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SOMACLONAL VARIATION IN BREAD WHEAT (TRITICUM AESTIVUM L.). V. MEIOTIC BEHAVIOR OF SOME GAMETOCLONES AND SOMACLONES

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ABSTRACT

The meiotic behavior of pollen mother cells (PMCs) in plants of the 3rd and 4th selfed generations of 11 wheat gametoclones in addition to 11th and 12th selfed generations of 11 wheat somaclones were studied in the present work as well as their 4 parental cultivars. Significant and non-significant differences in the percentage of total abnormal PMCs in both 3rd and 4th generations were found between the two parental cultivars Sids 5 and Giza 163 and their gametoclones. The highest percentage (35.71%) of total abnormal PMCs in the 3rd generation was found in DHS5.12 gametoclone, while the lowest percentage (0.72%) was found in the parent (Sids 5). The results also showed that, there were significant and non-significant differences in this respect between the somaclones and their parents (Sakha 8 and Sakha 69). The highest percentage (8.39%) of total abnormal cells in the 11th generation was found in S69.5 somaclone, while the lowest percentage (0.53%) was found in S69.4 somaclone in the 12th generation. Amongst all tested genotypes, S69.4 somaclone was more stable as it had the lowest value (0.53%) of total abnormal PMCs. Meiotic chromosome pairing studies showed that, the formation of 18.7 ring + 2.3 rod bivalents was the most common type in the PMCs of parental cultivars, whereas the formation of 18.78 ring + 2.22 rod bivalents was the most common type in the tested gametoclones and somaclones. Generally, somaclones at advanced generations (11th and

12th) were more cytologically stable than early generations (3rd and 4th) of gametoclones.

Key words: Meiotic behavior, Somaclones, Gametoclones, bread wheat (*Triticum aestivum* L.), Abnormal, Chiasma frequency

INTRODUCTION

Wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD) is the most widely grown cereal crop in the world. In Egypt, wheat is one of the oldest and most important cereal crops (Al-Naggar *et al.* 2015). The term somaclonal variation was first used by Larkin and Scowcroft (1981) to describe the induced changes in plants regenerated from *in vitro* cultured somatic tissues. While the term gametoclonal variation is describing variation in plants generated from *in vitro* gametes culture (Evans *et al.*, 1984, Morrison and Evans, 1987 and Karp, 1994).

Somaclonal variation is an important phenomenon that can be observed at varying levels in plant tissue culture in both dicot (Brar and Jain, 1998) and monocot families (Cheng *et al.*, 1992 and Chang *et al.*, 2003). It Somaclonal variation is attributed to pre-existing or *in vitro* induced genetic variation in somatic cells (Evans *et al.*, 1984). Lapitan *et al.*, (1984) found a high degree of chromosome structural changes in wheat by rye hybrids regenerated from tissue culture.

Haploid plant formation through androgenesis has been applied to several cereals and other crops such as, wheat (Simmonds *et al.*, 1993, Doghma, 2007, El-Hennawy *et al.*, 2011 and Al-Naggar *et al.* 2015), barley (Finnie *et al.*, 1991, Luckett and Smithard, 1992 and Munoz *et al.*, 2004), triticale (Charmet and Bernard, 1984), rice (Miah *et al.*, 1985), maize (Petolino and Thompson, 1987 and Bentolila *et al.*, 1992) and potato (Sarkar *et al.*, 2010).

A wider significance of chromosome variation in culture became apparent when plant regeneration was achieved from cultured cells and the regenerated plants were not true-to-type, as expected for asexual reproduction, but was subject to somaclonal variation (Larkin and Scowcroft, 1981). Frequently encountered types of somaclonal variation result from changes in chromosome number (aneuploidy, polyploidy or mixoploidy), abnormal structural changes of chromosomes (deletion, addition, transposition or inversion), single-gene mutations (Larkin and Scowcroft, 1981 and Lörz *et al.*, 1988) and DNA methylation (Kaeppler *et al.*, 2000).

The aim of the present investigation was to study the meiotic behavior of PMCs in bread wheat gametoclone and somaclone plants (the 3rd and 4th selfed generations of 11 wheat gametoclones and 11th and 12th selfed generations of 11 wheat somaclones) comparing with its parental cultivars.

MATERIALS AND METHODS

The present study was carried out at the Experimental Farm and Cytological Lab. of Dep. of Genetics, Faculty of Agriculture, Minia University during the two seasons 2012/2013 and 2013/2014.

Plant material

Seeds of 4 original commercial Egyptian bread wheat (*Triticum aestivum* L.) cultivars (Sids 5, Giza 163, Sakha 8 and Sakha 69) are used in this study together with their 22 *in vitro*-derived genotypes (11 gametoclones and 11 somaclones, Table 1) which were obtained from Minia Gene Bank, Genetics Department, Faculty of Agriculture, Minia University, El-Minia, Egypt to whom our thanks are due. Seeds at R3 of gametoclones and R11 of somaclones were planted in the Experimental Farm of Genetics Department, Faculty of Agriculture, Minia University during the winter seasons 2012/2013 and R4, R12 generations were planted at 2013/2014 with the 4 parental wheat cultivars as control in a randomized complete block design with three replicates.

Table (1): Original spring bread wheat (*Triticum aestivum* L.) cultivars and their driveled gametoclones and somaclones which used in this study.

Original cultivar	Gametoclones	Original cultivar	Somaclones
Sids 5	DHS5.1*, DHS5.4, DHS5.6, DHS5.9 and DHS5.12	Sakha 8	S8.2***, S8.4, S8.5, S8.10, S8.16 and S8.18
Giza 163	DHG163.2**, DHG163.3, DHG163.5, DHG163.8, DHG163.11 and DHG163.13	Sakha 69	S69.1****, S69.2, S69.3, S69.4 and S69.5

*DHS5 = Double haploid genotype derived from Sids 5 cultivar; **DHG163 = double haploid genotype derived from Giza 163 cultivar; ***S8 = Somaclone genotype derived from Sakha 8 cultivar;
****S69 = Somaclone genotype derived from Sakha 69 cultivar.

Sample collection

The spikes from at least 3-5 plants from each replicate at an appropriate stage of development were collected at 7-9 o'clock morning and fixed in fresh Farmer's liquid of 95% ethanol and glacial acetic acid (3:1, v/v) for a minimum of 24 hrs. and stored in 70% alcohol at 6°C until microscopic examination.

Cytological observation

For meiotic chromosome analysis at metaphase I and II, anaphase I and II, telophase I and II and tetrad stages slides were prepared by smearing the 3 anthers from a single floret, or in some cases from a single anther which were further macerated on the glass slid in a drop of aceto-carmine stain (1%). The total number of pollen mother cells (PMCs) analyzed had ranged from 350

to 450 per genotype. The percentages of abnormal cells i.e. showing different types of meiotic aberration including laggard chromosomes, micronuclei, outside chromosomes /chromatin and stickiness were calculated on the basis of means for each genotype.

Chiasma frequency

Chiasma frequencies (least chiasma number / cell) were determined from at least 30-40 PMCs per genotype at diakinesis, by counting the number of rod (one chiasma) and ring (two chiasma) bivalents.

Data Analyses

Data were analyzed using Statistical Package for the Social Sciences program (SPSS), SPSS (1990).

RESULTS AND DISCUSSION

Many types of chromosomal aberrations in PMCs of gameto-and somaclones such as chromosome fragment, stickiness, bridge, laggard, micronuclei and outside chromosome were observed in diferent meiotic stags in both generations of all tested genotypes (Plate 1).

Meiotic behavior of the gametoclones and somaclones

1- Gametoclones

The percentage of total abnormal PMCs in the two advanced generations 3rd "R3" and 4th "R4" generations of 5 gametoclones (DHS5.1, DHS5.4, DHS5.6, DHS5.9 and DHS5.12) which were obtained from the parental cultivar Sids 5 are showen in Table 2. The results showed that, there were significant differences in the percentage of total abnormal PMCs in both 3rd and 4th generations between Sids 5 (the parent cultivar) and all of its gametoclones, except gametoclones DHS5.1 and DHS5.6 in the 4th generation. The highest percentage of total abnormalities was found in gametoclone DHS5.12, i.e. 35.71 % and 7.33% in the R3 and R4 generations respectively, while the lowest percentage, 1.45% and 0.72%, were found in the parent Sids 5 in the two examined seasons, respectively.

Significant differences were also recorded between the parent cultivar (Sids 5) and all of its gametoclones in the percentage of cells with laggard, outside, stickiness, bridge, fragmented chromosome or micronuclei, except gametoclone DHS5.1 which showed non-significant differences in all chromosomal aberrations except outside chromosome.

Table (3) shows the percentage of abnormal PMCs in the R3 and R4 generations of five different gametoclones (DHG163.2, DHG163.3 DHG163.5, DHG163.8, DHG163.11 and DHG163.13) which were obtained from the parental cultivar Giza 163. The data showed that, there were significant differences in the percentage of abnormal PMCs in the 3rd generation between Giza 163 (2.64%) and all of its gametoclones, and also within gametoclones. Also significant differences were observed in the 4th generation between gametoclones comparing with the parental cultivar Giza 163, while non-

significant differences were found within gametoclones except DHG163.2 which was significantly different with all gametoclones.

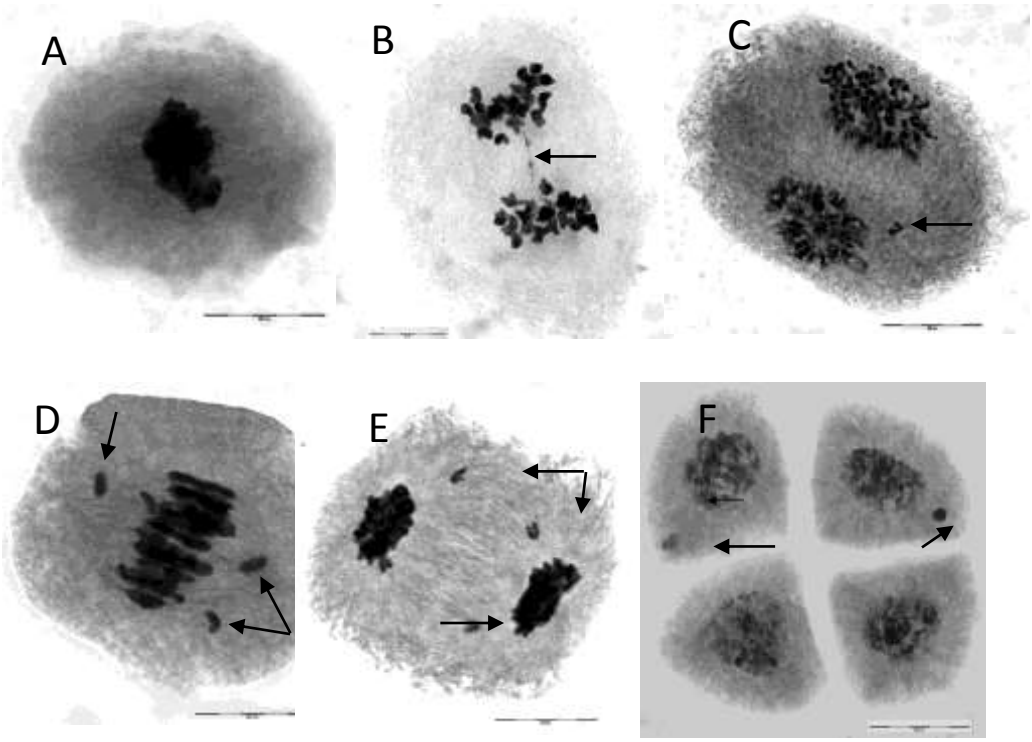

Plate (1): Meiotic aberrations in bread wheat (*Triticum aestivum* L.) cultivars, gametoclones and somaclones. A: sticky metaphase I in S69.3 somaclone; B: anaphase I with bridge (arrowed) in DHG163.2 gametoclone; C: telophase I with fragment (arrowed) in DHS5.9 gametoclone; D: metaphase I with three outside chromosomes (arrowed) in Sakha 69 cultivar; E: telophase I with three lagging chromosome (arrowed) in DHG163.13 gametoclone; F: tetrad with two micronuclei (arrowed) in DHS5.12 gametoclone.

Table (2) Percentage of PMCs showing meiotic chromosomal aberrations in five bread wheat gametoclones and their parental cultivar Sids 5 during season 2012-2013 (3rd generation) and season 2013-2014 (4th generation).

Generation	Genotype	Total tested cells	Lag* %	Outside* %	Stiknes %	Bridge %	Fragment %	Micro.* %	Total abnormal %
R3*	Sids 5	411	0.49	0.25	0.23	0.49	0.00	0.00	1.45
	DHS5.1	419	0.96	3.10	0.00	0.00	0.00	0.00	4.06
	DHS5.4	414	0.72	0.97	0.49	1.94	0.48	0.00	4.60
	DHS5.6	429	8.45	6.99	0.00	0.00	2.37	0.00	17.76
	DHS5.9	385	9.62	6.75	0.00	0.00	1.83	1.05	19.24
	DHS5.12	418	14.39	3.58	0.00	0.00	0.00	17.74	35.71
	LSD	-	1.34	1.03	0.42	0.45	0.67	0.90	2.27
R4*	Sids 5	418	0.25	0.00	0.24	0.23	0.00	0.00	0.72
	DHS5.1	407	0.99	1.47	0.00	0.24	0.00	0.00	2.70
	DHS5.4	367	3.97	0.84	0.00	0.00	0.00	0.00	4.82
	DHS5.6	394	1.09	0.53	0.82	0.00	0.00	0.00	2.44
	DHS5.9	372	1.88	1.88	0.00	0.00	0.00	0.00	3.77
	DHS5.12	396	2.02	1.78	0.00	0.00	0.00	3.53	7.33
	LSD	-	1.09	0.61	0.30	0.42	0.00	0.26	1.23

*R3= 3rd generation, R4= 4th generation, lag. = laggerd chromosome/ chromatide, Outside = outside chromosome/ chromatide and Micro. = micronuclei.

Table (3) Percentage of PMCs showing different meiotic chromosomal aberrations in six gametoclones and their parental cultivar Giza 163 during season 2012-2013 (3^{rd} generation) and season 2013-2014 (4^{th} generation).

Generation	Genotype	Total tested cells	Lag* %	Outside* %	Stiknes %	Bridge %	Fragment %	Micro.* %	Total abnormal %
R3*	Giza 163	418	1.20	1.19	0.00	0.24	0.00	0.00	2.64
	DHG163.2	425	4.23	6.83	0.00	0.47	0.00	0.00	11.53
	DHG163.3	372	6.51	7.57	0.00	0.00	1.36	1.35	16.79
	DHG163.5	357	3.37	1.95	0.00	0.00	0.00	0.00	5.33
	DHG163.8	424	5.44	5.67	0.00	0.25	0.46	2.61	13.99
	DHG163.11	407	2.95	2.95	0.00	0.25	0.00	0.49	6.64
	DHG163.13	389	9.78	3.09	0.00	0.00	0.25	0.00	13.11
	LSD	-	1.22	0.94	0.00	0.56	0.52	0.72	2.39
R4*	Giza 163	375	1.06	0.80	0.00	0.00	0.00	0.00	1.86
	DHG163.2	426	1.64	3.28	1.64	0.00	0.00	0.00	6.56
	DHG163.3	373	1.33	1.86	0.00	0.00	0.00	0.00	3.20
	DHG163.5	371	0.81	1.62	0.00	0.54	0.00	0.00	2.97
	DHG163.8	358	1.41	1.39	0.00	0.00	0.00	0.00	2.80
	DHG163.11	368	1.10	1.90	0.00	0.27	0.00	0.00	3.26
	DHG163.13	365	1.11	2.18	0.00	0.00	0.00	0.00	3.29
	LSD	-	0.77	0.60	0.25	0.43	0.00	0.00	0.84

*R3= 3^{rd} generation, R4= 4^{th} génération, lag. = laggerd chromosome/ chromatide, Outside = outside chromosome/ chromatide and Micro. = micronuclei.

2- Somaclones

Table (4) shows the percentage of total abnormal PMCs in two advanced generations (R11 and R12) of six different somaclones (S8.2, S8.4, S8.5, S8.10, S8.16 and S8.18) and their parental cultivar Sakha 8. The data showed that, there were significant differences in the percentage of total abnormal PMCs in the 11th generation between the parental cultivar and all of its somaclones except S8.16 somaclone. Also significant differences were found between the tested somaclones. Whereas in the 12th generation non-significant differences were observed in percentage of total abnormal PMCs between the parental cultivar and its somaclones, except S8.5 and S8.10 somaclones, and non-significant differences within most of somaclones of this generation.

The highest percentage value (6.46%) of total abnormal PMCs in the 11th generation was found in S8.2 somaclone, while the highest value (4.23%) in the 12th generation was found in S8.10 somaclone. However, the parental cultivar (Sakha 8) had the lower percentage 1.85 and 1.64% of total PMCs abnormalities in both seasons, respectively, indicating that it was more stable than its somaclones.

Table (5) shows the percentage of total abnormal PMCs of five bread wheat somaclones derived from Sakha 69 cultivar (S69.1, S69.2, S69.3, S69.4 and S69.5) as well as the parental cultivar in two advanced generations R11 and R12 during season 2012-2013 and 2013-2014, respectively. The data shows non-significant differences in total abnormal PMCs between the parent and all of its somaclones in 11th and 12th generations except somaclones S69.3 and S69.5 at both generations.

The higher percentage of total abnormal PMCs was found in the somaclone S69.5 in both 11th and 12th generations (8.39 and 5.25%, respectively). While the lowest percentage of total abnormal PMCs was observed in the somaclone S69.4 (0.85% and 0.53%) in both generations, respectively. Therefore, genotype S69.4 can be considered the best cytological tested genotype among all 26 cultivars and *in vitro* derived clones. The present our results showed that, somaclones at advanced generation (11th and 12th) were more cytological stable than gametoclones at early generations (3rd and 4th).

Our results were in a good agreement with Mortada (2014) who found significant differences in total abnormal PMCs between some wheat (*Triticum aestivum,* L.) cultivars and their somaclones. In plant tissue cultures and its derivatives, the whole range of genetic changes may occur, from point mutations to chromosome aberrations, in addition to epigenetic i.e. nonheritable variation (Karp and Bright, 1985). It has been shown however that somaclonal and gametoclonal variation could be induced by chromosomal changes and/or point mutations (D'Amato, 1977, Bayliss, 1980, Pring *et al.*, 1981, Karp and Bright 1985, Lee and Phillips 1988, Karp 1989 and Logue 1996).

Table (4) Percentage of PMCs showing different meiotic chromosomal aberrations in six bread wheat somaclones as well as their parental cultivar Sakha 8 during season 2012-2013 (11^{th} generation) and season 2013-2014 (12^{th} generation)

Generation	Genotype	Total tested cells	Lag* %	Outside* %	Stiknes %	Bridge %	Fragment %	Micro.* %	Total abnormal %
R11*	Sakha 8	434	1.38	0.47	0.00	0.00	0.00	0.00	1.85
	S8.2	381	1.69	3.09	1.40	0.29	0.00	0.00	6.46
	S8.4	445	1.12	0.91	0.65	0.21	0.24	0.24	3.36
	S8.5	405	1.30	2.61	0.00	0.51	0.00	0.00	4.42
	S8.10	410	2.69	2.67	0.00	0.49	0.00	0.00	5.86
	S8.16	386	0.74	1.23	0.00	0.25	0.00	0.00	2.23
	S8.18	356	1.80	0.00	0.00	0.78	0.24	0.00	2.82
	LSD	-	0.52	0.78	0.53	0.65	0.38	0.26	0.93
R12*	Sakha 8	427	1.17	0.47	0.00	0.00	0.00	0.00	1.64
	S8.2	360	1.38	0.55	0.00	0.00	0.00	0.00	1.66
	S8.4	387	0.78	1.80	0.00	0.00	0.00	0.00	2.58
	S8.5	359	1.40	1.95	0.00	0.00	0.00	0.00	3.34
	S8.10	401	2.74	1.25	0.00	0.00	0.00	0.24	4.23
	S8.16	368	1.09	1.09	0.00	0.00	0.00	0.00	2.18
	S8.18	396	1.26	0.76	0.25	0.00	0.00	0.00	2.27
	LSD	-	0.71	0.72	0.29	0.00	0.00	0.28	0.94

*R11= 11^{th} generation, R12= 12^{th} generation, lag. = laggerd chromosome/ chromatide, Outside = outside chromosome/ chromatide and Micro. = micronuclei.

Table (5) Percentage of PMCs showing different meiotic chromosomal aberrations in five bread wheat somaclones and their parental cultivar Sakha 69 during season 2012-2013 (11^{th} generation) and season 2013-2014 (12^{th} generation).

Generation	Genotype	Total tested cells	Lag %	Outside %	Stiknes %	Bridge %	Fragment %	Micro. %	Total abnormal %
R11	Sakha 69	354	0.00	0.58	0.29	0.56	0.00	0.00	1.43
	S69.1	371	1.06	0.52	0.00	0.29	0.00	0.00	1.87
	S69.2	407	0.74	0.48	0.00	0.00	0.00	0.00	1.22
	S69.3	352	1.41	1.44	0.85	0.27	0.30	0.00	4.27
	S69.4	355	0.85	0.00	0.00	0.00	0.00	0.00	0.85
	S69.5	381	1.83	5.25	0.00	0.00	0.00	1.31	8.39
	LSD	-	0.51	0.76	0.36	0.61	0.38	0.33	0.79
R12	Sakha 69	373	0.00	0.53	0.28	0.26	0.00	0.00	1.07
	S69.1	370	0.81	0.53	0.00	0.28	0.00	0.00	1.62
	S69.2	379	0.53	0.26	0.00	0.00	0.00	0.00	0.79
	S69.3	370	1.34	1.90	0.00	0.28	0.00	0.00	3.52
	S69.4	368	0.27	0.27	0.00	0.00	0.00	0.00	0.53
	S69.5	382	2.87	1.85	0.00	0.00	0.00	0.53	5.25
	LSD	-	0.63	0.86	0.35	0.59	0.00	0.33	0.95

*R11= 11^{th} generation, R12= 12^{th} génération, lag. = laggerd chromosome/ chromatide, Outside = outside chromosome/ chromatide and Micro. = micronuclei.

It has been shown that, primary cytological changes observed among regenerated plants and their progeny include both chromosome rearrangements and changes in chromosome number (Logue 1996 and Kaeppler *et al.,* 2000). The observed chromosomal aberrations were chromosome laggard, bridges, fragments, stickiness and outside chromosome. Similar kinds of meiotic chromosomal aberrations were observed in the regenerated wheat plants (Karp and Maddock, 1984, Ahloowalia and Sherington, 1985, Youssef *et al.,* 1989, Whelan 1990, Shang and Wong 1991, Ata *et al.,* 1998 and Chrzastek 2003).

It has also been shown in wheat that, although chromosomal abnormalities were found in somaclonal plants of all lines, there were some differences in the nature and frequency of the abnormalities among the studied lines, the higher frequency of chromosomal abnormalities in the most somaclone progenies as compared to the parental cultivars (control) implies that structural change in the affected chromosomes had occurred during the tissue culture process and had persisted through several selfed generations (Ata *et al.,* 1998).

Laggards and outside chromosomes represented the most common types of mitotic and meiotic abnormalities. The induction of laggard could be attributed to the failure of the spindle apparatus to organize and function in a normal way rather than inhibition of these spindle fibers and this may lead to irregular orientation of chromosomes (Grant 1978, Mansour 1984 and Patil and Bahat 1992), The bridges may result from chromosome stickiness (Abraham and Koshy 1979 and Badr 1983). Due to such stickiness the separation of daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus they remain connected by chromatin bridges (Kabarity *et al.,* 1974).

Bridges may also result from breakage of chromosomes followed by proximal chromatid reunion, which evidently results in dicentric chromosomes (Tomkins and Grant 1972 and Grant, 1978). The stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross-linkage of chromoproteins (Kong and Ma 1999), this led to subchromatid connections between chromosomes and thus they remained connected by bridges (McGill *et al.,* 1974, Klasterska *et al.,* 1976 and Badr *et al.,* 1992). Micronuclei are true mutagenic aspects and lead to a loss of genetic material. This mutagenic effect was estimated as a percentage of micronuclei formed in interphase (Ronchi *et al.,* 1986). It could originate from precocious ascension at the metaphase, or from laggards at the anaphase, while chromosome segments of broken bridges can also form micronuclei (Masoumeh *et al.,* 2010). Similar meiotic chromosomal aberrations (chromosome laggard, bridges, fragments, stickiness,

micronuclei, and outside chromosome) were also observed by many other works in different regenerated wheat plants (Karp and Maddock, 1984, Ahloowalia and Sherington, 1985, Youssef *et al.*, 1989, Whelan, 1990, Shang and Wong, 1991, Ata *et al.*, 1998 and Chrzastek 2003).

Chiasma frequency in gameto- and somaclones

The mean chiasma frequency per cell and the mean number of ring bivalents per cell of the four parental cultivars (Sids 5 and Giza 163, Sakha 8 and Sakha 69), 11 gametoclones and 11 somaclones were shown in Tables (6, 7, 8 and 9). The chiasma frequency was examined in pollen mother cells (PMCs) at diakinesis stage. The results showed that, the regular number of bivalents in each cell were 21 bivalent (Plate 2 A, B and C) while, Fig D showed stickiness in bivalents. Most of observed bivalents were of the ring shape and few bivalents were of the rod shape.

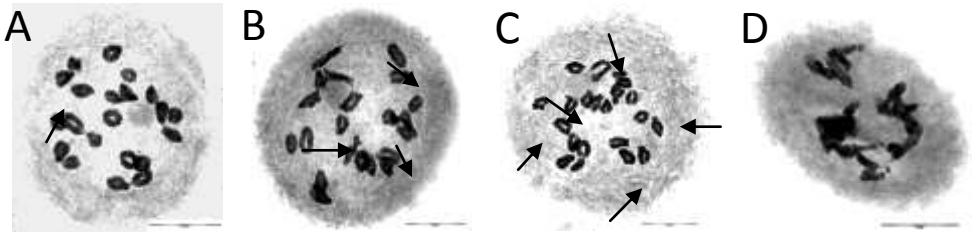

Plate (2): Different figures of diakinesis of tested bread wheat (*Triticum aestivum* L.) genotypes (2n= 42). A: PMCs at normal diakinesis showing 21 bivalents = 20 ring + 1 rod (arrowed) in S69.3 somaclone; B: normal diakinesis showing 21 bivalents = 18 ring + 3 rod (arrowed) in DHS5.6 gametoclone; C: normal diakinesis showing 21 bivalents = 16 ring + 5 rod (arrowed) in S69.3 somaclone; D: sticky chromosomes in PMCs at diakinesis in DHG163.2 gametoclone.

Gametoclones

Table (6) shows significant variances in the mean number of chiasma frequency, ring and rod bivalents per cell, between the parent cultivar Sids 5 and its gametoclones in both seasons (2012/2013 and 2013/2014) except mean number of chiasma frequency per cell in the 4th generation. The highest numbers of chiasma frequency and ring bivalents per cell in the 3rd generation (40.19) and (19.19), respectively were observed in DHS5.12 gametoclone, while the lowest number of chiasma frequency (39.13) and ring bivalent per cell (18.13) were found in gametoclone DHS5.9. Whereas in the 4th generation the higher mean number of chiasma frequency and ring bivalent were found in DHS5.12 gametoclone 40.24 and 19.24, respectively, the lowest mean number of chiasma frequency (39.21) and ring bivalent per

cell (18. 21) were found in gametoclone DHS5.9.

The mean number of chiasma frequency of Giza163 and its 6 gametoclones as well as the mean number of ring bivalents and mean number of rod bivalent were shown in Table (7). Significant differences were found between the parent cultivar and its gametoclones and also within most tested gametoclones in the mean number of chiasma frequency per cell in both 3rd and 4th generation. The highest mean number of chiasma per cell was observed in DHG163.5 genotype 40.18 and 40.05 in both generations, respectively, while the lowest mean was found in DHG163.13 genotype 39.1 and 39.36 in both 3rd and 4th generations, respectively.

On the other side, the mean number of ring bivalents shows significant differences between the parent and its gametoclones and also within the tested gametoclones. The highest mean ring bivalents number was found in genotype DHG163.5 (19.18 and 19.05) in both 3rd and 4th generations, respectively, while the lowest number of ring bivalents per cell was found in genotype DHG163.13 (18.1 and 18.36) in the 3rd and 4th generations, respectively.

Somaclones

The data of Table (8) showed that, there were non-significant differences in the mean number of chiasma frequency per cell between the parental cultivar Sakha 8 and its 6 somaclones except genotype S8.5 which has significant differences with the parent cultivar in the 11th generation. Genotype S8.5 showed the highest mean number of chiasma frequency (40) and ring bivalents (19) in the 11th generation, while the lowest means number of chiasma frequency and ring bivalents (39.43 and 18.43) were found in genotype S8.16, respectively. Whereas in the 12th generation, non-significant differences were found in the mean number of chiasma frequency per cell between the parental cultivar Sakha 8 and its somaclones. However, the highest chiasma frequency mean number (40.42) and ring bivalents (19.42) was observed in somaclone S8.2, while the lowest mean number of chiasma frequency (39.38) and ring bivalent (18.38) was found in somaclone S8.18.

The data of Table (9) shows significant differences between the parent and its 5 somaclones in the mean numbers of ring bivalents and chiasma frequency per cell in both generations except somaclone S69.1 in the 11th generation and somaclone S69.1 and S69.2 in the 12th generation. The highest numbers of ring bivalents and chiasma frequency per cell in the 11th and 12th generations were found in genotype S8.5 and S69.2, respectively. While the lowest numbers of ring bivalents and chiasma frequency per cell in the 11th and 12th generations were found in somaclone S69.2 and the parent cultivar (Sakha 69) respectively.

Table (6): Mean numbers of ring, rod bivalents and chiasma frequency per cell in the parent cultivar Sids 5 and its 5 gametoclones in the 3rd and 4th generations.

Generation	3rd generation			4th generation		
Genotype	Ring	Rod	Chiasma Freq.	Ring	Rod	Chiasma Freq.
Sids 5	18.82	2.18	39.82	18.72	2.28	39.72
DHS5.1	18.91	2.09	39.92	18.80	2.20	39.80
DHS5.4	18.57	2.43	39.57	18.25	2.75	39.25
DHS5.6	19.12	1.88	40.12	19.14	1.86	40.14
DHS5.9	18.13	2.87	39.13	18.21	2.79	39.21
DHS5.12	19.19	1.81	40.19	19.24	1.76	40.24
LSD	0.22	0.22	0.22	0.18	0.18	1.71

Table (7): Mean numbers of ring, rod bivalents and chiasma frequency per cell in the parent cultivar Giza 163 and its 6 gametoclones in the 3rd and 4th generations.

Generation	3rd generation			4th generation		
Genotype	Ring	Rod	Chiasma Freq.	Ring	Rod	Chiasma Freq.
Giza 163	18.54	2.46	39.54	18.82	2.18	39.82
DHG163.2	19.00	2.00	40.00	18.61	2.39	39.61
DHG163.3	18.66	2.34	39.66	18.67	2.33	39.67
DHG163.5	19.18	1.82	40.18	19.05	1.95	40.05
DHG163.8	18.81	2.19	39.81	18.83	2.17	39.83
DHG163.11	18.72	2.28	39.72	18.72	2.28	39.72
DHG163.13	18.1	2.9	39.1	18.36	2.78	39.36
LSD	0.22	0.22	0.22	0.34	0.28	0.34

Table (8): Mean numbers of ring, rod bivalents and chiasma frequency per cell in the parent cultivar Sakha 8 and its 6 somaclones in the 11th and 12th generations.

Generation	11th generation			12th generation		
Genotype	Ring	Rod	Chiasma Freq.	Ring	Rod	Chiasma Freq.
Sakha 8	18.62	2.38	39.62	18.75	2.25	39.75
S8.2	18.93	2.07	39.93	19.42	1.58	40.42
S8.4	18.65	2.35	39.65	18.60	2.40	39.60
S8.5	19.00	2.00	40.00	18.75	2.25	39.75
S8.10	18.8	2.2	39.80	18.87	2.13	39.87
S8.16	18.43	2.57	39.43	18.83	2.17	39.83
S8.18	18.90	2.10	39.90	18.38	2.25	39.38
LSD	0.33	0.33	0.33	0.44	0.41	0.43

Table (9): Mean numbers of ring, rod bivalents and chiasma frequency per cell in the parent cultivar Sakha 69 and its 5 somaclones in the 11th and 12th generations.

Generation	11th generation			12th generation		
Genotype	Ring	Rod	Chiasma Freq.	Ring	Rod	Chiasma Freq.
Sakha 69	18.69	2.31	39.69	18.61	2.39	39.61
S 69.1	18.96	2.04	39.96	19.07	1.93	40.07
S 69.2	18.57	2.56	39.57	19.05	1.95	40.05
S 69.3	18.94	2.06	39.94	18.67	2.33	39.67
S 69.4	18.96	2.04	39.96	18.77	2.23	39.77
S 69.5	19.00	2.00	40.00	18.81	2.19	39.81
LSD	0.24	0.29	0.24	0.28	0.26	0.28

Meiotic chromosome pairing data showed that, the form (18.7 ring bivalents + 2.3 rod bivalents) is the most common types in the PMCs of parental cultivars, whereas the form (18.78 ring bivalents + 2.22 rod bivalents) was the highly observed in their gametoclones and somaclones genotypes. The number of chiasmata/cell and types of chromosome pairing in wheat and its allies were early studied by several workers (Molnar-Lang *et al.*, 1991 and Chrzastek, 2003). Data reported herein exhibited some similarities and dissimilarities with those of Naranjo and Lacadena (1980) and Stefanowska (1988). Our results were similar to the result of Mortada, (2014) and Stefanowska, (1988) who found significant and non-significant differences in mean chiasma frequency between the tested genotypes.

Chiasma frequency per cell was significantly increased or decreased depending upon the studied wheat lines. A clear example given by Chrzastek, (2003) who observed differences in mean number of chiasma/cell between additional and substitutional wheat lines. Numerous studies have revealed that heterochromatin negatively affects the formation and terminalization of chiasmata (Thomas and Kaltsikes, 1976; Roupakias and Kaltsikes, 1977; Schlegel, 1979; Naranjo and Lacadena, 1980 and Attia and Lelley, 1987).

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الملخص العربي

التباينات الجسدية في قمح الخبز 5- السلوك الميوزي لبعض السلالات الجاميطية والجسدية

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تم دراسة السلوك الميوزي للخلايا الامية المولدة للجاميطات لنباتات الجيل الثالث والرابع لـ 11 سلالة جاميطية والجيل الحادي عشر والثاني عشر من التلقيح الذاتي لـ 11 سلالة جسدية لقمح الخبز (*Triticum aestivum* L.) بالاضافة الى الاربعة آباء التجارية المصرية المنتجة لهم (سدس 5 ، جيزة 163، سخا 8 و سخا 69). وقد اظهرت النتائج أن هناك اختلافات معنوية وغير معنوية في النسبة المئوية للتغيرات الكروموسومية الكلية بين التراكيب الوراثية المختلفة (الاباء الاربعة و السلالات الجاميطية والجسدية الناتجة منهم) فى كلا من الجيلين الثالث والرابع (للسلالات الجاميطية) والجيلين الحادي عشر والثاني عشر (للسلالات الجسدية)، وأظهرت السلالة الجاميطية DHS5.12 اعلى قيمة (35.71%) في التغيرات الكروموسومية الكلية فى الجيل الثالث بينما اظهر الاب سدس 5 أقل قيمة (0.72%) للتغيرات الكروموسومية الكلية بين السلالات الجاميطية وابائهم في الموسم الزراعي الثاني 2014/2013م. أما بالنسبة للسلالات الجسدية فأظهرت السلالة S69.5 اعلى قيمة (8.39%) للتغيرات الكروموسومية الكلية فى الجيل الحادي عشر بينما اظهرت السلالة الجسدية S69.4 أقل قيمة (0.53%) لها فى الجيل الثاني عشر. وكانت السلالة الجسدية S69.4 هي أفضل الطرز الوراثية التى تم دراستها متفوقة على الاباء الاربعة وكذلك الـ 21 سلالة الاخرى حيث اظهرت أقل معدل من التغيرات الكروموسومية (0.53%). واظهرت دراسة تزاوج الكروموسومات خلال الطور التشنتي والاستوائي الاول أن متوسط عدد الوحدات الثنائية في الوضع الحلقي Ring الى الوضع العصوي Rod كانت في الاباء الاربعة بمعدل 18.7 الى 2.3 على الترتيب، بينما كانت أعلى نسبة للشكل الحلقي الي الشكل العصوي هي 18.78 الى 2.22. وقد لوحظ ان السلالات الجسدية وهي المتقدمة في العمر كانت اكثر ثباتا عن السلالات الجاميطية الاصغر عمرا، وربما يعود هذا لكون السلالات الجسدية في أجيال متقدمة عن السلالات الجاميطية.