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VARIATION OF KARYOTYPE FORMULA, AND ASYMMETRY BETWEEN INDIVIDUAL PLANTS OF GARLIC AND ITS RELATIONSHIP WITH *AC* TRANSPOSON

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ABSTRACT

To study the karyotypic variation between the roots generated from cloves and bulbs within one genotype and among two different garlic accessions (Baladi and Egaseed1), the karyotype formula and asymmetry estimated from chromosome criteria (short arm, long arm, total length, centromeric index, arm ratio and relative length) were analyzed. Almost all examined cells of the two studied genotypes showed 8 chromosome pairs (four large and four medium sized). In Baladi genotype, four categories of karyotypic formula (8m, 7m+ 1sm, 6m+ 2sm and 5m+ 3sm) could be determined. The former two (8m and 7m+ 1sm) were frequently predominant, while the other two categories (6m+ 2sm and 5m+ 3sm) were found in rare frequency. Data showed variation in karyotypic formula among different cells of the same clove as well as among the different cloves of the same bulb. Values of this parameter ranged from 52.22% to 58.26% and were evidently different among the three examined cells of the same clove as well as between the cloves and subsequently between bulbs of Baladi genotype. However, Egasseed1 showed three categories of karyotypic formula (8m, 7m+ 1sm and 6m+ 2sm). Karyotypic asymmetry indices of the examined cells in this genotype ranged from 54.91% to 58.64%. In addition, the number of satellite (SAT) bearing chromosomes varied from zero to three chromosomes. According to the existence and number of SAT chromosomes (chromosomes with secondary constrictions, SC) per genome of cloves and bulbs of the two studied genotypes, the examined cells have been divided into five forms (cells with zero, one, two, three

or four SC chromosomes). All five SC chromosome forms are found in cloves and bulbs of Baladi genotype but with different percentages. Data also showed considerable differences of the percentages of these forms within and between cloves and bulbs of the two studied genotypes. SC and SAT could be observed in all 8 chromosome pairs but with different numbers and distributions. Data obtained after conventional PCR amplification of *Ac* transposable element clearly showed the existence of common monomorphic base fragment (100 bp) in all examined 18 cloves of the two studied garlic genotypes (Baladi and Egaseed1). In addition, one polymorphic band of ~500 bp was detected in one clove from the first bulb and two cloves from the second bulb of Baladi genotype. Likewise, this band (~500 bp) has appeared in two cloves from the first bulb of Egaseed 1 genotype. The finding that there is additional 500 bp fragment of *Ac* transposable element in some cloves might be due to molecular relationship between autonomous and non-autonomous (truncated) transposition and may reflect the effect of transposition on chromosome structure and consequently on the karyotypic formula and asymmetry.

Keywords: Garlic, Karyotype, Secondary constrictions and Transposable element

INTRODUCTION

Allium is the largest genus of the family Amaryllidaceae which comprises more than 800 species of monocotyledonous perennial, mostly bulbous flowering and economically important plant species (Fritsch *et al.*, 2001 and Ricroch *et al.*, 2005; Anwar *et al*., 2017), including bulb onion (*Allium cepa,* 2n=2x=16), Japanese onion (*Allium fistulosum*, 2n=2x=16), leek (*A. porrum,* 2n=4x=32), and garlic (*A. sativum*, 2n=2x=16). Chromosome numbers are known for only about one-third of them and detailed cytological data are very limited (Ramesh, 2015). A single garlic accession frequently displays a high degree of phenotypic plasticity that is likely to be dependent upon soil type, moisture, and altitude (Fritsch and Friesen, 2002, Usman *et al.,* 2016 and Anwar *et al*., 2017). Karyotype of *A. sativum* comprises 16 chromosomes and could be, with difficulties, arranged into 8 pairs (Verma and Mittal, 1978; Elmamlouk *et al.*, 2002; Ata, 2005; Osman *et al*., 2007; Ata and Osman, 2009; Anwar and Ata, 2017 and Mahmoud *et al*., 2017).

The eight pairs of chromosomes tend to fall into three distinct groups, A) 5 "long" pairs, B) 2 satellited pairs, and C) the shortest pair. Examinations of the meiotic chromosomes pairing particularly those with nucleolar regions have clearly shown that several types of structural aberrations were *frequently* occurred during gametogenesis (Anwar and Ata, 2017). Surprisingly, almost all examined PMCs couldn't exhibit normal or regular chromosome

pairing (primary association of the eight chromosome pairs). In the same manner, Arisuryanti *et al.* (2018) detected two different karyotypic formula of the two local garlic clones (Tawangmangu Baru with 2n = 16 = 12m + 4sm and Bawang Jawa with 2n = 16 = 14m + 2sm) with similar diploid chromosome number (2n = 16). Karyotypic Differences attributed to chromosome size (the length of short (p) and long (q) arm) divergence between the two studied garlic clones.

On the other hand, heterochromatin is often composed of heterogeneous sequences, which are usually composed of transposable elements or tandem repeat arrays. The mobility of transposable elements may play an important role in garlic genome instability by means of point mutation and chromosomal changes induction (Lamb *et al.*, 2007). Moreover, several morphological, cytological and molecular analyses clearly supported the findings that genetic variation in garlic has arisen from accumulation of mutations resulted in clonal and/or varietal diversity. Indeed, genetic variation in garlic is found not only between clones but also between cloves derived from one head and/or bulb. This hypothesis has been argued cytologically (Helmy, 2020) and molecularly (Anwar *et al.*, 2020). Therefore, the analysis of karyotypic diversity using different chromosome criteria and sources of the chromosome variation among sister cells in the same tissue and between individual cloves of two garlic clones (Baladi and Egaseed1) is the main goal in the present study. It is extended to clarify the relationship between occurrence of chromosomal changes and existence of *Ac* transposable element.

MATERIALS AND METHODS

1. Plant materials

Two garlic (*Allium sativum* L.) genotype called Baladi or Egyptian (White bulbs) and Egaseed1 (purple bulbs) were kindly obtained from Seds Agriculture Research Station, Agriculture Research Center (ARC), Egypt. Three bulbs of each clone were selected and three individual cloves of each bulb were germinated. The root tips of each individual clove were separately collected. Three root tips of each clove were used for mitotic examination and karyotype analysis.

2. Mitotic preparations of root tips and karyotype analysis.

To study some mitotic parameters, the growing root tips (1-2 cm long) of each single garlic clove were collected and pretreated separately with 0.05% colchicines at room temperature for three hours. The colchicines-treated roots were immediately fixed with Farmer's solution (absolute Ethyl alcohol and Glacial acetic acid 3:1 v/v) for 24h and stored in 70% ethanol at 4ºC. Before cytological examination roots were hydrolyzed with 1 N HCl at 60ºC for six minutes then transferred to 70% ethanol. Acetocarmin-squashed preparations were made and the stained metaphase plates with well-spread chromosomes were chosen. Good metaphase spreads (At least 9 good metaphase plats/clove) were photographed microscopically

using CCD camera (Olympus C-4040). Chromosomes were measured under the microscope using the Soft Imaging System (SIS) program (Version 3.0) edited in 1999 by Soft Imaging System GmbH, Germany.

Chromosome measurements were analyzed using the software Micro-measure (Altınordu *et al.*, 2016). Karyotype Asymmetry (AsK) index was estimated according to the method reported by Altınordu *et al*. (2016) as shown in Table (1). Percentage of cells contained Sat-chromosomes at chromosome complement in the individual cloves of the two garlic clones was tested. Similarly, the percentage of cells with sat-chromosomes existing in different chromosome pairs was recorded.

Table (1): Karyological parameters and symbols

Short arm length	**S**
Long arm length	L
Basic chromosome number	x
Chromosome length	CL = L + S
Arm ratio	Ar = L/S
Relative length of chromosome	RL% = (CL/ΣCL) × 100
Centromeric index	CI% = S/(L + S)
Karyotype Asymmetry index	AsK% = Length of long arms in chromosome set / Total chromosome length in set X 100

3. Ac transposon detection

3. 1. Extraction of genomic DNA

DNA was isolated from 18 individual cloves (2 clones X three bulbs X three cloves) according to the method of (Anwar *et al.*, 2016). Cornell extraction buffer (500 mM NaCl; 100 mM Tris-HCl, pH 8.0; 50 mM EDTA and 0.84% SDS, equilibrated to 65ºC and mixed with 0.38 g sodium bisulfite/100 ml buffer before adjusting the pH to 7.8-8.0 with NaOH) was used. Concentration and purity of DNA were spectro-photometrically assessed according to Sambrook *et al.* (1989).

3. 2. PCR conditions

The amplification of DNA was carried out using *Ac* primer (*Ac*-1, 5'-GCCTCTACTGGCAAAACAAA-3' and *Ac*-2, 5'-GCTGCTACTGCCTACACT CTGG-3'). PCR reaction was performed in a final volume of 25µl containing 12.5µl 2X master mix [0.05 units/µl *Taq* DNA polymerase in 2X PCR buffer (4mM MgCl2 and 0.4mM of each 4dNTPs)], 10 µM of each primer and 1ng/µl of DNA template. The final reaction volume was completed to 25 µl using sterilized double distilled water. DNA amplifications were carried out in a thermal cycler (Thermo Hybaid) programmed for initial preheating period in one step of 5 min at 94º C; subsequent 30 cycles of 3 steps: DNA denaturation at 94º C for 45 sec, followed by primer annealing at 56 ºC for 45 sec and then primer extension at 72º C for 45 sec; subsequent the final cycle in one step of post

extension at 72ºC for 10 min. Amplified products were resolved by gel electrophoresis on 2% agarose gel, stained with Ethidium bromide (0.1g Ethidium bromide dissolved in 10 ml 1X TAE buffer) for 30 min, visualized on UV light and photographed using Gel Doc. (GBOX 230V). Size of DNA fragments were estimated by comparison with the standard marker of 100 bp ladder.

RESULTS

1. Variation of Karytypic formula between cells, cloves and bulbs of the two garlic genotypes

To study the karyotypic variation between the roots generated from cloves and bulbs within one genotype and among the two different garlic genotypes (Baladi and Egaseed1), the chromosome criteria (short arm, long arm, total length, centromeric index, arm ratio and relative length) of three random cells, obtained from three different roots within the same clove were analyzed using the software Micro-measure (Altınordu *et al.*, 2016). Generally, almost all examined cells of the two studied genotypes showed 8 chromosome pairs (four large and four medium size). Arranging them in gradient sizes were carried out after entering data of chromosome measurements of three random cells representing the three different roots within the same clove.

1.1. Karyotypic variation among cells of cloves and bulbs of Baladi genotype.

The karyotypic formula and asymmetry at metaphase cells of meristematic tissues that found at three levels (roots, cloves, bulbs of Baladi genotypes) are shown in Table (2). Four categories of karyotypic formula (8m, 7m+ 1sm, 6m+ 2sm and 5m+ 3sm) could be determined. Out of them, two (8m and 7m+ 1sm) were frequently predominant, while the other two categories (6m+ 2sm and 5m+ 3sm) were found in rare frequency. In fact, (8m) category might be found in other form (1M+7m). Similarly, such categories of these karyotypic formula (7m+1sm, 6m+2sm and 5m+3sm) were observed in different forms depending upon whether the chromosome numbering is meta- or sub-metacentrics in the karyotype. Karygrams of these categories and forms of all four karyotypic formula are represented in plate (1).

Data in Table (2) also exhibited clear differences in karyotypic formula among different cells of the same clove as well as among the different cloves of the same bulb. For instance, three karyotypic formulas (8m, 1M+ 6m+ 1sm and 5m+ 3sm) were found in clove number two of the first bulb at cells no. 1, 2 and 3, respectively. Two different karyotypic formulas (8m in cells no. 1 and 3 and 1M + 6m +1sm in cell no. 2) were also observed in clove number three of the same bulb.

Karyotypic asymmetry is a ratio between the total lengths of long arms in haploid set and total lengths of all chromosomes of haploid number indicating dominancy of either meta- or sub-metacentric. Values of this parameter ranged from 52.22% to 58.26% and were evidently different among the three examined cells in the

same clove as well as between the cloves and subsequently between bulbs of Baladi genotype (Table 2). Furthermore, data in Table (2) demonstrated that there are clear differences in the numbers and position of the satellited (SAT) chromosomes among different bulbs and cloves in the same bulb as well as among cells in the same clove. The number of SAT chromosomes varied from zero to three chromosomes. SAT bearing chromosomes were mostly no. 5, 6 and 7, while SC and SAT were rare in chromosomes 1, 2, 3, 4 and 8 (Table 2).

1.2. Karyotypic variation among cloves of the same bulb of Egaseed 1

Three categories of karyotypic formula (8m, 7m+ 1sm and 6m+ 2sm) were observed at metaphase cells of roots germinated from one bulb in Egaseed1 genotype, while only two categories (8m and 7m+ 1sm) could be detected in the other two bulbs as shown in Table (3). As rule, the representative karyograms are shown in Plate (1). The karyotype asymmetry indices of the examined cells in this clone ranged from 54.91% to 58.64% (Table 3). On the other hand, data in Table (3) showed clear alterations in the number and position of SAT chromosomes among the examined cells of the same clove, as well as among cloves within the same bulb and thus among different bulbs. The number of SAT chromosomes varied from zero to three chromosomes (Table 3). Detailed analysis of SAT chromosomes in cells, cloves and bulbs of two garlic genotypes will be explained in the following paragraphs.

2.1. Percentages of Secondary constrictions (SC chromosomes) bearing cells in cloves and bulbs of both Baladi and Egaseed1 genotypes

According to the existence and number of chromosomes with secondary constrictions (SC) per genome of cloves and bulbs of the two garlic genotypes (Baladi and Egaseed1), the examined cells have been divided into five forms (cells without or 0.0 SC chromosomes, with only one, with two, with three and with four SC chromosomes). Data in Table (4) showed the percentages of cells with these five different SC chromosome forms in cloves and bulbs of both Baladi and Egaseed1 genotypes. SC chromosome could be also called satellite (SAT) chromosomes. All five SC chromosome forms are found in cloves and bulbs of Baladi genotype but with different percentages. The fourth form (cells with three SC chromosomes) showed the highest percentage (33%) in Baladi, while the second form (cells with one SC chromosomes) was the highest (45.7%) in Egaseed1 genotype. It could be noted that the fifth form (cells with four SC chromosomes) were absolutely absent in Egaseed1 genotype. Data also showed a considerable difference of the percentages of these forms within and between cloves and bulbs of the two studied genotypes. Representative cells with different SC chromosome forms are shown in Fig. (1).

Table (2): karyotype formula, karyotypic **asymmetry (AsK) and SAT chromosomes of 27cells representing three cloves derived from three bulbs of garlic genotype (Baladi)**

Baladi Genotype		Bulb 1			Bulb 2			Bulb 3		
		Clove 1	Clove 2	Clove 3	Clove 1	Clove 2	Clove 3	Clove 1	Clove 2	Clove 3
	formula	7m + 1sm	8m	8m	7m + 1sm	7m + 1sm	1M + 7m	7m + 1sm	8m	8m
Cell 1	AsK	58.26%	58.26%	57.17%	55.60%	57.26%	52.22%	57.27%	55.26%	55.26%
	SAT	2 (No. 3,5)	2 (No. 3,7)	3 (No. 1,2,3)	-	1 (No. 6)	1 (No.6)	2 (No .5,6)	2 (No. 6,7)	2 (No. 6,7)
	formula	8m	1M + 6m +1sm	1M + 6m +1sm	1M + 7m	7m + 1sm	6m + 2sm	8m	8m	8m
Cell 2	AsK	57.88%	56.45%	55.42%	56.36%	55.16%	57.50%	55.26%	55.26%	55.26%
	SAT	2 (No. 3,7)	3 (No .5,6,8)	2 (No .5,7)	1 (No .3)	3 (No. 5,7,8)	1 (No .5)	2 (No. 6,7)	2 (No. 6,7)	2 (No .6,7)
	formula	7m + 1sm	5m + 3sm	8m	1M + 6m+ 1sm	8m	8m	8m	8m	7m + 1sm
Cell 3	AsK	56.01%	56.62%	54.86%	56.36%	56.37%	57.14%	55.26%	55.26%	55.26%
	SAT	2 (No. 6,7)	2 (No. 4,7)	2 (No. 3,5)	2 (No. 4,6)	1 (No. 5,7)	3 (No. 4,5,6)	2 (No. 6,7)	2 (No. 5,6)	2 (No. 6,7)

M = Metacentric with 1.00 arm ratio, m = metacentric with 1.10-1.20 and sm = sub-metacentric with 1.21-2.00
Formula = karyotypic formula, SAT =Satellite bearing chromosomes and AsK= a ratio between the total lengths of long arms in haploid set and total lengths of all chromosomes of haploid number indicating dominancy of either meta- or sub-metacentric.

Table (3): karyotype formula, karyotypic asymmetry (AsK) and SAT chromosomes of 27cells representing three cloves derived from three bulbs of garlic genotype (Egaseed1)

Egaseed1Genotype		Bulb 1			Bulb 2			Bulb 3		
		Clove 1	Clove 2	Clove 3	Clove 1	Clove 2	Clove 3	Clove 1	Clove 2	Clove 3
	formula	8m	7m + 1sm	6m + 2sm	8m	8m	7m + 1sm	7m + 1sm	7m + 1sm	8m
Cell 1	AsK	54.91%	56.98%	56.67%	56.93%	54.91%	56.43%	57.09%	56.75%	56.19%
	SAT	2 (No.5,6)	1 (No.5)	1 (No.7)	1 (No.3)	3 (No.4,7,8)	1 (No.5)	2 (No.2,6)	1 (No.4)	1 (No.4)
	formula	7m + 1sm	7m + 1sm	7m + 1sm	8m	7m + 1sm	8m	8m	7m + 1sm	7m + 1sm
Cell 2	AsK	56.11%	55.37%	58.28%	55.65%	58.64%	56.11%	56.17%	57.07%	56.79%
	SAT	1 (No.5)	1 (No.6)	2 (No.3)	-	2 (No. 2,7)	1 (No.7)	3 (No.4,5,8)	2 (No.4,7)	2 (No.3,6
	formula	8m	8m	7m + 1sm	8m	8m	8m	8m	7m + 1sm	8m
Cell 3	AsK	56.37%	57.12%	57.08%	55.49%	56.39%	56.12%	56.44%	55.58%	55.72%
	SAT	2 (No.2,8)	1(No.7)	2 (No.3,6)	1 (No.4)	3 (No.4,7,8)	1 (No.5)	3 (No.3,6,7)	2 (No.4,6)	2 (No.4,6)

M = Metacentric with 1.00 arm ratio, m = metacentric with 1.10-1.20 and sm = sub-metacentric with 1.21-2.00
Formula = karyotypic formula, SAT =Satellite bearing chromosomes and AsK= a ratio between the total lengths of long arms in haploid set and total lengths of all chromosomes of haploid number indicating dominancy of either meta- or sub-metacentric.

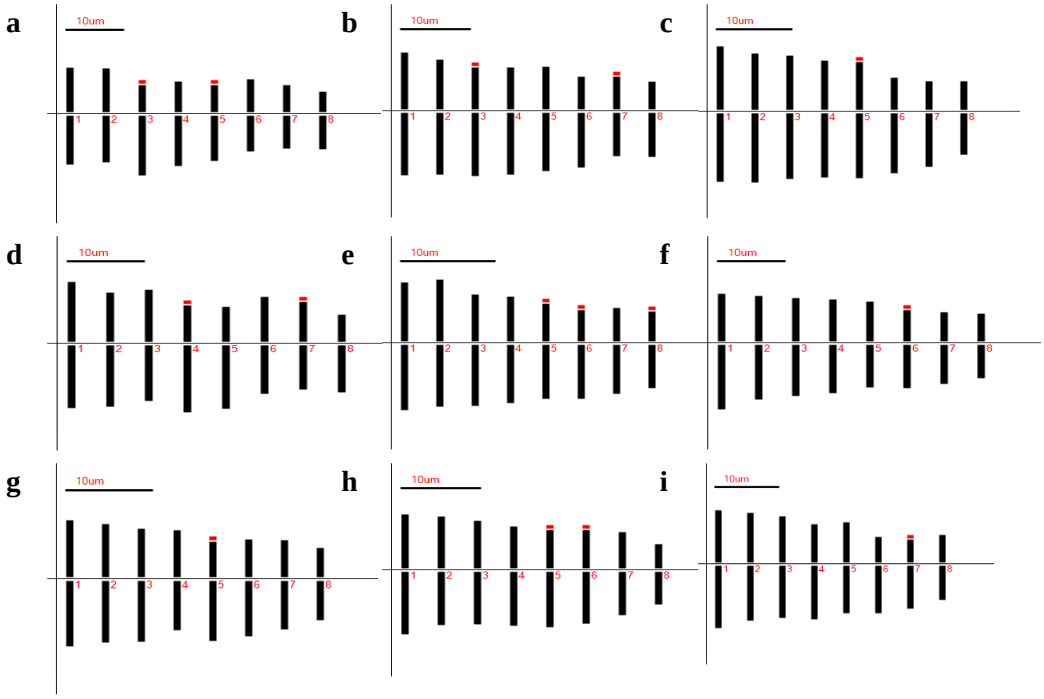

Plate (1): Karyograms showing the different categories of karyotypic formula in both Baladi and Egaseed1clone. a: 7m + 1sm, b: 8m, c: 6m + 2sm, d: 5m + 3sm, e: 1M + 6m +1sm f: 1M + 7m, g: 7m + 1sm, h: 8m and i: 6m + 2sm; bar= 10 microns.

Table (4): Percentage of five SC chromosome forms bearing cells of cloves and bulbs of both Baladi and Egaseed1 garlic genotypes.

Genotypes	Bulbs	Cloves	% SC chromosomes bearing cells				
			Without (0) SC chromosome	**With (One) SC chromosome**	**With (two) SC chromosomes**	**With (Three) SC chromosomes**	**With Four SC chromosomes**
Baladi	First bulb	Clove1	0.00	11.11	11.11	44.44	33.33
		Clove2	22.22	22.22	22.22	22.22	11.11
		Clove3	0.00	22.22	44.44	22.22	11.11
		Mean	7.41	18.52	25.93	29.63	18.52
	Second bulb	Clove1	11.11	44.44	0.00	22.22	22.22
		Clove2	11.11	11.11	33.33	44.44	0.00
		Clove3	11.11	22.22	0.00	55.56	11.11
		Mean	11.11	25.93	11.11	40.74	11.11
	Third bulb	Clove1	0.00	22.22	22.22	22.22	33.33
		Clove2	0.00	22.22	22.22	33.33	22.22
		Clove3	11.11	0.00	33.33	33.33	22.22
		Mean	3.70	14.81	25.93	29.63	25.93
	Mean of Clone Baladi		7.40	19.70	21.00	33.00	18.50
Egaseed1	First bulb	Clove1	0.00	11.11	55.56	33.33	0.00
		Clove2	0.00	100.00	0.00	0.00	0.00
		Clove3	0.00	44.44	44.44	11.11	0.00
		Mean	0.00	51.85	33.33	14.81	0.00
	Second bulb	Clove1	0.00	55.56	11.11	22.22	0.00
		Clove2	11.11	22.22	22.22	44.44	0.00
		Clove3	0.00	44.44	55.56	0.00	0.00
		Mean	3.70	40.74	29.63	22.22	0.00
	Third bulb	Clove1	0.00	11.11	55.56	33.33	0.00
		Clove2	22.22	55.56	22.22	0.00	0.00
		Clove3	11.11	66.67	22.22	0.00	0.00
		Mean	11.11	44.44	33.33	11.11	0.00
	Mean of Clone Egaseed1.		7.40	45.70	32.00	16.00	0.00

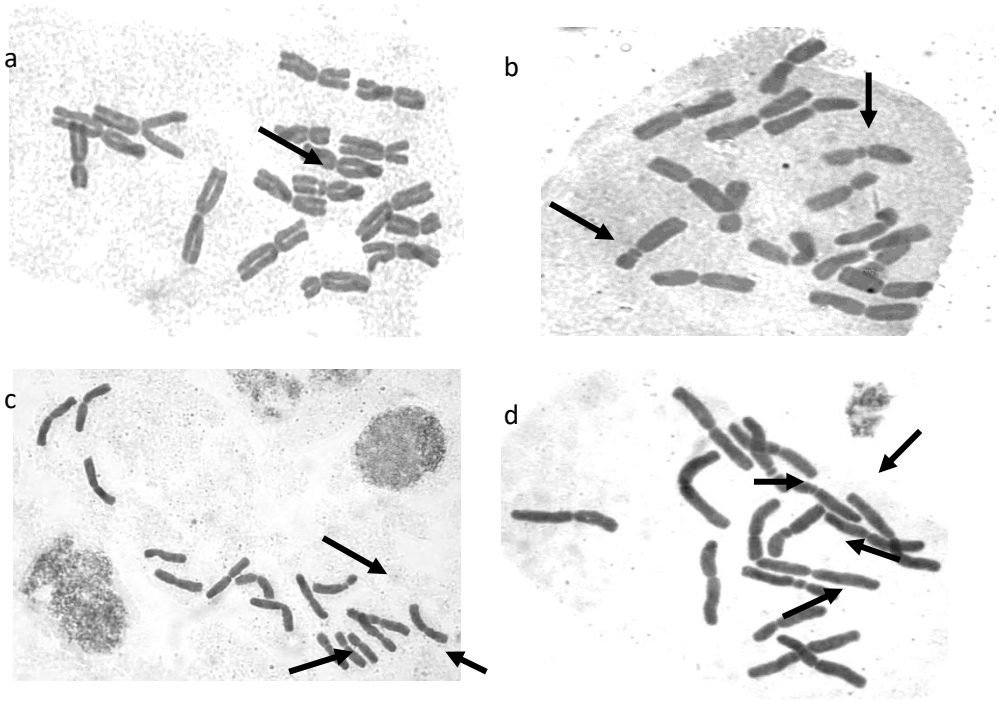

Fig. (1): metaphase cells of both Baladi and Egaseed1garlic genotypes showing four different forms of SC chromosomes a: with one SC chromosome (arrow) b: with two SC chromosomes (arrows), c: with three SC chromosomes (arrows), b: with four SC chromosomes (arrows)

2. 2. The number and distribution of SC and SAT on the chromosomes of cloves and bulbs of both Baladi and Egaseed1 genotypes.

Data in Table (5) showed the distribution of satellites (SAT) and secondary constrictions (SC) along with the eight chromosome pairs in cells from cloves and bulbs of both Baladi and Egaseed1 genotypes. It is difficult to determine which chromosome of the 8 pairs could be called as SAT chromosomes. SC and SAT could be observed in all 8 chromosome pairs but with different percentages. For instance, chromosome pair nos.5, 6 and 7 exhibited the highest percentages with SC and SAT, while chromosome pair nos.1 and 8 showed lowest percentages in both Baladi and Egaseed1 genotypes. Sometimes, chromosome nos.3 and 4 exhibited a high percentage of SC and SAT as those found in cloves of bulb 2 and 3 in Egaseed1 genotype. These data reflected the variation of SC and SAT positions on the eight chromosomes even between sister cells and within cloves derived from the same bulb.

Table (5): Distribution of satellites (SAT) and secondary constrictions (SC) along with the eight chromosome pairs in cells from cloves and bulbs of both Baladi and Egaseed1 genotypes.

Genotype	Clove and bulbs	Chromosome number							
		1	2	3	4	5	6	7	8
Baladi	Clove 1	**7.70**	**0.00**	**7.70**	**7.70**	**11.50**	**26.90**	**26.90**	**11.50**
	Clove2	0.00	0.00	12.50	6.25	18.75	31.25	18.75	12.50
	Clove 3	10.53	5.26	26.32	10.53	31.58	10.53	5.26	0.00
	Mean of bulb 1	6.08	1.75	15.51	8.16	20.61	22.89	16.97	8.00
	Clove 1	0.00	0.00	5.56	27.78	16.67	27.78	22.22	0.00
	Clove2	0.00	0.00	5.26	5.26	15.79	26.32	31.58	15.79
	Clove 3	4.76	4.76	9.52	19.05	14.29	33.33	9.52	4.76
	Mean of bulb 2	1.59	1.59	6.78	17.36	15.58	29.14	21.11	6.85
	Clove 1	8.70	4.30	0.00	8.70	21.74	21.74	30.43	4.35
	Clove2	0.00	4.17	0.00	25.00	33.33	20.83	16.67	0.00
	Clove 3	0.00	13.04	0.00	4.35	17.39	30.43	30.43	4.35
	Mean of bulb 3	2.90	7.17	0.00	12.68	24.15	24.33	25.84	2.90
	Mean of Baladi genotype	**3.52**	**3.50**	**7.43**	**12.73**	**20.11**	**25.45**	**21.31**	**5.92**
Egaseed1	Clove 1	10.00	5.00	5.00	5.00	35.00	10.00	5.00	25.00
	Clove2	0.00	11.11	11.11	0.00	22.22	22.22	33.33	0.00
	Clove 3	0.00	0.00	33.33	20.00	20.00	0.00	20.00	6.67
	Mean of bulb 1	3.33	5.37	16.48	8.33	25.74	10.74	19.44	10.56
	Clove 1	0.00	0.00	23.08	7.69	23.08	23.08	7.69	15.38
	Clove2	0.00	5.56	5.56	33.33	5.56	5.56	27.78	16.67
	Clove 3	0.00	0.00	7.14	7.14	42.86	14.29	21.43	7.14
	Mean of bulb 2	0.00	1.85	11.93	16.05	23.83	14.31	18.97	13.06
	Clove 1	0.00	5.00	15.00	10.00	25.00	25.00	15.00	5.00
	Clove2	0.00	0.00	11.11	44.44	11.11	22.22	11.11	0.00
	Clove 3	0.00	0.00	11.11	22.22	33.33	33.33	0.00	0.00
	Mean of bulb 3	0.00	1.67	12.41	25.55	23.15	26.85	8.70	1.67
	Mean of Egaseed1 genotype	**1.11**	**2.96**	**13.61**	**16.64**	**24.24**	**17.30**	**15.70**	**8.43**

3. Molecular analysis via AC element

Data obtained from the agarose gel after conventional PCR amplification of *Ac* transposable element clearly showed the existence of common monomorphic base fragment (100 bp) in all examined 18 cloves of the two studied garlic genotypes (Baladi and Egaseed1). In addition, one polymorphic band of ~500 bp was detected in clove 3 (from the first bulb) and cloves 5 and 6 (from the second bulb) of Baladi genotype. Likewise, this band (~500 bp) has appeared in cloves 10 and 11 clove (from the first bulb) of Egaseed 1 genotype (Fig. 2).

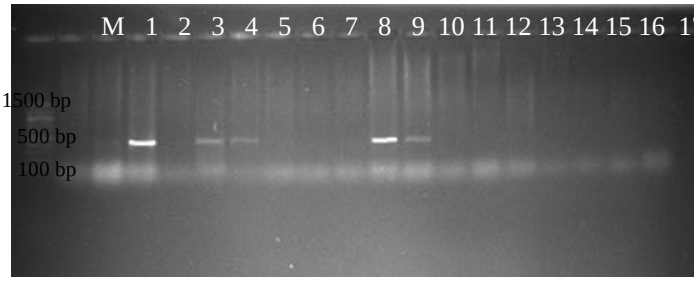

Figure (2): Electrophoretic pattern of PCR amplification product using *Ac* primer. M: ladder, 1- 9: individual cloves of Baladi clone (3 cloves/bulb), 10-18: individual cloves of Egaseed 1 clone (3 cloves/bulb),

DISCUSSION

The diversity of karyotype formula, karyotype asymmetry, percentages of secondary constrictions and SAT bearing chromosomes among sister cells in same tissue and between individual cloves of two garlic clones (Baladi and Egaseed1) is analyzed in the present work. Data reported herein assured that the variable karyotypic formula and asymmetry were recorded not only between genotypes but also within and between the cloves and the bulbs of the same genotypes. Four categories of karyotypic formula (8m, 7m+1sm, 6m+2sm and 5m+3sm) were found in the two studied genotypes called Baladi and Egaseed1. Out of them Two (8m and 7m+1sm) were predominantly appeared, while the other two (6m+2sm and 5m+3sm) were found in rare frequencies. Likewise, variation of values of karyotype asymmetry (which indicate to the predominance of either metacentrics or submetacentrics) within and between cloves and bulbs of garlic genotypes assured the changes of karyotypic formula. In spite of using the conventional chromosome staining, the applying of fine and digital micro-photography with recent karyogram analysis might allow to detect the minute differences of karyotypic formula and asymmetry within and between cloves and bulbs of the same genotype. The hypothesis that accumulation of somatic chromosome mutations (particularly those of structural aberrations) caused

alterations of chromosome morphology (that might be quantified in terms of karyotype formula and asymmetry) between garlic genotypes was previously accepted (Bozzini and De Luca, 1991; Elmamlouk *et al*., 2002; Yüzbaşioğlu and Unal, 2004; Ata, 2005; Osman *et al*., 2007; Ata and Osman, 2009; Ata *et al*., 2010; Mukherjee and Roy, 2012; Ramesh, 2015; Anwar and Ata, 2017; Mahmoud *et al.,* 2017 and Helmy, 2020). However, the interpretation of the given variation in karyotype formula and karyotypic asymmetry within and between cloves and bulbs in this study is still problematic. Increasing asymmetry can arise either through the move of centromere position (from medium/sub-medium to terminal of subterminal) or through the accumulation of changes in the relative size between the chromosomes of the complement. More confusion is occurring when data of SC and SAT chromosome were considered. It was known that mitotic cells of garlic plants have two SAT or SC chromosome pairs (no.6 and 7). Otherwise, the examined cells even within the same root exhibited five different SC or SAT chromosome forms with different percentages and approximately distributed in all chromosome pair. These data is in accordance with those previously reported (Elmamlouk *et al*., 2002; Yüzbaşioğlu and Unal, 2004; Ata, 2005; Osman *et al*., 2007; Ata and Osman, 2009; Anwar and Ata, 2017; Mahmoud *et al.,* 2017 and Helmy, 2020).

Data reported herein are in accordance with those of Anwar and Ata (2017) and Helmy (2020) in which they also determined four different nucleoli attached-chromosomes (pair nos. 5, 6, 7 and 8) during meiotic drive. In addition, Anwar and Ata (2017) reported that these four chromosomes pairs have associated in a chain or ring configurations during meiotic MI. it could be noted that these aberrations caused the changes in the position and size of these satellites along with chromosomes nos,5, 6, 7 and 8. The secondary constrictions may be involved in the nucleolus organization; hence the intra and inter-individual variation in number and size of secondary constrictions could be due to the shifting of nucleolar organizer in the chromosome arm brought by deletion and unequal translocation or inversion (Verma and Raina, 1981). These structural aberrations have been recognized to various mechanisms such as transposon-mediated transposition events and chromosomal rearrangements, such as locus duplication/deletion (Altinkut *et al.*, 2006; Datson and Murray, 2006 and Raskina *et al.,* 2008).

In the present work, 100 bp monomorphhic fragment of *Ac* transposable element is found in almost all examined cloves, while a fragment of about 500 bp appeared in some cloves and absent in others. These data are in agreement with those reported of Helmy and Anwar (2018), in which they confirmed the changes of karyotypic formula within and between the same examined garlic cloves. Recently, Molecular variation within and between cloves

and bulbs in garlic genotypes were also reported (Anwar *et al*., 2020). The finding that there is additional 500 bp fragment of Ac element in some cloves might be due to molecular relationship between autonomous and non-autonomous ((truncated) transposition (Ata, 1994).

These data may reflect the effect of transposition on chromosome structure and consequently on the karyotypic formula and asymmetry (Zhang and Peterson, 1999). Large deletions and inverted duplications could be generated via transposition reactions involving Ac/Ds termini located on sister chromatids [sister-chrom transposition (SCT)]. Reinsertion of the excised transposon ends into the chromatid bridge generates structurally altered sister chromatids containing a reciprocal deletion and inverted duplication. Examined cells in this study showed changes of karyotypic formula and asymmetry which may reveal the effect of transposons on cohesion complexes of mitotic chromosomes. Sister chromatids are held together by multisubunit complexes called cohesions. Disruption of cohesion can lead to genome instability, such as aneuploidy, defects in DNA repair, and chromosomal translocations (Brooker and Berkowiz, 2014). Mutations in cohesins have also been shown to result in an increased distance between sister centromeres (Brooker and Berkowiz, 2014). Likewise, Ac transposition may play an important role in garlic genome instability through induction of point mutation and chromosomal changes induction.

CONCLUSION

Data obtained in the present study revealed, the presence of many changes in garlic karyotypic formula (including chromosome length, arm ratios, centromeric index and so on) even between individual sister cells in the same tissue. Current study also sheds light on the source and clarification of these variations. Additionally, more studies are required at the molecular cytogenetical level using FISH technique to detect satellite movement. Also, more transposon families and their molecular sequences should be studied on garlic to explain the main sources of garlic karyotypic variations.

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الملخص العربي

تباين معادلات الطرز الكروموسومية وعدم التناسق بين النباتات الفردية للثوم وعلاقتها بالترانسبوزون Ac

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لقد تم تحليل الإختلاف فى الطرز الكروموسومى بين الجذور المستنبتة من الفصوص والبصيلات داخل الطرز الوراثي الواحد وبين طرزين مختلفين من الثوم (بلدي وإيجاسيد 1)، وذلك بحساب معادلات عدم التناسق ورصد الأشكال المتعددة للطرز الكروموسومى والمقاسة بالمعايير الكروموسومية المعروفة وهي طول الأذرع الكروموسومية والطول الكلى لكل كروموسوم على حده ومعامل الأذرع الكروموسومية ومعامل السنترومير. أظهرت جميع الخلايا التى درست فى سلالتى الثوم والتي تم فحصها أنها تحتوي على 8 أزواج كروموسومية (أربعة كبيرة وأربعة متوسطة الحجم). وفى السلالة البلدى أمكن تحديد أربع فئات من أشكال الطرز الكروموسومية (8 وسطى السنترومير، 7 وسطى السنترومير + 1 تحت وسطى السنترومير، 6 وسطى السنترومير + 2 تحت وسطى السنترومير، 5 وسطى السنترومير + 3 تحت وسطى السنترومير). كانت الفئتين الأوليين (8 وسطى السنترومير، 7 وسطى السنترومير + 1 تحت وسطى السنترومير) متواجدتين بتكرار أكبر في أحيان كثيرة، بينما ظهرت الفئتين الأخريين (6 وسطى السنترومير + 2 تحت وسطى السنترومير، 5 وسطى السنترومير + 3 تحت وسطى السنترومير) بنسب نادرة. وأظهرت النتائج تباينًا في معامل الطرز الكروموسومية بين الخلايا المختلفة من نفس الفص وكذلك بين الفصوص المختلفة من نفس البصيلة. تراوحت قيم هذا المعامل من 52.22٪ إلى 58.26٪ واختلفت بشكل واضح بين الخلايا الثلاث المفحوصة من نفس الفص وكذلك بين الفصوص وبالتالي بين البصيلات من التركيب الوراثي البلدي. ومع ذلك، أظهر التركيب الوراثي إيجاسيد 1 ثلاث فئات فقط من الصيغ الكروموسومية (8 وسطى السنترومير، 7 وسطى السنترومير + 1 تحت وسطى السنترومير، 6 وسطى السنترومير + 2 تحت وسطى السنترومير). تراوحت مؤشرات عدم تناسق الطرز الكروموسومية للخلايا التي تم فحصها في هذا التركيب الوراثي من 54.91٪ إلى 58.64٪. بالإضافة إلى ذلك، كان هناك تفاوت في عدد الكروموسومات الحاملة للحزوز الثانوية ومنطقة التابع ما بين صفر إلى ثلاث كروموسومات. وفقًا لوجود وعدد الكروموسومات الحاملة لمنطقة التابع (الكروموسومات ذات الحزوز الثانوية) لكل جينوم من الفصوص والبصيلات للسلالتين تحت الدراسة، ولقد تم تقسيم الخلايا المفحوصة إلى خمسة أشكال: خلايا ذات صفر، واحد، اثنان، ثلاثة أو أربع كروموسومات محتوية على تابع. ولقد تم العثور على جميع الأشكال الخمسة في فصوص وبصيلات السلالة البلدي وإيجاسيد 1 ولكن بنسب مختلفة، ولقد أمكن ملاحظة الحزوز الثانوية ومناطق التابع في جميع أزواج الكروموسومات الثمانية

ولكن بأرقام وتوزيعات مختلفة. وأظهرت البيانات التي تم الحصول عليها بعد كلونة المادة الوراثية (DNA) الخاصة بالعنصر المتحرك (Ac) من خلال تفاعل البلمرة المتسلسل (PCR) وجود حزمة واحدة من الـ DNA يبلغ حجمها 100 زوج من القواعد في جميع الفصوص الثمانية عشر المستخدمة من الطرزين الوراثيين المدروسين من الثوم (بلدي وإيجاسيد 1). وبالإضافة إلى ذلك، تم العثور على حزمة واحدة من حوالي 500 زوج من القواعد في فص واحد من البصلة الأولى واثنين من البصلة الثانية من التركيب الوراثي البلدي. وبالمثل، ظهرت هذه الحزمة في فصين من البصلة الأولى من الطرز الوراثي إيجاسيد 1. وقد يكون اكتشاف وجود حزمة إضافية بمقدار 500 زوج من القواعد للعنصر المتنقل ***Ac*** في بعض الفصوص ناتجًا عن العلاقات الجزيئية بين العناصر المتحركة الكاملة والناقصة وقد ينعكس تأثير هذا النوع من الحركة الجينية على بنية الكروموسومات وبالتالي على الصيغ الكروموسومية وعدم التناسق.