A. halimus subsp. halimus.

B: Non typical form of A. halimus subsp. halimus.

C: Typical form of A. halimus subsp. schweinfurthii.

D: Non typical form of A. halimus subsp. schweinfurthii.

5. DISCUSSION

The taxonomic revision of *Atriplex halimus* L. in Egypt in this study revealed the presence of two subspecies namely: subsp. *halimus* L. and subsp. *schweinfurthii* Boiss. This treatment was based on the differences in morphology, with respect to habit, size, leaf shape and fruit morphology. This assumption was confirmed earlier by Walker *et al*. (2004), who seperated *A. halimus* into two groups diploid (2X), named *A. halimus* subsp. *halimus* and tetraploid (4X), named *A. halimus* L. subsp. *schweinfurthii* Bioss. Microscopic examination of *A. halimus* populations revealed that each of the two subspecies has two forms one form has all male flowers with five tepals (T_5) and five stamens named typical and the other form has some male flowers with four tepals and four stamens and other male flowers with five tepals and five stamens (T_4+T_5) named non typical. The high degree of morphological variations noted in the studied *A. halimus* 52 populations (subsp. *halimus* and subsp. *shweinfurthii*) in this study was supported by the earlier works cited by Franclet & LeHouerou (1971), LeHouérou (1992), Haddioui & Baaziz (2001) and Abbad *et al*., (2003), they mentioned that *A. halimus* L. populations showed considerable variability at both the morphological and isozyme polymorphism levels.

The relation between soil characters and pollen sterility

For the typical and the non typical forms of AHH, the higher soil salinity, EC, and the higher concentration of salts (Ca^{2+}, CO_3^{2-}, HCO_3^-, and SO_4^{2-}; Figs. (1 & 2); Table 4) were positively linked to higher degree of pollen sterility, and the increase in sterility become notable if the increase in the concentration of salts was accompanied with the increase in aridity (high temperature & drought in upstream wadis) as in Wadi Digla populations (Table 4). This effect of salinity and high temperature was reported earlier by Abdullah *et al*., (2001) who claimed that viability of pollens in rice was reduced in all the cultivars under salinity, while the temperature increase decrease the pollen fertility was also reported by Dane *et al*., (1991) and Sharafi (2010). In addition Saini & Aspinall (1982), mentioned that pollen formation is one of the most heat-sensitive developmental stages in cereals. Similar results were reported in wheat by Saini *et al*., (1984) and in barley by Sakata *et al*., (2000), in these species, high-temperature treatment for several days caused abnormal pollen development and complete sterility.

On the opposite side, the results retrieved from both typical and non typical forms of AHS showed higher adaptability to higher degrees of soil salinity and high EC (Table 3 & Figs. 1 &

2), these factors have no significant effect on the pollen fertility, also this form prefers the arid environmental conditions (high temperature & low water content, accordingly when the temperature increase and moisture decrease, pollen fertility increase as in Wadi Digla and Wadi Hof populations in the upstream as shown in Figs. (1 & 2) and Table (4), this conclusion was supported by Hcini *et al*., (2007) who mentioned that *A. halimus* is dominant in the semi-arid and sub-humid areas while *A. schweinfurthii* is more common in arid areas. The higher adaptability of the subsp. *schweinfurthii* is may be due to its polyploidy form, and according to Soltis & Soltis (2000), polyploid may have a much better adaptability to diverse ecosystems.

The results of the study showed some pollen characters of high taxonomic value, which indicate the relation between polyploidy and pollen morphological variation in *Atriplex halimus* L., the most significant characters are exine feature, number of pores, number of worts on pore membrane, pore shape, pore margin, number of pores/mm, spinulae density, spinule shape (Table 5 & Figs 5 & 6).

In the studied AHS populations (tetraploid populations) the exine has numerous evenly distributed spinules, in diploid populations in addition to these spinules, the exine is wavy, and have punctate tectum (Table 5 & Fig 6). Tetraploid populations have smaller number of pores than diploid populations, as the number of pores/mm in tetraploid populations is 16-17, and in diploid populations the number of pores/mm is 23-26 (Table 5 & Fig 5), but the number of worts on pore membrane is larger in tetraploid populations (18-23) than in diploid populations (up to 14) as shown in Table 5 & Fig 6. However (Kessler & Larson, 1969; Cartier, 1971; Dajoz, 1990) provide evidence that there is no clear relation between aperture numbers and polyploidy, (Cramer, 1991 and Philippi, 1961) claimed that pollen grains from tetraploid plants should have more pores than those from diploid plants.

In tetraploid populations the pores are spherical with distinct margin, but in diploid the pores have irregular shape with non distinct margin (Table 5 & Fig 6). Tetraploid populations have higher spinulae density than diploid populations, as number of spinulae in 1 μm^2 in tetraploid populations is 6-8, but the number of spinulae in 1 μm^2 in diploid populations is 2-5, these spinulae have rounded tip in tetraploid populations, but in diploid populations the spinulae have acute or rounded tip (Table 5 & Fig 6).

6. CONCLUSION

The results of the study different *Atriplex halimus* L. populations in Egypt showed that pollen characters has high taxonomic value, in infra-specific level. There was a relation between polyploidy and pollen morphological variations in *Atriplex halimus* L., the most significant characters were: exine feature, number of pores, number of worts on pore membrane, pore shape, pore margin, number of pores/mm, spinulae density, spinule shape. Soil analysis and calculation the percentage of pollen grain sterility and the correlation between the studied items revealed that in case of AHH the pollen sterility increase with the increase of salinity and aridity. AHS showed more adaptive character to arid and saline soil, and the pollen fertility increased under these harsh environmental conditions.

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