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Chromosome count and karyotype study on endemic species *Silene schimperiana* Boiss. (Caryophyllaceae), Sinai, Egypt.

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Abstract:

Chromosome count and karyotype study were performed on the available nine populations of the endangered endemic species *Silene schimperiana* Boiss., section—Sclerocalycinae (Family: Caryophyllaceae), recorded only in a few localities in Sinai Peninsula, Egypt. The species studied showed chromosome number 2*n*=2*x*=24 for the first time in Egypt. The populations studied of *S. schimperiana* showed significant values of Pearson's correlation according to karyotypic characters. The cluster analysis based on karyotypic characters divided the studied populations into two groups with 92% similarity coefficient.

Key words: Karyotype, *Silene schimperiana*, Chromosome number, Chromosome count, Sinai, Egypt

Introduction

The genus *Silene* L. is one of the largest genera of flowering plants in the world (Bari, 1973). *Silene* L. (Caryophyllaceae) consists of about 700 species worldwide. These species are mainly distributed in the Northern hemisphere, Europe, Asia and Northern Africa (Bari 1973 and Greuter 1995). This genus is of particular interest in evolutionary and ecological studies, as highlighted by Bernasconi *et al.* (2009).

Silene species have been placed in 44 sections based on the characters of inflorescence, calyx (shape, hairiness and venation), shape of leaves, characters of capsule and seeds (Chowdhuri, 1957). Recently, molecular studies do not support such sectional classifications particularly for the endemic North American taxa (Oxelman and Liden 1995; Oxelman and Berglund 1997; Oxelman *et al* 2000; Burleigh and Holtsford 2003).

In Africa, Over 90 species are recorded, the vast majority in North Africa, with very few extending southwards into sub-Saharan Africa. Turrill (1956) and Wild (1961) recorded three native and one introduced species of *Silene* in the flora of south tropical Africa,

In Egypt, 29 species of *Silene* L. are distributed in the Mediterranean, Suez and Aqaba Gulfs, coastal plains in Sinai, the Nile Valley, Oases and Gebel Elba massive (Täckholm, 1974 and Hosny *et al.*, 1993). Out of these, 4 species, namely *S. leucophylla*, *S. odontopetela*, *S. oreosinaica* and *S. schimperiana* are endemic to Sinai, whereas *S. biappendiculata* is near-endemic in Egypt and Libya, Boulos (2009). In other words, the endemism ratio of *Silene* L. is 13.8 % in Egypt.

In general, *Silene* species are annual, biennial, or perennial herbs. Diploid species, which are more frequent, have 2*n*=18, 20 or 24. Triploid, hexaploid and even higher polyploidy levels that have 2*n*=*c*. 96, 120 and 192, are also known in the genus (Swank 1932; Heaslip 1951; Bari 1973; Sopova and Sekovski 1982; Zhang 1994 and Oxelman *et al.* 1997), while 2*n*=3*x*=30 is reported in *S. fortunei* (Heaslip, 1951). So, *x*=9, 10, 12 or 23 are the known basic chromosome numbers in *Silene* (Melzheimer 1978; 1988; Markova *et al*. 2006; Sheidai *et al.*, 2011).

Many investigators in Iran and Turkey, where the species of Silene L. is more abundant, have made chromosome counting and karyological studies. Nine *Silene* species belong to section *Sclerocalycinae* grow in Iran, showed 2*n*=2*x*=24 (Sheidai *et al*., 2009). Eighteen *Silene* L. species, subspecies and varieties belong to section *Auriculatae* showed 2*n*=2*x*=24 in 9 species, 2n=4*x*=48 in 8 species and 2*n*=6*x*=72 in

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one species (*S. hirticalyx*), Gholipour and Sheidai (2010a). Other studies showed 2*n*=2*x*=24, 2*n*=4*x*=48 and 2*n*=8*x*=96 (Gholipour and Sheidai, 2010b; Gholipour *et al.*, 2010; Sheidai *et al.*, 2011 and Atzazadeh *et al.*, 2014). In turkey; the chromosome numbers of the investigated species counted 2*n*=24 and *x*=12 (Martin *et al*., 2008a; Martin *et al.*, 2008b; Kiliç and Özçelik 2008; Yildiz *et al.*, 2008; Minareci *et al.*, 2009 and Yildiz*et al.*, 2009). Other studies in the worldwide showed 2*n*=20, 2*n*=2*x*=24, 2*n*=40, 2*n*=48 (Luo D. *et al.*, 2011; Rautenberg *et al.*, 2012; Peruzziand Carta 2013; Draghia *et al*., 2013; Rani *et al.*, 2014; Nersesian and Goukasian1995; Kamari *et al.*, 2015; Jeelani *et al.*, 2011 and Ghazanfar, 1983).

In Egypt, the only study on *Silene* L. was carried out by Badr *et al.*(1987) who recorded diploid number 2*n*=24 in four species viz. *Silene succulenta*, *S. rubella*, *S. ligulata*, and *S. biappendiculata*.

The present study was carried on the endemic species *Silene schimperiana.* This species is rare and endangered species, known from Sinai, Egypt; belongs to section: *Sclerocalycinae* (Hosny *et al.*, 1993).

It is a perennial glaucous herb, woody at the base; stems 50-80 cm, erect, rigid, branching, thicker at the nodes; lower leaves 4-10 x 0.3-0.5 cm, narrowly linear-spathulate, acute, with a prominent midrib on the lower surface; upper leaves shorter, narrowly linear; flowers in lax paniculate cymes, solitary or rarely 2 together, subtended by 2 minute bracteoles; pedicel 1.5-2 cm; calyx 2.2-2.7 cm, to 3 cm in fruit, 10-nerved, cylindric, glabrous, coriaceous; calyx-teeth 2-2.5 mm, dimorphic, triangular, acute (2.5 mm), and broadly triangular, obtuse (2 mm); petals 2-fid; capsule 1.3-1.5 cm, oblong, glabrous: carpophore equaling the capsule; seeds 1 x 1.5 mm, deeply grooved. (Boulos 1999). Fig.1(a).

Material and methods:

Cytological studies were performed on 9 populations of *Silene schimperiana*: 1- Tinia, 2- Abu Tweta, 3- Farsh Em Sella, 4- Shegif Em Sella, 5-Errommana, 6- Abu-Alie, 7 - Maarofia, 8- Abu-Qasaba and 9- Shag Saqr that are shown in Fig. 1 (b). These populations are named after their localities.

Chromosome count and karyotype studies

Seeds of *Silene schimperiana were* collected from natural habitats, Saint Catherine protectorate, South Sinai, Egypt. The seeds have been germinated in Petri dishes at room temperature in the laboratory. The root tips were soaked in 0.1 % colchicine for 3 hours. Afterwards, the root tips were placed in fresh mixture of absolute alcohol: glacial acetic acid (3:1) for 24 hours in a refrigerator. Then the root tips were stored in 70% ethyl alcohol in a refrigerator until examination. The root tips were hydrolyzed in 1N HCl for 12 minutes at 60^{o}c. The root tips were stained according to Feulgen technique.

The counting, measuring of chromosomes lengths, and the karyotype analysis were taken place by using slides contain the chromosomes at the metaphase stage of the mitosis. The photographs, enlarged 9x60, were taken using a camera attached to the microscope. Only the slides with good spread and clearly observable morphologies are considered. Afterwards, the procedures for the location of the centromere, determination of the arm index, chromosome arms and total length, were conducted after the transfer of the images for the computer using karyotype 2 software **(**Altinordu *et al.*, 2016).

Karyotype description

The chromosomes were identified according to Levan *et al.* (1964) as indicated in Table (1). Karyotype asymmetry (ST) was determined according to Stebbins (1971) as given in Table (2), while other karyotype parameters like haploid total chromosome length, mean chromosome length (Peruzzi *et al.*, 2009), total form percentage (TF%) (Huziwara, 1962)**,** coefficient of variation of the centromeric index (CVCI) and coefficient of variation of the chromosome length (CVCL) (Paszko, 2006) as well as the intra-chromosomal asymmetry index (A1) and the inter-chromosomal asymmetry index (A2) (Romero-Zarco, 1986) were determined. The following formulae are used to calculate the TF, A1 and A2:

$$TF\% = \frac{\text{length of short arms in chromosome set}}{\text{total chromosome length in set}} \times 100$$

$$A_1 = 1 - \frac{\sum_{i=1}^{n} \frac{q_i}{p_i}}{n},$$

Where qi is the mean length for short, and pi for long arms in every homologous chromosome pair or group; n is the number of homologous chromosome pairs or groups.

$$A_2 = \frac{s_{CL}}{x_{CL}}.$$

A2) is the ratio between the standard deviation (S_{CL}) and the mean chromosome length (X_{CL}).

Statistical analysis

For comparison between karyotypic features among different populations of *S. schimperiana*, we used Pearson's correlation coefficient by SPSS (Version 19), and principal components analysis PCA plot and Intra-chromosomal asymmetry index (A1) against the inter-chromosomal asymmetry index (A2) by PC-ORD (Version 5.0) respectively. Primer (Version 7.0.11) was used to show the similarity of karyotype features in Hierarchical Cluster analysis and Non-metric Multi-Dimensional Scaling (nMDS).

Results and Discussion

The karyotype analyses showed that the chromosomes of studied populations of *Silene schemperiana* were 2*n*=2*x*=24, Table (3) and Fig. (2). The chromosomes were mostly sub-metacentric. Anyhow, the 9 populations differed in their karyotype formulae. Six populations (Tinia, Shegif Em Sella, Errommana, Abu-Alie, Maarofia and Shag Saqr) had both meta sub-metacentric and sub-telocentric chromosomes; while two populations (Abu Tweta and Abu-Qasaba) had metacentric and sub-metacentric chromosomes. Meanwhile, only one (Farsh Em Sella) had sub-metacentric chromosomes.

The highest value of total and mean haploid chromosome lengths are recorded in Abu-Alie population (82.65 & 6.89 µm, respectively). While, the lowest value of the same parameters occurred in Farsh Em Sella population (42.7 & 3.47 µm, respectively). The size of the longest chromosome varied from 2.76 µm in Farsh Em Sella population to 6.7 µm in Abu-Alie population, Table (3).

The highest coefficient of Variation of Centromeric Index (CVCI) is 21.62 monitored in Abu-Alie population, while the lowest value (10.59) is monitored in Maarofia population. The highest Coefficient of Variation of Chromosome Length (CVCL) is 24.07 monitored in Shag Saqr population, while the lowest value (8.64) is monitored in Farsh Em Sella population. Total form percentage (TF%) varied from 13.1 in Farsh Em Sella population to 33.37 in Maarofia population (Table, 3). The highest intra-chromosomal asymmetry index (A1) is 0.64 recorded in Abu Tweta population, while the lowest value (0.5) in Maarofia population. The highest inter-chromosomal asymmetry index (A2) is 0.24 recorded in Shag Saqr population, while the lowest value (0.13) in Farsh Em Sella population (Table, 3).

The Pearson's correlation among the karyotype features (Table, 4) showed positive significant correlations between the total haploid length (THL), mean chromosome length, size of the longest chromosome (Correlation is significant at 0.01 level), the size of the shortest chromosomes and coefficient of Variation of Chromosome Length **(**CVCL) at the 0.05 level. In addition, the size of the longest chromosomes showed positive significant correlations with mean chromosome lengths at the 0.01 level. As well as, the size of the shortest chromosomes and the inter-chromosomal asymmetry indices (A2) at 0.05 level. The arm ratio (L/S) showed positive significant correlations with three asymmetrical indices CVCL, A1 and A2 at 0.05 level. In addition, there were other positive significant correlations between CVCL and A2 at 0.01 level. Mean chromosome lengths showed positive significant correlations with the size of the longest chromosomes at 0.01 level, and with the size of the shortest chromosomes at 0.05 level.

PCA analysis (data not given) showed that the first 2 axes comprised about 78% of the total variation. In the first axis with about 56% of total variance. The intra-chromosomal symmetry index (A1) and the inter-chromosomal asymmetry index (A2) were the most variable characters (Fig. 5).

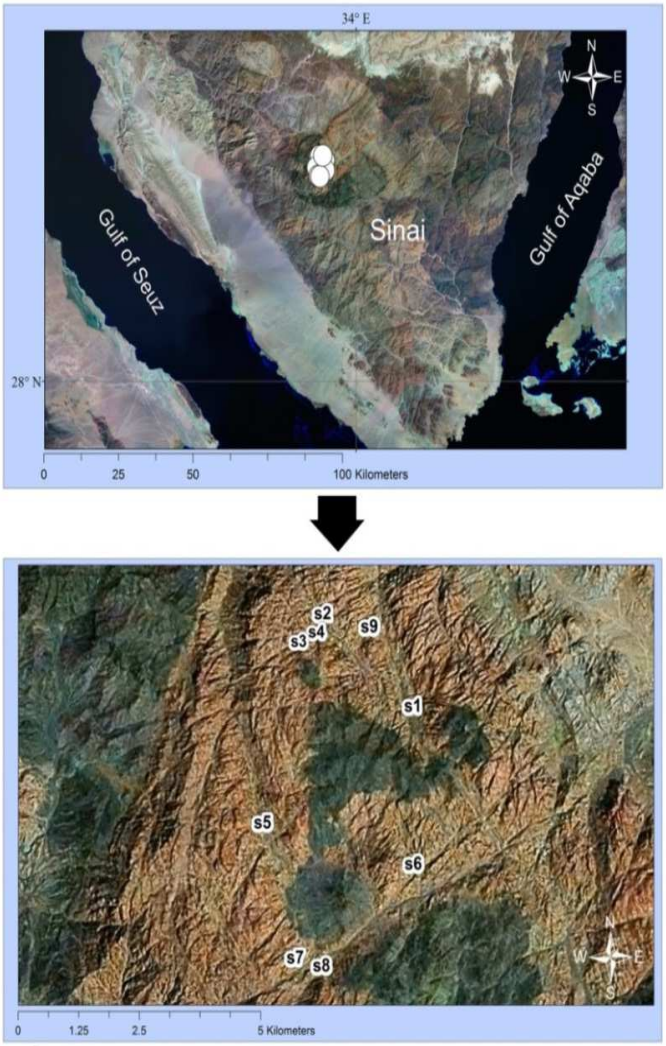

Fig. 1a:

Map of study area

s1-Tinia,

s2-Abu Tweta,

s3-FarshEmSella,

s4-ShegifEmSella,

s5-Errommana,

s6- Abu-Alie,

s7-Maarofia,

s8-Abu-Qasaba

s9-Shag Saqr

Figure 1b: *Silene schimperiana* photo from Sinai, Egypt

Table 1. Karyotype formula according (Levan *et al*., 1964)

Term	Centromeric	Arm ratio	Cl x 100	Chromosome designation	
M	Median point	1	50	Atelocentric	Metacentric[1]
M	Median region	1 – 1.7	50 – 39.5		
Sm	Submedian region	1.7 – 3	39.5 - 25		Submetacentric
St	Subterminal region	3 – 7	25 - 12.5		Subtelocentric
T	Terminal region	7 - ∞	12.5 - 0		Acrocentric
T	Terminal point	∞	0	Telocentric	

[1] Not a recommended term.

Table 2. The classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971)

Ratio	**Proportion of chromosomes with arm ratio<2:1**			
L/S	**1.00(1)**	**0.99–0.51(2)**	**0.50–0.01(3)**	**0.00(4)**
<2:1(A)	1A	2A	3A	4A
2:1–4:1(B)	1B	2B	3B	4B
>4:1 (C)	1C	2C	3C	4C

Table 3. Karyotype features in the populations studied of *Silene shimperiana*. For abbr.: THL = Total chromosome length, L = Longest chromosome, S = Shortest chromosome, Ratio (L/s) = Longest/shortest chromosome, X = Mean chromosome length, A1 and A2 =Romero-Zarco indices, TF = Total form percentage, Coefficient of Variation of Centromeric Index (CVCI) and Coefficient of Variation of Chromosome Length (CVCL).

Loc Code	Location	x	2n	THL	L(µm)	S(µm)	L/S	*X*(µm)	TF%
S1	Tinia	12	24	57.04	4.08	0.81	5	4.75	30.05
S2	Abu Tweta	12	24	43.83	3.36	0.45	7.5	3.65	26.38
S3	FarshEmSella	12	24	41.7	2.76	0.74	3.7	3.47	13.1
S4	ShegifEmSella	12	24	79.87	5.7	1.02	5.6	6.66	30.79
S5	Errommana	12	24	77.27	5.55	1.04	5.3	6.44	29.59
S6	Abu-Alie	12	24	82.65	6.7	1.05	6.4	6.89	27.69
S7	Maarofia	12	24	49.12	3.4	0.79	4.3	4.09	33.37
S8	Abu-Qasaba	12	24	72.51	6.64	1	6.6	6.04	29.27
S9	Shag Saqr	12	24	70.98	5.48	0.69	7.9	5.92	30.88

Loc Code	Location	CVCI	CVCL	A1	A2	ST	Karyotype formula
S1	Tinia	17.56	15.82	0.57	0.16	3B	2m +7sm + 3st
S2	Abu Tweta	19.72	14.85	0.64	0.15	3A	6sm + 6st
S3	FarshEmSella	17.1	8.64	0.51	0.13	2A	12sm
S4	ShegifEmSella	16.77	14.46	0.56	0.14	3A	3m + 8sm +1st
S5	Errommana	17.58	16.87	0.57	0.17	3B	2m + 7sm + 3st
S6	Abu-Alie	21.62	21.91	0.61	0.22	3B	1m + 4sm + 7st
S7	Maarofia	10.59	13.94	0.5	0.14	3A	2m + 9sm + 1st
S8	Abu-Qasaba	15.14	22.49	0.58	0.22	3B	9sm + 3st
S9	Shag Saqr	18.75	24.07	0.56	0.24	3B	2m + 7sm + 3st

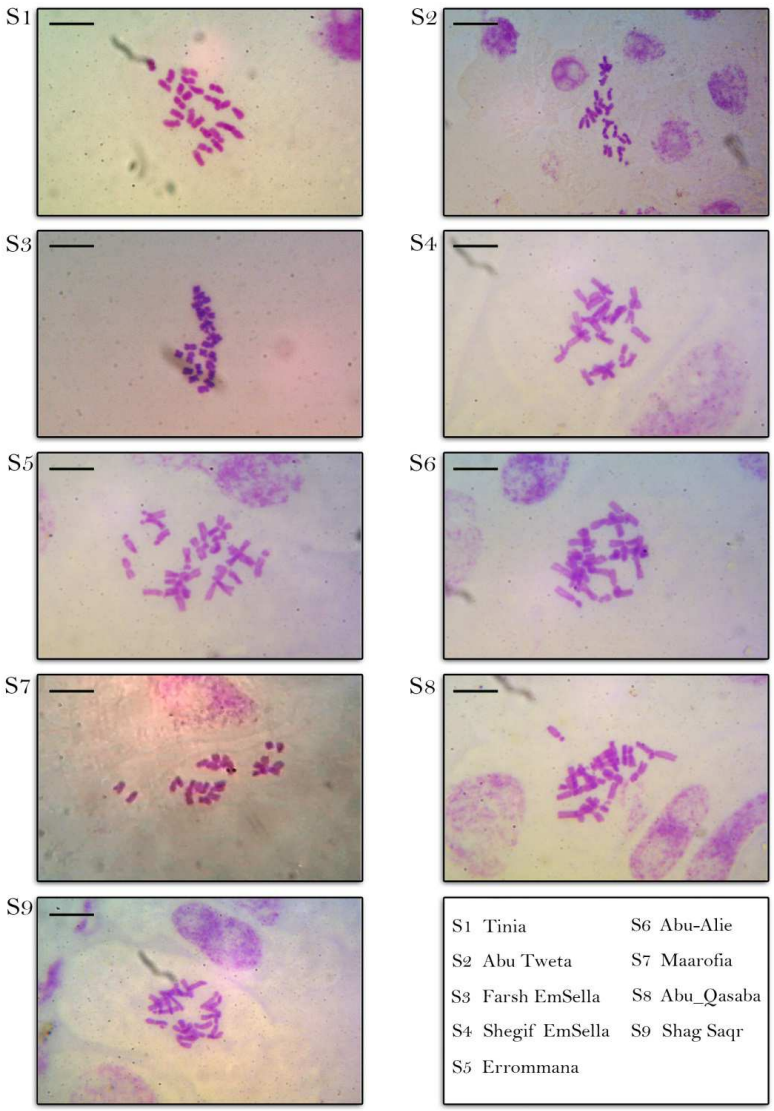

Fig. 2. Mitotic metaphase chromosomes of *Silene schimperiana*. Scale bar =10 µm

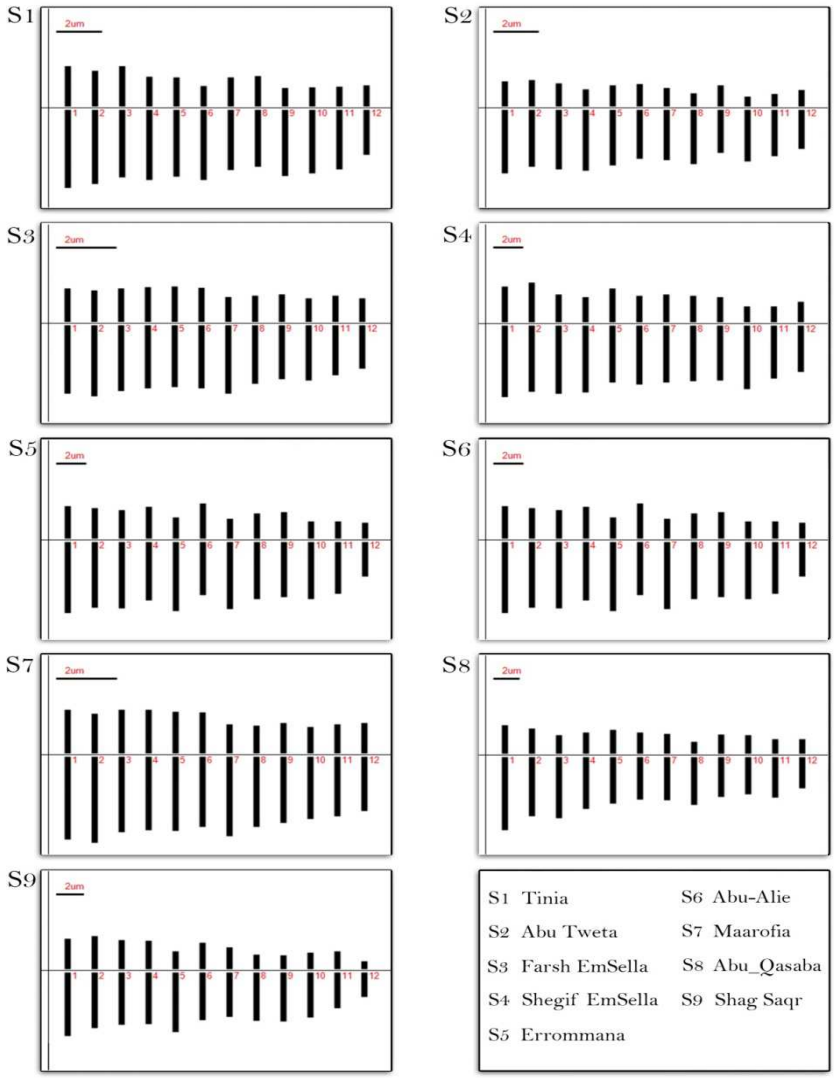

Fig. 3. Representative ideograms of karyotypes in the nine populations of *Silene schimperiana*. Scale bar is different.

In our study *Silene schimperiana*, section Sclerocalycinae, is reported for the first time in Egypt with chromosome number counts for 2n=2*x*=24 and this result supports those of Sheidai *et al*. (2009) on nine species of *silene* in Iran. Also, it supports the results of Yildiz *et al.* (2009) that reported 2n=24 and *x*=12 for fifteen taxa of *Silene* sect. *Sclerocalycinae* from Turkey.

Pearson's correlation gave significant correlation between mean chromosome lengths, size of the longest chromosomes and size of the shortest chromosomes; this is in line with the conducted by Gholipour and Sheidai (2010b). Therefore, the significant quantitative change in the chromosomes could be occurred in the size of both chromosomes arms during the species diversification.

PCA plot of *Silene schimperiana* based on karyotypic data, Fig. (4) supports the clustering results. PCA also showed that A1 and A2 are the most variable characters and accordingly we have made A1 against A2.

Fig. (5) showed the intra-chromosomal asymmetry index (A1) against the inter-chromosomal asymmetry index (A2). It is obvious that showed that Abu-Alie, Abu-Qasaba and Shag Saqr populations are separated in a group, while the other populations in the other one. The latter is subdivided into two subgroups, one of them includes Farsh Em Sella and Maarofia populations, while the other includes Tinia, Abu Tweta, Shegif Em Sella and Errommana populations. Also, a same division is recorded in the Hierarchical Cluster analysis and Non-metric Multi-Dimensional Scaling (nMDS), Figs. (6) & (7). The studied nine populations were separated into two main groups with similarity 92% (Fig. 6). Although the high similarity values between the studied *S. schimperiana* populations (Fig. 6), there are differences between the karyotype characters. These differences may be due to the occurrence of different ecotypes for *S. schimperiana* that needs further study on wide range of populations.

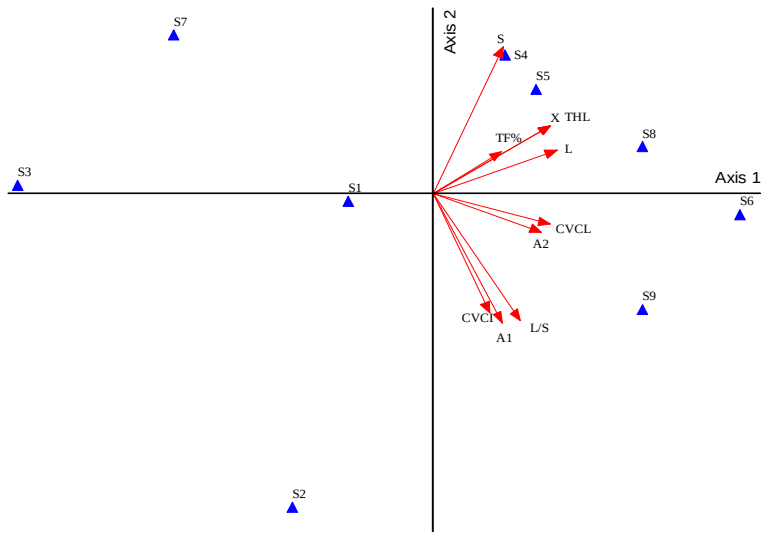

Figure 4. PCA plot of *Silene* species based on karyotypic data. Species code as in Table (3).

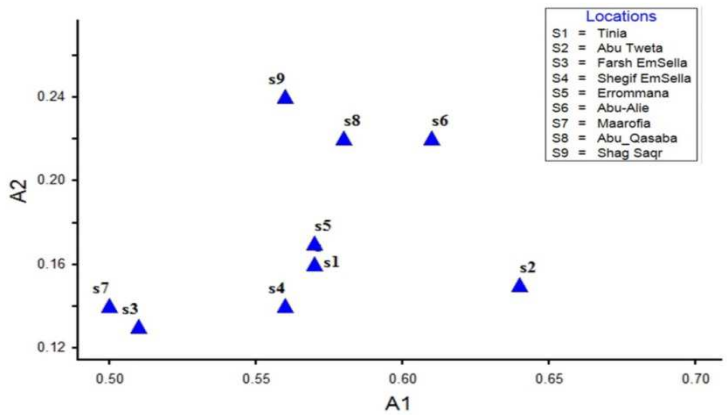

Fig. 5. Two-dimensional plot based on intra-chromosomal asymmetry index (A1) against the inter-chromosomal index (A2).

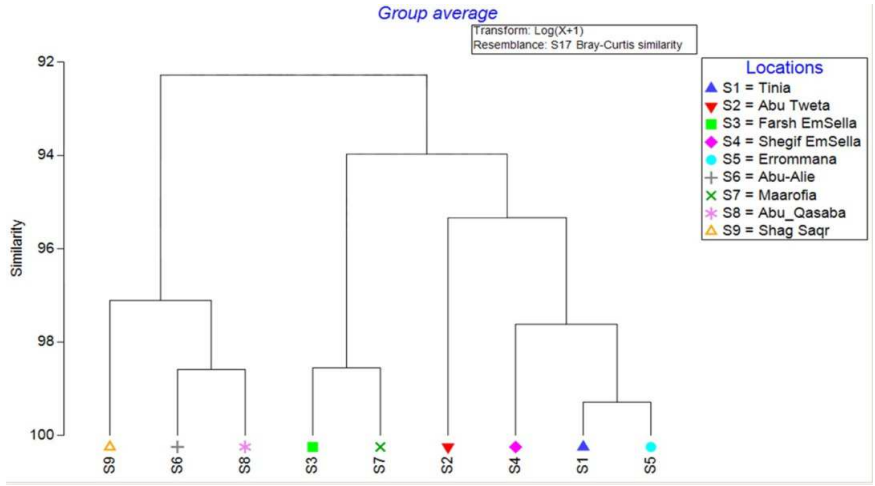

Figure 6. Hierarchical Cluster analysis in the studied populations of *Silene schimperiana*

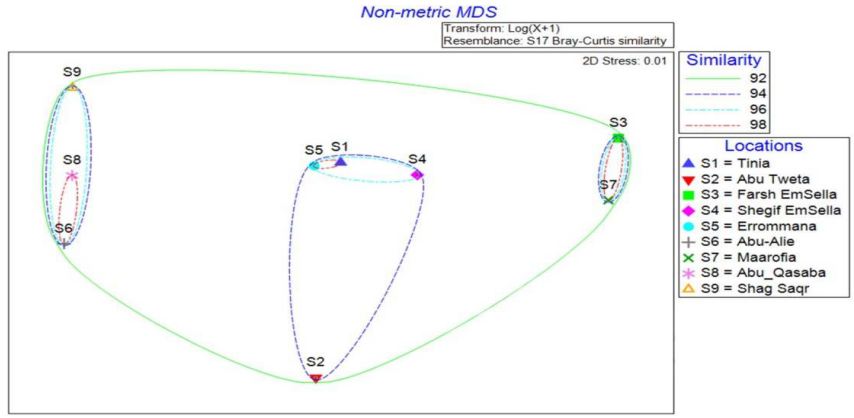

Figure 7. Non-metric Multi-Dimensional Scaling (nMDS) in the studied populations of *Silene schimperiana* populations.

Table 4. Pearson's coefficient of correlation among karyotype parameters, for abbreviations, see table (3).

		THL	L (µm)	S (µm)	L/S	X (µm)	CVCI	CVCL	TF%	A1	A2
THL	Pearson's Correlation	1	**.948****	**.795***	.351	**1.000****	.304	**.674***	.476	.279	.600
	Sig. (2-tailed)		.000	.010	.355	.000	.426	.047	.195	.467	.088
	N	9	9	9	9	9	9	9	9	9	9
L (µm)	Pearson's Correlation	**.948****	1	**.732***	.479	**.947****	.304	**.804****	.447	.387	**.747***
	Sig. (2-tailed)	.000		.025	.192	.000	.427	.009	.228	.303	.021
	N	9	9	9	9	9	9	9	9	9	9
S (µm)	Pearson's Correlation	**.795***	**.732***	1	-.241	**.795***	-.057	.277	.242	-.113	.240
	Sig. (2-tailed)	.010	.025		.533	.010	.883	.470	.530	.772	.535
	N	9	9	9	9	9	9	9	9	9	9
L/S	Pearson's Correlation	.351	.479	-.241	1	.351	.524	**.760***	.367	**.734***	**.705***
	Sig. (2-tailed)	.355	.192	.533		.354	.147	.017	.332	.024	.034
	N	9	9	9	9	9	9	9	9	9	9
X (µm)	Pearson's Correlation	**1.000****	**.947****	**.795***	.351	1	.305	**.674***	.476	.279	.600
	Sig. (2-tailed)	.000	.000	.010	.354		.425	.047	.195	.467	.088
	N	9	9	9	9	9	9	9	9	9	9
CVCI	Pearson's Correlation	.304	.304	-.057	.524	.305	1	.307	-.257	**.732***	.379
	Sig. (2-tailed)	.426	.427	.883	.147	.425		.422	.505	.025	.314
	N	9	9	9	9	9	9	9	9	9	9
CVCL	Pearson's Correlation	**.674***	**.804****	.277	**.760***	**.674***	.307	1	.547	.447	**.962****
	Sig. (2-tailed)	.047	.009	.470	.017	.047	.422		.127	.228	.000
	N	9	9	9	9	9	9	9	9	9	9
TF%	Pearson's Correlation	.476	.447	.242	.367	.476	-.257	.547	1	.168	.317
	Sig. (2-tailed)	.195	.228	.530	.332	.195	.505	.127		.666	.406
	N	9	9	9	9	9	9	9	9	9	9
A1	Pearson's Correlation	.279	.387	-.113	**.734***	.279	**.732***	.447	.168	1	.371
	Sig. (2-tailed)	.467	.303	.772	.024	.467	.025	.228	.666		.326
	N	9	9	9	9	9	9	9	9	9	9
A2	Pearson's Correlation	.600	**.747***	.240	**.705***	.600	.379	**.962****	.317	.371	1
	Sig. (2-tailed)	.088	.021	.535	.034	.088	.314	.000	.406	.326	
	N	9	9	9	9	9	9	9	9	9	9

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

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