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Cytogenetic and molecular studies of the Egyptian *Capsella bursa-pastoris* (Brassicaceae)

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Abstract. *Capsella bursa-pastoris* (Brassicaceae) is one of the most successful tetraploid species in the world. It showed high morphological diversity within Egyptian populations. Morphological investigations of herbarium specimens and fresh collected populations grouped them under three distinguished morphotypes (Lobed "L"; Simple "S" and Lobed-Simple "LS") depending mainly on the basal leaves structure. This high degree of phenotypic variation has received our critical attention. Until recently, the previous studies on *C. bursa-pastoris* attributed its phenotypic variation to environmental factors. But in Egypt, these three morphotypes were traced in mixed populations along with the species geographical range, so the environmental factors have no influence on their distribution or phenotypic variation. Accordingly, our primary concern in this study was to determine the factors controlling this variation. The cytogenetic studies revealed that the three identified morphotypes are three distinct genotypes with three different chromosome numbers: 2n=2x=16 (diploid) for "L"; 2n=3x=24 (triploid) for "S"; 2n=4x=32 (tetraploid) for "LS". The triploid genotype "S" showed rare occurrence among the studied populations and is postulated to be a new record of a hybrid in Egypt. Karyotyping of the three genotypes showed significant differences in the genome and chromosomes relative lengths. Molecular study using cpSSR technique supported the cytogenetic results and differentiated the three studied genotypes. The retrieved results revealed that the phenotypic diversity within the Egyptian *C. bursa-pastoris* populations is genetically controlled.

Keywords: *Capsella bursa-pastoris*, cytogenetic studies, genotypes, karyotyping, molecular study, phenoplasticity.

INTRODUCTION

Brassicaceae (Cruciferae) or mustard family is one of the largest Angiosperm families, it comprises 3977 species and 341 genera and 52 tribes (Kiefer *et al.*, 2014). One of the most important genera in the Brassicaceae is genus *Capsella* Medik. Molecular systematic studies confirmed that genus *Capsella* belongs to the tribe Camelineae (Neuffer *et al.*, 2014). This genus is an excellent model for molecular evolutionary studies due to its phylogenetic relations within the Brassicaceae. Studying its genetics, speciation and sympatric distribution is important for agricultural matters.

Genus *Capsella* is represented worldwide by five species: the two self-compatible tetraploid *C. bursa-pastoris* (L.) Medik and *C. thracica* Velen

(2n=4x=32); the self-incompatible diploid *C. grandiflora* (Fauche & Chaub) Boiss; the two self-compatible diploid *C. rubella* Rent. and *C. orientalis* Klokov (Hurka *et al.*, 2012; Neuffer *et al.*, 2014). These species differ greatly in their geographical distribution, where, *C. grandiflora* is limited to northwestern Greece and Albania; *C. rubella* has a broad Mediterranean / central European distribution; *C. orientalis* is found from Eastern Europe to Central Asia (Hurka *et al.*, 2012); *C. thracica* is endemic to Bulgaria (Neuffer *et al.*, 2014).

Capsella bursa-pastoris (Shepherd's Purse) is an annual to biennial species, extremely variable in size and leaf form, distinguished by terminal and axillary raceme inflorescences. Its silicula fruit is obcordate-obtriangular in shape (Amer *et al.*, 2019). This species is the second most common flowering plant in the world (Zhou *et al.*, 2001), grows as a common weed of agriculture in almost all countries of the world from tropical to subarctic habitats (Holm *et al.*, 1979), and shows a high phenotypic plasticity (Korsmo, 1954; Holzner and Numata, 1982). Accordingly, the evolution of polyploidy and weediness in *C. bursa pastoris* is interesting to agricultural research (St Onge, 2010).

Many taxonomic studies were carried out on this species depending on morphological characters and resulted into many species, subspecies, varieties, microspecies, biotypes, and segregates (Aksoy *et al.*, 1998; Aksoy *et al.*, 1999; Neuffer, 2011).

A considerable amount of literature has attributed the phenotypic variation in *C. bursa-pastoris* to environmental or geographical factors like seasonality, temperature, shade, rainfall, latitudinal and altitudinal gradients (Almquist, 1929; Neuffer, 1989; Neuffer and Bartelheim, 1989; Stace, 1989; Neuffer, 1990; Aksoy, 1996; Aksoy *et al.*, 1999; Neuffer and Hoffrogge 2000).

In Egypt, *Capsella* is a monospecific genus represented by *C. bursa-pastoris* (Boulos, 1999). The field study and morphological investigations of this species – based on the leaves, inflorescence and fruit characters – showed the presence of high degree of phenotypic variation and revealed the presence of three morphotypes namely: Lobed "L", Simple "S" and Lobed-Simple "LS" (Amer *et al.*, 2019). These three morphotypes were traced in mixed populations along with the species geographical range, so the environmental factors have no influence on their distribution or phenotypic variation.

Therefore, this study aims to determine the factors controlling the phenotypic variation of *C. bursa-pastoris* morphotypes through cytogenetic and molecular studies to clarify the impact of genetic diversity on their phenoplasticity.

MATERIALS AND METHODS

Morphological study

The morphological investigations of *C. bursa-pastoris* were carried on 36 old populations deposited as herbarium specimens in Cairo University Herbarium (CAI) and Assiut University Herbarium (ASTU). In addition to 66 fresh populations collected from Menoufia (Abu Sleem village), Faiyum (Sinnuris district, El Siliene) and El Saff regions during our field work conducted in 2016-2018. The studied specimens (old & fresh) from different distribution localities are shown in Table 1. From 66 fresh populations, 25 specimens/ population were undergone morphological investigations using different morphological criteria of leaves, inflorescence and fruit. Acronyms of herbaria follow Thiers (2019).

The morphological investigations distinguished three morphotypes of *C. bursa-pastoris* in all the studied populations namely: (L) Lobed, (S) Simple and (LS) Lobed-Simple (Amer *et al.*, 2019).

Cytogenetic studies

Sample preparation

For cytogenetic studies, the specimens collected from Faiyum region (marked with * in Table 1) were selected to nullify the environmental factors. Seeds of 30 specimens representing the three morphotypes (10/ morphotype) were collected, soaked in distilled water for 2 hours, and germinated at room temperature. Root tips of about 1 cm length were treated with colchicine ($C_{22}H_{25}NO_6$, 0.025 %) for 2 hours at room temperature, and washed thoroughly with distilled water. Fixation was done using ethyl alcohol: glacial acetic acid (3:1, v/v). Samples were washed thoroughly with water and hydrolyzed using 1 N HCl at 64° C for 5 minutes. The slides were prepared by squashing the root tips using 45% acetic acid and stained with aceto orcein solution.

Chromosome count and karyotyping

Chromosome count was performed on mitotic metaphase cells. For each morphotype, ten clearly observable metaphase cells from ten individuals were selected and photographed using standard and high resolution automated karyotyping software processing (Leica CW4000). Metaphase chromosomes of each morphotype were placed in pairs, arranged and numbered in order of size, with keeping in view the centromere position to consti-

tute a karyotype. The length of the short arm (p) and the long arm (q) was measured for each chromosome, and the total length (TL=p+q) was calculated. The relative length (RL) of the chromosomes (TL / sum TL × 100) and the mean relative length (MRL) of each chromosome pair were calculated. The centromeric index (CI) was estimated by (P / TL x 100), the mean centromeric index (MCI) was calculated to represent the centromeric index value of a particular chromosome pair, then the chromosomes were classified according to Levan *et al.* (1964).

Molecular study

From the same 30 specimens collected from Faiyum region (Table 1) for cytogenetic studies, 14 specimens representing the three morphotypes were chosen for molecular study (4 Lobed, 4 Simple and 6 Lobed-Simple).

DNA extraction

A total genomic DNA was extracted from 1 g young leaves using CTAB (cetyl-trimethyl ammonium bromide) extraction buffer procedure described by Doyle and Doyle (1990) and modified by Allen *et al.* (2006).

PCR reactions and data analysis

For each 25 µl PCR reaction, add 12.5 µl Dream Taq Green PCR Master Mix (2X), 1 µl Forward primer (5′-GCC TAC CGC ATC GAA ATA GA-3′), 1 µl Reverse

Table 1. Collected specimens of *Capsella bursa-pastoris* (L.) Medik. in Egypt with their geographical distribution (arranged from North to South).

Collection & Herbarium	Date	Longitude	Latitude	Locality
Amer 8312 (CAI)	18.1.1987	30°26'33"	31°25'15"	Beheira Province, Rosetta
Fahmy 963 (CAI)	2.5.1988	27°14'55"	31°21'23"	Mersa Matruh, El Sallum road
Fayed & El Naggar s.n. (ASTU)	15.3.1984	30°00'44"	31°17'00"	Alexandria, El Montazha
Amer 16225 (CAI)	6.3.1988	30°32'58"	31°12'17"	Beheira Province, Mahmudiya
Abdel Fattah & Abdel Aziz s.n. (CAI).	19.3.1974	31°23'00"	31°01'53"	El Mansoura
Gun Romée 443 (CAI)	12.3.1968	30°55'33"	30°47'13"	Tanta
Amer 1515 (CAI)	18.3.1982	31°48'59"	30°43'19"	Sharkiya, Faqus
G. Täckholm s.n. (CAI)	7.1.1927	31°12'46"	30°41'40"	Barrage (Zifta)
El Naggar s.n. (ASTU)	30.1.1985	31°11'11"	30°27'19"	Banha, Kafor Mousa
El Bakry 2708 (CAI)	29.4.1981	31°33'43"	30°24'57"	Bilbeis
Chrtek, Kosinova & Slavikova s.n. (CAI)	4.4.1977	31°17'00"	30°12'01"	Bahtim
El Batanony s.n. (CAI)	7.2.1957	31°14'14"	30°07'26"	El Menoufia
Amer *et al.* s.n. (CAI)	27.1.2017	31°12'54"	30°06'45"	El Menoufia, Abu Sleem village
El Hadidy s.n. (CAI)	17.1.1952	31°11'58"	30°04'52"	Imbaba
El Hadidy s.n. (CAI)	12.1.1956	31°12'27"	30°01'39"	Giza, Faculty of Science farm
Taher El sayed s.n. (CAI)	19.11.1926	31°11'45"	30°01'12"	Giza, in clover fields
Chrtek & Kosinova s.n. (CAI)	13.4.1971	31°12'29"	30°01'05"	Giza, Faculty of Agriculture farm
Chrtek & Kosinova s.n. (CAI)	1.4.1971	31°13'12"	30°00'35"	Giza, El Harraniya village
Chrtek, Kosinova & Imam s.n. (CAI)	27.4.1967	31°15'48"	29°35'21"	El Saff, fields along the road
Amer *et al.* s.n. (CAI)	23.4.2018	31°15'17"	29°34'57"	El Saff
Abd El Ghani 5820 (CAI)	13.3.1983	30°51'27"	29°24'48"	Faiyum, Sinnuris district, El Siliene
Amer *et al.* s.n. (CAI)	27.1.2017	30°51'27"	29°24'48"	*Faiyum, Sinnuris district, El Siliene
Abd El Ghani 5234 (CAI)	8.3.1983	30°48'56"	29°21'20"	Faiyum district, Beni Saleh
Abd El Ghani 5320 (CAI)	8.3.1983	30°27'11"	29°19'16"	Faiyum district, in clover fields
Fayed *et al.* s.n. (ASTU)	2.5.2010	34°18'25"	27°56'48"	Southern Sinai, Farsh Elias
Zareh & Fayeds.n. (ASTU)	5.12.1990	31°12'05"	27°10'20"	Assiut, Sohag East road
Zareh s.n. (ASTU)	5.12.1990	31°20'18"	27°02'44"	Assiut, El- Matmar
Zareh & Fayed s.n. (ASTU)	30.1.1991	31°22'02"	26°57'05"	Assiut, Sedfa
Zareh s.n. (ASTU)	28.2.1962	32°00'10"	26°14'08"	El-Balliana, Sohag

*Specimens subjected to cytogenetic and molecular studies.

primer (5′- CAA GAA AGT CGG CCA GAA TC-3′), 2 µl Template DNA, and complete to 25 µl by water (nuclease-free).

The PCR was performed using the recommended thermal cycling conditions: one cycle of initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 45 seconds followed by annealing at 57°C for 45 seconds then extension at 72°C for 60 seconds, and one cycle of final extension at 72°C for 10 minutes.

The reaction products were separated by electrophoresis on 1.6 % agarose gel in 1x TBE buffer and run in the same buffer at 100 V for 1 hour, and visualized by staining with 0.5 µg/ml of ethidium bromide and photographed under UV light.

The cpSSR locus ATCP31017 was sequenced and DNA was amplified using the previously mentioned primers (Castro *et al.*, 2014). The amplified fragments were sequenced in ABI377 DNA sequencer (ABI, USA). Then BLAST programs were used for searching DNA databases for sequence similarities. Mega software was used to carry out multiple sequence alignment and calculate genetic distances among studied taxa. Neighbour-joining dendrogram was constructed showing the genetic relationships among 14 specimens of the three studied genotypes.

RESULTS

Morphological diversity

The morphological investigations and taxonomic revision of 36 old herbarium specimens and 66 recently collected populations of *C. bursa-pastoris* based on 25 morphological characters including plant height, basal and cauline leaves features, as well as inflorescence and fruit characters (Amer *et al.*, 2019). The most differential characters were that of the basal leaves. The results of that study (Amer *et al.*, 2019) revealed the presence of three morphotypes in Egypt namely: Lobed "L" with all basal leaves are lobed, Simple "S" in which all basal leaves are simple, and Lobed-Simple "LS" in which basal leaves are mixed lobed with simple (Figure 1).

The three identified morphotypes were co-distributed and traced in the field as mixed populations along with the species geographical range. Where, the "LS" morphotype was the most common type and showed the highest phenotypic variation, while the "S" morphotype showed rare occurrence in all studied localities. The environmental factors such as shading, temperature, soil type and rainfall showed no influence on the distribution of these morphotypes (Amer *et al.*, 2019).

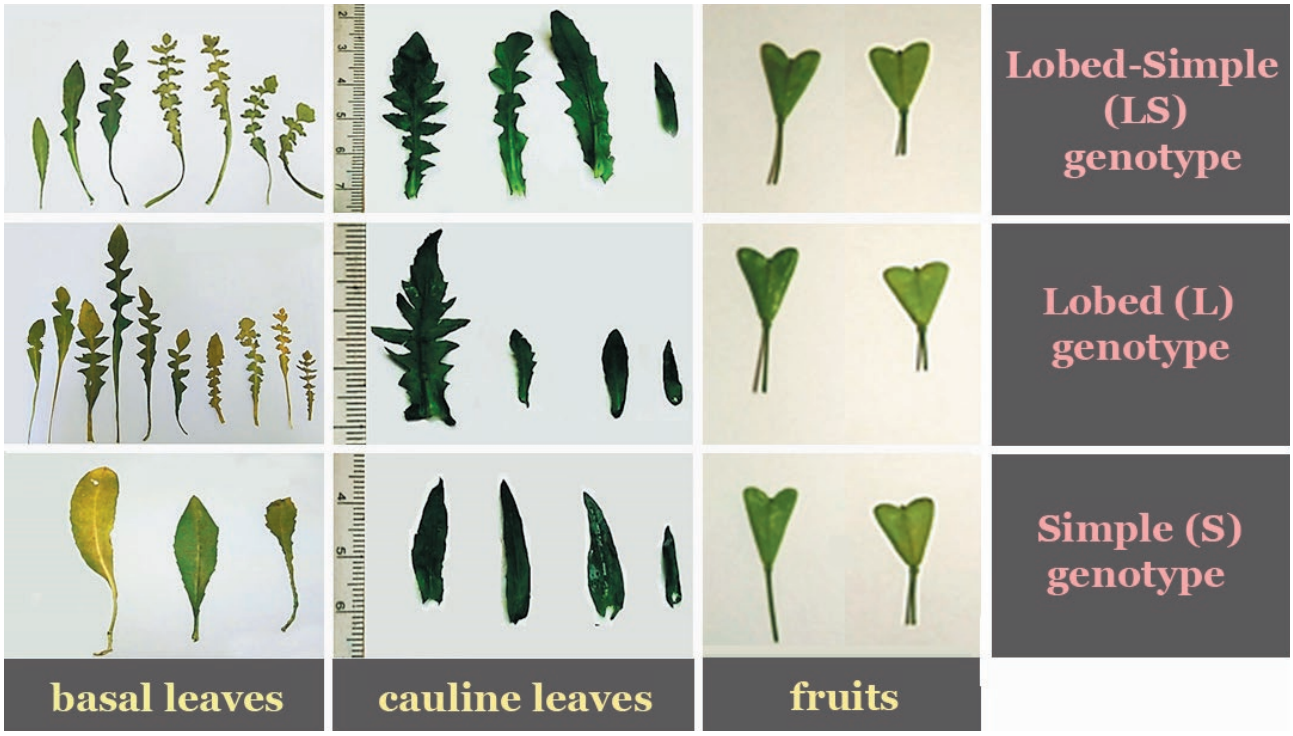

Figure 1. Morphological diversity within the Egyptian *C. bursa pastoris* genotypes; L: Lobed, **S:** Simple, **LS:** Lobed-Simple.

Cytogenetic analysis

Chromosome number

Chromosome count for each morphotype of *C. bursa-pastoris* was done in the mitotic metaphase (Figure 2). The three studied morphotypes recorded three different chromosome numbers. Accordingly, they are treated as three distinct genotypes. The Lobed "L" genotype has diploid chromosome set of 2n=2x=16. The Simple "S" genotype has triploid chromosome set of 2n=3x=24 with minor aneuploidy in chromosome no. 6 and 8 (2n=3x-2=22). This triploid genotype is recorded for the first time in Egypt. While the Lobed-Simple "LS" genotype recorded the presence of tetraploid chromosome set of 2n=4x=32 (Figure 2).

Karyotype analysis

The karyotyping data of these three genotypes are provided in Figure 3 and Table 2. The retrieved results showed that the chromosomes are small in size, the total genomic length ranges from 43.48 µm in the Lobed "L" genotype to 67.76 µm in Lobed-Simple "LS" genotype, while the Simple "S" genotype has an intermediate value of 61.46 µm.

Furthermore, the chromosomes are highly variable referring to their mean relative length (MRL), as shown in Figure 4. The chromosome pair 1 is the longest in the three genotypes, its length ranges from 3.21 µm in Lobed-Simple "LS" genotype to 4.48 µm in Simple "S" one, and its mean relative length (MRL) ranges from 4.74% in Lobed-Simple "LS" genotype to 7.99% in Lobed "L" one. The length of the shortest chromosome pair 8 ranges from 1.54 µm in Simple "S" to 1.74 µm in Lobed "L, and its mean relative length (MRL) ranges from 2.35% in Simple "S" genotype to 3.99% in Lobed "L" with an intermediate value of 2.52% in Lobed-Simple "LS" genotype.

The eight chromosome pairs were grouped based on the centromere position into four types: acrocentric, metacentric, submetacentric and subtelocentric (Table 2). The chromosome pairs from 1 to 4 are metacentric in all the studied genotypes. The chromosome pair no. 5 is metacentric in genotypes "L" and "S", while it is acrocentric in "LS" genotype. The chromosome pair 6 is acrocentric in "L" genotype, while it is submetacentric in "S" and "LS" genotypes. The chromosome pair 7 appeared acrocentric in "L" genotype, submetacentric in "S" genotype, and metacentric in "LS" genotype. The chromosome pair 8 is metacentric in "L" and "S" genotypes, while subtelocentric in "LS" genotype.

Molecular analysis

Gel electrophoresis of the PCR amplification products of 14 studied specimens (4 Lobed, 4 Simple and 6 Lobed-Simple) produced 14 bands of good quality.

The statistical results of cpSSR locus ATCP31017 sequences developed a Neighbour-joining dendrogram (Figure 5) that separated the genotypes "L", "S", and "LS" into three genetic clusters. The first cluster included four specimens (L1-4) that represented the Lobed "L" genotype (2n=16). In this cluster, specimens L1 and L3 showed high genetic similarity to each other. The second cluster included also four specimens (S5-8) that represented the Simple "S" genotype (2n=24). In this cluster, specimens S5 and S7, and specimens S6 and S8 showed high genetic similarity to each other. The third cluster included six specimens (LS9-14) that represented the Lobed-Simple "LS" genotypes (2n=32). In this cluster, specimen LS9 showed the lowest genetic similarity

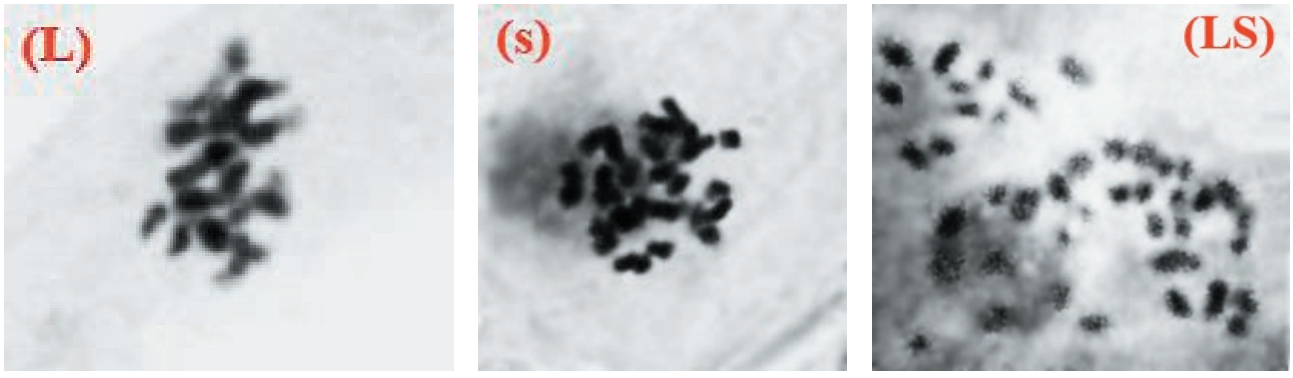

Figure 2. Photomicrographs of well spread mitotic metaphase in the three genotypes of *C. bursa pastoris*: **L:** Lobed diploid with 2n=2x=16; **S:** Simple triploid with 2n=3x=24; **LS**: Lobed-Simple tetraploid with 2n=4x=32.

Table 2. Karyotyping data for the studied *C. bursa pastoris* genotytpes, **L:** lobed, **S:** simple, **LS:**lobed-simple, **A:** Acrocentric, **M:** Metacentric, **SM:** Submetacentric, **ST:** Subtelocentric.

	L	S	LS
Long arm (q) µm			
1	1.74±0.05	2.74±0.63	1.63±0.05
2	1.58±0.00	1.97±0.12	1.29±0.16
3	1.58±0.10	1.37±0.10	1.10±0.26
4	1.37±0.10	1.23±0.04	1.05±0.10
5	1.37±0.00	1.19±0.09	1.63±.38
6	2.32±0.00	1.51±0.25	1.34±0.24
7	2.21±0.00	1.23±0.23	0.95±0.15
8	1.08±0.50	0.84±0.49	1.35±0.22
Short arm (p) µm			
1	1.74±0.05	2.10±0.32	1.58±0.11
2	1.58±0.00	1.86±0.16	1.21±0.16
3	1.53±0.05	1.33±0.11	0.95±0.11
4	1.32±0.05	1.19±0.04	1.00±0.11
5	1.21±0.05	1.16±0.10	0.22±0.10
6	0.26±0.15	0.70±0.39	0.45±0.44
7	0.21±0.00	0.74±0.35	0.84±0.06
8	0.66±0.34	0.70±0.39	0.36±0.15
Total length (p+q) µm			
1	3.48±0.10	4.84±0.95	3.21±0.16
2	3.16±0.00	3.83±0.28	2.5±0.32
3	3.11±0.15	2.70±0.20	2.05±0.37
4	2.69±0.15	2.42±0.08	2.05±0.21
5	2.58±0.05	2.35±0.19	1.84±0.48
6	2.58±0.15	2.21±0.64	1.79±0.58
7	2.42±0.00	1.97±0.55	1.79±0.21
8	1.74±0.54	1.54±0.79	1.71±0.37
Mean relative length (MRL)			
1	7.99	7.38	4.74
2	7.27	5.83	3.69
3	7.14	4.12	3.03
4	6.18	3.69	3.03
5	5.94	3.59	2.72
6	5.94	3.37	2.64
7	5.57	2.99	2.64
8	3.99	2.35	2.52
Mean centromeric index (MCI)			
1	50	44.41	49.13
2	50	48.55	48.41
3	49.23	49.18	45.67
4	49.02	49.17	48.82
5	46.88	49.25	12.3
6	9.94	30.69	25.01
7	8.68	36.63	47.16
8	40.11	47.53	21.93
Type			
1	M	M	M
2	M	M	M
3	M	M	M
4	M	M	M
5	M	M	A
6	A	SM	SM
7	A	SM	M
8	M	M	ST

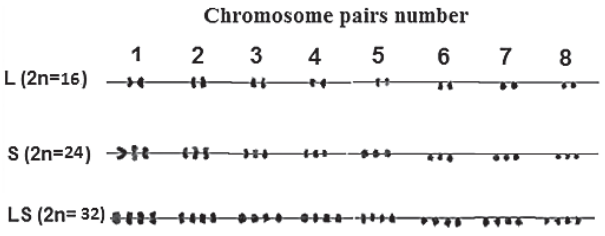

Fig. 3. Karyotypes of *C. bursa-pastoris* genotytpes. L: Lobed diploid (2n=2x=16); **S:** Simple triploid (2n=3x=24); **LS:** Lobed-Simple tetraploid (2n=4x=32).

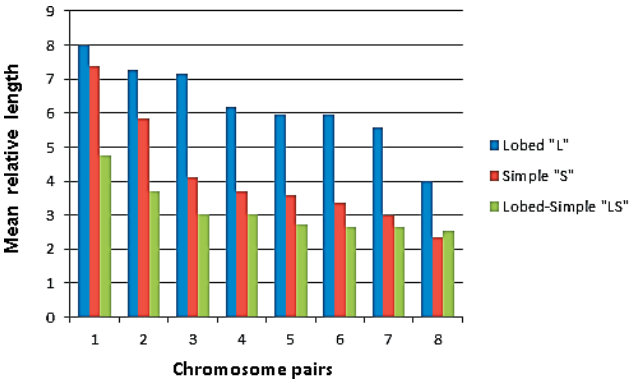

Fig. 4. Mean relative length (MRL) of each chromosome pair in the three studied genotypes of *C. bursa-pastoris.*

with other specimens. While specimens LS10 and LS12, also LS13 and LS14 showed high genetic similarity to each other.

The DNA sequences of the studied 14 specimens of *C. bursa-pastoris* were registered on the National Center for Biotechnology Information (NCBI) under the following accession numbers MN602606, MN602607, MN602608, MN602609, MN602610, MN602611, MN602612, MN602613, MN614131, MN614132, MN614133, MN614134, MN614135 and MN614136.

	L1	L2	L3	L4	S5	S6	S7	S8	LS9	LS19	LS11	LS12	LS13	LS14
L1														
L2	0.0258													
L3	0.0205	0.0361												
L4	0.0255	0.0415	0.0279											
S5	0.0361	0.0685	0.0457	0.0602										
S6	0.0306	0.0515	0.0415	0.0531	0.0395									
S7	0.0363	0.0685	0.0462	0.0609	0.0315	0.0536								
S8	0.0306	0.0495	0.0402	0.0531	0.0395	0.0351	0.0541							
LS9	0.1682	0.1636	0.1651	0.1835	0.1553	0.1591	0.1532	0.1622						
LS10	0.0573	0.0856	0.0797	0.0797	0.0721	0.0756	0.0735	0.0921	0.1429					
LS11	0.0567	0.0822	0.0736	0.0689	0.0602	0.0715	0.0621	0.0852	0.1441	0.0622				
LS12	0.0575	0.0861	0.0807	0.0802	0.0725	0.0763	0.0742	0.0932	0.1296	0.0563	0.0672			
LS13	0.0518	0.0755	0.0696	0.0672	0.0567	0.0631	0.0546	0.0818	0.1468	0.0714	0.0702	0.0815		
LS14	0.0515	0.076	0.0685	0.0664	0.0565	0.0625	0.0541	0.0812	0.1521	0.0718	0.0711	0.0822	0.0363	

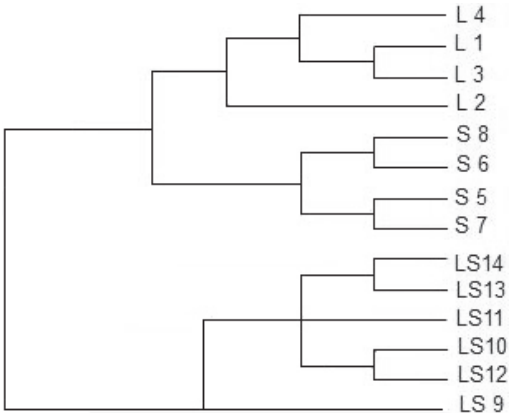

Fig. 5. Diagonal matrix of the genetic distances and Neighbour-joining dendogram showing the genetic relationship among 14 specimens of the three studied genotypes (L, S and LS).

DISCUSSION

The taxonomic revision of *Capsella bursa-pastoris* in Egypt revealed the presence of a high degree of phenoplasticity in all the studied populations (old and fresh) and resulted in three distinctive morphotypes ("L" Lobed, "S" Simple and "LS" Lobed-Simple) based mainly on the basal leaves structure (Amer *et al.*, 2019). These morphotypes were traced as mixed populations in all the studied localities (Table 1), so the environmental factors have no influence on their distribution or phenoplasticity (Amer *et al.*, 2019). However, positive correlation between the degree of genetic heterogeneity and environmental variability was reported by Aksoy *et al.* (1998), Neuffer *et al.* (1999), Neuffer and Hurka (1999) and Castro *et al.* (2014). In addition to Han *et al.* (2015) who reported that *C. bursa-pastoris* populations from different regions are polymorphologically different.

The study of genetic diversity of *C. bursa-pastoris* in Egypt has received little attention so far. In this study, we tried to elucidate the role of genetic diversity within the Egyptian morphotypes in their phenoplasticity.

The chromosome counting of the identified morphotypes recorded three different chromosome numbers (2n=16 for Lobed "L", 3n=24 for Simple "S", 4n=32 for Lobed-Simple "LS"), so they are treated here as genotypes. Shull (1909) was the first to distinguish between four biotypes with different leaf types (simplex, rhomboidea, tenuis, and heteris) and cleared the correlation between leaf shape and chromosome number. He recorded the tetraploid number for simplex and rhomboidea biotypes, while heteris and tenuis were diploid. Nonetheless, the Egyptian morphotypes are not equivalent to Shull's biotypes (Amer *et al.*, 2019).

Worldwide, the tetraploid *C. bursa-pastoris* (2n=4n=32) is one of the most successful plants that have high polymorphic level (Han *et al.*, 2015). Similarly in Egypt, the tetraploid "LS" genotype is the most common and diverse type with a wide range of leaf forms on the same plant (Amer *et al.*, 2019). This is supported by Shull (1929), Löve and Löve (1956), Davis (1965), Raj (1965), Hsu (1968), Svensson (1983) and Hurka (1984).

The diploid chromosome number (2n=2x=16) recorded in Lobed "L" genotype, was early recorded in Europe by Bosbach and Hurka (1981), in Greece by Svensson (1983), and in Kashmir by Jeelani *et al.* (2013).

Although *C. bursa-pastoris* is a self-compatible species, different percentages of outcrossing were recorded by many authors: Aksoy *et al.* (1998) recorded 1-2%, Hurka *et al.* (1989) recorded 3-12%, and Hurka and Neuffer (1997) recorded up to 20%.

In reviewing the literature, no data were found on the triploid "S" genotype (2n=3x=24) which is recorded for the first time in Egypt. Its rare presence within *C. bursa-pastoris* populations comparing with the other two genotypes (Amer *et al.*, 2019) may support its hybrid origin. The lack of chromosome no. 6 and 8 (aneuploidy, 2n=3x-2=22) in some individuals of this genotype supports our postulation. Bretagnolle and Thompson (1995) cleared that the triploid offspring are typically sterile due to problems in chromosomal pairing and segregation during meiosis, which may cause aneuploid gametes and result in sterility.

Karyotype analysis showed that the chromosomes are small in size, this result agrees with Schmidt and Bancroft (2011) who reported that mitotic chromosomes of crucifer species are generally very small in size. The karyotyping of the three genotypes (Fig. 3 & Table 2) showed distinctive variations within genotypes in the

genome and chromosomes mean relative lengths (MRL) in addition to the centromere position. Our results are supported by Guerra (2008) who claimed that the karyological data in taxonomy contribute to evaluate the genetic relationships among species or populations and lead to better understanding of the way they diverged from each other.

As reported by Amer *et al.* (2019), the "LS" populations were large in size, up to 80 cm length with inflorescence up to 75 cm, while "L" and "S" populations were small in size (up to 50 cm long with inflorescence up to 40 cm). These results indicate that the growth rate of tetraploid populations is greater than that of other genotypes, and agree with Neuffer's finding (1989) that the rate of growth was greater for the tetraploid groups. On the other hand, the small size of the diploid "L" and the triploid "S" genotypes can be explained by deletion of redundant genes which can result in downsizing of the genome, as reporteded early by Devos *et al.* (2002), Blanc and Wolfe (2004), Vitte and Bennetzen (2006).

The molecular results achieved by sequencing the cpSSR locus ATCP31017 of 14 specimens of *C. bursa-pastoris* genotypes support the cytogenetic results, where the neighbour-joining dendrogram (shown in Figure 5) separated the studied genotypes "L", "S", and "LS" into three distinctive genetic clusters. The first cluster included four specimens for the diploid "L" genotype. In this cluster, specimens L1 and L3 showed high genetic similarity with each other, this result may be reflected from high morphological similarity (both specimens had plant length up to 50 cm and inflorescence up to 40 cm and similar cauline leaves). The second cluster included four specimens for the triploid "S" genotype. In this cluster, specimens S5 and S7 showed high genetic similarity reflected from morphological similarities with each other (plant length up to 50 cm, its inflorescence up to 40 cm). Moreover, specimens S6 and S8 showed high genetic similarity as both samples had very small plant size (stem length was up to 20 cm, its inflorescence was up to 15 cm). The third cluster included six specimens for the tetraploid "LS" genotype. In this cluster, specimen LS9 had the lowest genetic similarity with the other specimens. This may be due to its very large size (stem length was up to 80 cm, its inflorescence was up to 75 cm) and presence of both simple and lobed cauline leaves on the same plant. On the other hand, specimens LS10 and LS12 were genetically similar (both specimens had large plant size and simple cauline leaves). Also, specimens LS13 and LS14 were genetically similar, as both specimens had small plant size and simple cauline leaves. The Lobed-Simple "LS" genotype with the highest phenotypic plasticity (as shown in Fig. 1), also showed high genetic diversity (Fig. 5). The position of the triploid "S" genotype in the obtained dendrogram is supporting our postulation that it is a hybrid between "LS" and "L" genotypes. Together these results provide important insights about the correlation of the phenotypic diversity within the Egyptian *C. bursa-pastoris* and the genetic diversity.

CONCLUSION

The phenotypic diversity within the Egyptian *C. bursa-pastoris* populations is genetically controlled. The three studied morphotypes represent distinct genotypes. The environmental factors have no effect on their phenoplasticity. The postulated hybrid (triploid genotype) may suffer from sterility and disappear in the near future.

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