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CYTOLOGICAL STABILITY OF R_2 GENERATION OF EGYPTIAN BREAD WHEAT (*TRITICUM AESTIVUM* L.) GAMETOCLONES

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ABSTRACT

In this study, plants of six Egyptian bread wheat (*Triticum aestivum* L.) commercial cultivars (i.e. Giza139, Giza163, Giza164, Giza165, Sids2 and Sids5) and their 16 R_2 derived gametoclones were meiotically analyzed to identify gametoclonal variation and check their stability. Data showed that all of the examined pollen mother cells (PMCs) in plants of all studied genotypes (six wheat cultivars and their 16 derived R_2 gametoclones) were diploid (2n = 6X = 42 chromosomes) and possess 21 chromosome bivalent units. At diakinesis, most of these bivalents are ring-shaped and few were in rod-shaped. Two or three satellite-chromosome bivalents were also clearly observed. Statistical analysis using LSD value clearly showed that there were significant differences of mean values of chiasma frequency per cell and per chromosome between plants of parental cultivars and their derived gametoclones. Chiasma frequencies per cell and per chromosome of gametoclone S5-39 were the highest values (54.7 and 2.6, respectively), whereas the donor parent plants of variety Sids5 had the lowest values (35.0 and 1.67, respectively).

It was clearly observed that, R_2 plants of gametoclone G164-25 were cytologically instable because they had the highest percentage (53.79%) of chromosomal abnormalities when compared with those of the almost all studied genotypes, while G165-26 has the lowest abnormalities (12.33%) and were cytologically more stable. Chromosome lagging, fragment, bridges and outside chromosomes as well as micronuclei and microcytes were the common types of abnormalities observed in tested gametoclones. The continuity of cytological stability through advanced generation of wheat gametoclones is discussed.

Keywords: Chiasma Frequency; Chromosomes; Egyptian wheat Cultivars; Gametoclonal Variation; Genetic Stability; Meiotic analysis; Wheat (*Triticum aestivum* L.).

INTRODUCTION

Wheat is the most important human diet worldwide and it is one of the major staple foods in world. In Egypt, wheat is one of the oldest and most important cereal crops (Ahmed, 1996), but unfortunately, Egypt is one of the largest importers of wheat (FAO, 2017). The genetic improvement of wheat has received considerable attention from plant breeders with purpose of increasing the grain yield, and minimizing crop loss due to unfavorable environmental conditions and attack by various pests and pathogens. Producing new varieties which have highly productive using modern biotechnological techniques in plant breeding, could contribute, to a great extent, in the induction of novel genetic variation, which are not existed in the gene pool (Khan *et al.,* 2014). The development of *in vitro* technologies has complemented the conventional methods of wheat breeding in generating genetic variability necessary for creating novel cultivars with desirable characters (Ahmed and Sagi, 1993).

The variation induced through plant tissue culture (gametoclonal and somaclonal variation) offers an opportunity to broaden the genetic variation of crops. Many workers reported that a wide range of plant characters could be altered as a result of plant regeneration from cell and tissue cultures (Karp, 1994; Vasil, 1994). However, genetic stability of regenerated plants (clones) are out most important issue forwarding using these clones in plant breeding programs.

The term gametoclonal variation is describing heritable genetic variation observed among and within regenerated plants from *in vitro* culture of haploid germ cell (gametes), and has been applied to several cereals and other crops such as, wheat (Simmonds *et al.,* 1993; Karp**,** 1994; Doghma, 2007; El-Hennawy *et al.,* 2011**;** Al-Naggar *et al.,* 2015**).** Another culture is one of the most

common methods used to produce haploids both in dicotyledonous and monocotyledonous plants (Yildirim *et al.*, 2008). Cytological characterizations of gametoclonal variation in bread wheat to determine the genetic stability of the produced gametoclone lines were carried out by many research group (Ahmed *et al.*, 1997, 1998, 1999; Gordeeva *et al.*, 2009). Chromosome behavior and aberrations at somatic and germ tissues of plants of wheat parent cultivars and successive generations of its gametoclones were the most common test (D'Amato, 1977; Karp, 1994; Ahmed *et al.*, 1999).

Gordeeva *et al.* (2009) showed that in the cytologically stable wheat lines, chromosome configuration at the MI stage of meiosis was mostly bivalent (21II) with small proportion of defect cells (almost 10%), which at most contained two univalent (20II + 2I). Cytologically unstable plants contained high proportions of PMCs with abnormal chromosome pairing as well as the cells with multivalents, and aneuploid. Likewise, Youssef *et al*. (1989) evaluated of 14 anther-derived doubled-haploid hexaploid wheat lines after at least three generations of selfing. There were no deviations from the hexaploid chromosome number (2n = 42) in root tips. Meiotic chromosome pairing was as stable as that in the control (Centurk) in most progenies. Chromosomal structural changes and (or) behavioral deviations were detected at the metaphase I, anaphase I, telophase I, and quartet stages of meiosis in a minor proportion of the cells. The frequencies of multivalents, lagging bivalents and univalents, bridges, and micronuclei were higher in some progenies than in the control. Chromosomal fragments were infrequent. Rezaei *et al.* (2010) analysed PMCs in plants of some wheat gametoclones derived from tetra and hexaploid wheat and explained the chromosomal behaviour and irregularities. An *et al.* (2013) studied the chromosome meiotic behavior of PMCs of the hybrid progenies from *Triticum timopheevi* x *Avena fatua* L. var. glabrata Pat. The results showed that the PMCs meiotic index of the F3 line was 87.46 indicating some genetic instability.

In Egypt, the first report described and evaluated the androgenetic capacity of some Egyptian wheat genotypes for embryoid induction and for plant regeneration was carried out by Ali and Gouda (1992), but remarkable success was recoded with Ahmed *et al.* (1996, 1997, 1998, 1999). Therefore, assessing gametoclonal variation cytologically at the PMCs in plants of six wheat cultivars (Giza139, Giza163, Giza164, Giza165, Sids2 and Sids5) and their 22 derived R_2 gametoclones was the main goals of this study.

MATERIALS AND METHODS

Plant material

The current studies followed anther culture program of Genetics Department,

Faculty of Agriculture, Minia University (Ahmed *et al.*, 1996) and used materials (16 gametoclones) herein were kindly obtained from Minia Gene Bank, Genetics Dept., Fac. Agric., Minia Univ. Those 16 gametoclones were obtained via regeneration of plants from callus derived from anther culture of 6 commercial Egyptian hexaploid spring bread wheat (*Triticum asetivum* L. cvs. Giza 139, Giza 163, Giza 164, Giza165, Sids 2 and Sids 5; Ali, 1998).

Gametoclones was derived from a single R_0 regenerated plants. The notation mentioned by Chaleff (1981) was adopted, where the tissue culture-regeneration plant is termed the R_0 generation and subsequent generations of selfed progeny are R_1, R_2, R_3, R,….etc. Grains of six Egyptian spring hexaploid wheat cultivars and their 16-regenerated derived gametoclones (R_2) were used as shown in Table (1).

Table (1**)**: Six Egyptian spring bread wheat cultivars ((*Triticum asetivum* L.) and their 16 derived R_2 gametoclones versus each cultivars used in meiotically analysis.

cultivars	symbol	Number of derived tested gametoclones	Code of tested gametoclones
Giza 139	G139	2	G139-1, G139-2
Giza 163	G163	3	G163-4, G163-8, G163-13
Giza 164	G164	3	G164-23, G164-24, G164-25
Giza 165	G165	3	G165-26, G165-27, G165-28
Sids 2	S2	3	S2-29, S2-30, S2-32
Sids 5	S5	2	S5-39, S5-41

Meiotic preparation

For meiotic chromosome analysis at metaphase I, II, anaphase I, II, telophase I and tetrad stages, the spikes from at least 3 plants from each of the 3 replicates of randomized complete block design at an appropriate stage of development were collected and immediately fixed with fresh Farmer's solution (3 ethyl alcohol: 1 acetic acid) for a minimum of 24 hrs. at 5°C and stored in 70% alcohol at 5°C until microscopic examination. Primary micro-sporocytes (pollen mother cells "PMCs") were prepared from the fixed anthers and stained with aceto-carmine. Temporary squash preparations of pollen mother cells were made in one percent aceto-carmine. Slides were prepared by smearing the 3 anthers from a single floret and macerated on a glass slide in a drop of aceto-carmine stain.

At least 100 diakinesis were examined for estimating and calculating chiasma frequencies according to the method of **Ata *et al.* (2003)** depending on the bivalent shape either rod or ring. Moreover, hundreds of PMCs were

scored at different stages to determine uni- or bivalents outside the equatorial plate at metaphase I, or metaphase II., laggard chromosomes at ana-telophase I, or ana-telophase II. The rest of R_2 gametoclones plants were allowed to produce seeds. Cytological data of R_2 plants were obtained using the above described methods. Data were statically analyzed (in RCBD) using MSTAT program (Version 4). PMCs were examined and photographed using an Olympus BX 51 microscope at initial magnification of X 2000. Photomicrographs of suitable meiotic cells were taken for illustration using C-4040 digital camera through the microscope eyepiece.

RESULTS AND DISCUSSION

In this study, plants of six hexaploidy spring bread wheat cultivars (*Triticum aestivum* L.) and their 16 of R_2 derived gametoclones were meiotically analyzed to identify gametoclonal variation for cytologically viewpoint. All of the examined PMCs in plants of six wheat cultivars (Giza139, Giza163, Giza164, Giza165, Sids2 and Sids5) and their derived 16 R_2 gametoclones were diploid (2n = 6X = 42) and possessed 21 chromosome bivalent units. At diakinesis, most of these bivalents are ring-shaped and few were in rod-shaped as shown in Fig.1 (a, b and c). Two or three satellite chromosome bivalents were also clearly observed.

Chiasma frequency:

Means of chiasma frequencies per cell and chromosome at diakinesis of 6 parental cultivars and their selected 14 gametoclones are shown in Table (2). Statistical analysis using LSD test clearly showed that there were significant differences of mean values of chiasma frequency per cell and per chromosome between plants of parental cultivars and their derived gametoclones. For instance, chiasma frequencies per cell and per chromosome of gametoclone G139-1 (40.00 and 1.90, respectively) were significantly lower than those of its donor parental plants (44.00 and 2.10). While, chiasma frequencies per cell and per chromosome of gametoclone S5-39 was highest (54.7 and 2.6) than its donor parent plants of variety Sids5 which had the lowest values (35.0 and 1.67) among all tested 20 genotypes. We must mention that the gametoclone S5-39 had the highest and significant value of the chiasma frequencies per cell and per chromosome compare with all other 19 tested genotypes (6 parents and 13 gametoclones).

Meiotic aberrations:

Frequencies and types of chromosomal aberrations of six wheat cultivars (G139, G163, G164, G165, Sids2 and Sids5) and their 16 R_2 gametoclones (i.e. G139-1, G139-2, G163-4, G163-8, G163-13, G164-23, G164-24, G164-25, G165-26, G165-27, G165-28, S2-29, S2-30, S2-32, S5-39 and S2-41) are shown in Fig (1) and Table (3). Generally, frequencies of

PMCs having chromosomal aberrations were very high in tested gametoclones comparing to their donor cultivars. For example, in group of G139 genotypes, the total abnormal PMCs bearing different abnormalities types were higher in plants of gametoclones G139-1 (41.82%) and G139-2 (33.30%) than those of its original G139 parent (9.37%). The microcytes and chromosome stickiness were the most common abnormalities in this G139 group. For the second tested cultivar (Giza 163) and their derived 3 gametoclones, considerable high values of total abnormalities were found in PMCs of gametoclones than those of the donor parent plants (Table 3). R_2 Plants of gametoclone G163-8 exhibited the highest percentage (37.63%) of chromosome abnormalities when compared with those of her parent and other sister G163 gametoclones. The most common abnormalities in this group were the lagging chromosomes and chromatids and outsides as shown in Fig. (1).

Table (2): Mean frequencies of chiasmata per cell and chromosome at diakinesis of PMCs in plants of six Egyptian wheat (*Triticum aestivum* L.) cultivars and their 14 derived R_2 gametoclones.

Genotypes	Mean of chiasma frequency/cell	Mean of chiasma frequency/chromosome
G139	44.0	2.00
G139-1	40.0	2.40
G139-2	42.0	2.14
G163	39.4	2.46
G163-4	42.6	1.99
G163-8	43.7	2.36
G164	46.3	1.85
G164-23	51.0	2.08
G164-24	47.1	2.02
G164-25	42.0	1.82
G165	42.2	2.08
G165-26	45.3	1.95
G165-27	43.7	1.78
G165-28	42.5	2.12
S2	46.9	2.22
S2-29	45.0	2.12
S2-30	42.3	2.00
S5	35.0	1.65
S5-39	54.7	2.60
S5-41	53.7	2.56
LSD 0.05	3.38	0.11

Moreover, R_2 plants of gametoclone G164-25 had the highest percentage of chromosomal abnormalities (53.79%) ever when compared with those plants of the 6 parental cultivars (even G164), and all checked 16 gametoclones (esp. sisters G164-23 and G164-24). It is very interest that the original parent plants had the lowest value (7.06%, Table 3). Lagging and outside chromosomes and chromatids were the common types of abnormalities in this G164 group. Moving to G165 group, data also showed that, the gametoclone G165-27 display the highest percentage of meiotic aberrations (20.18%) as compared with the donor parental cultivar G165 (14.81%) and the other sister gametoclones G165-28 and G165-26 (13.04 and 12.33, respectively). However, the gametoclone G165-27 displayed the highest percentages of PMCs containing laggards (3.56%), bridges (2.56%) and outside (11.21%) when compared with those of its parent and most of other tested gametoclones. On the other hand, gametoclone G165-28 displayed the highest PMCs with stickiness (3.62%).

The gametoclone S2-29 of Sids 2 group displayed the highest percentage of total meiotic aberration (44.95%) than those of parental cultivar Sids2 (7.08%) and the other peer gametoclones S2-30 and S2-32 (34.55 and 20.17%, respectively). Gametoclone S2-29 exhibited the highest percentage of PMCs containing bridge (7.56%), fragment (6.41%), micronucli (4.81%) and outside (4.00%), whereas PMCs of gametoclone S2-30 exhibited the highest percentages of laggards and microcyte (21.59 and 5.26%, respectively) when compared with their parental cultivar and the other gamentoclones. For the last group Sids5, the two derived gametoclones (S5-39 and S5-41) showed high percentages of abnormal PMCs except those containing the outside which was higher in the parental cultivar Sids5 (6.20%). The gametoclone S5-41 exhibited the highest percentage of PMCs with total meiotic aberrations (42.19%) when compared with those of its parental cultivar S5 (20.14%) and the other gametoclone S5-39 (14.19%). Gametoclone S5-41 had the highest percentages of PMCs with chromosomal Laggard (15.28%), Bridge (4.57%), Fragment (3.47%), micronuclei (14.41%), and microcyte (1.77%), while peer gametoclone S5-39 had the highest percentage of PMCs with stickiness (3.27%)

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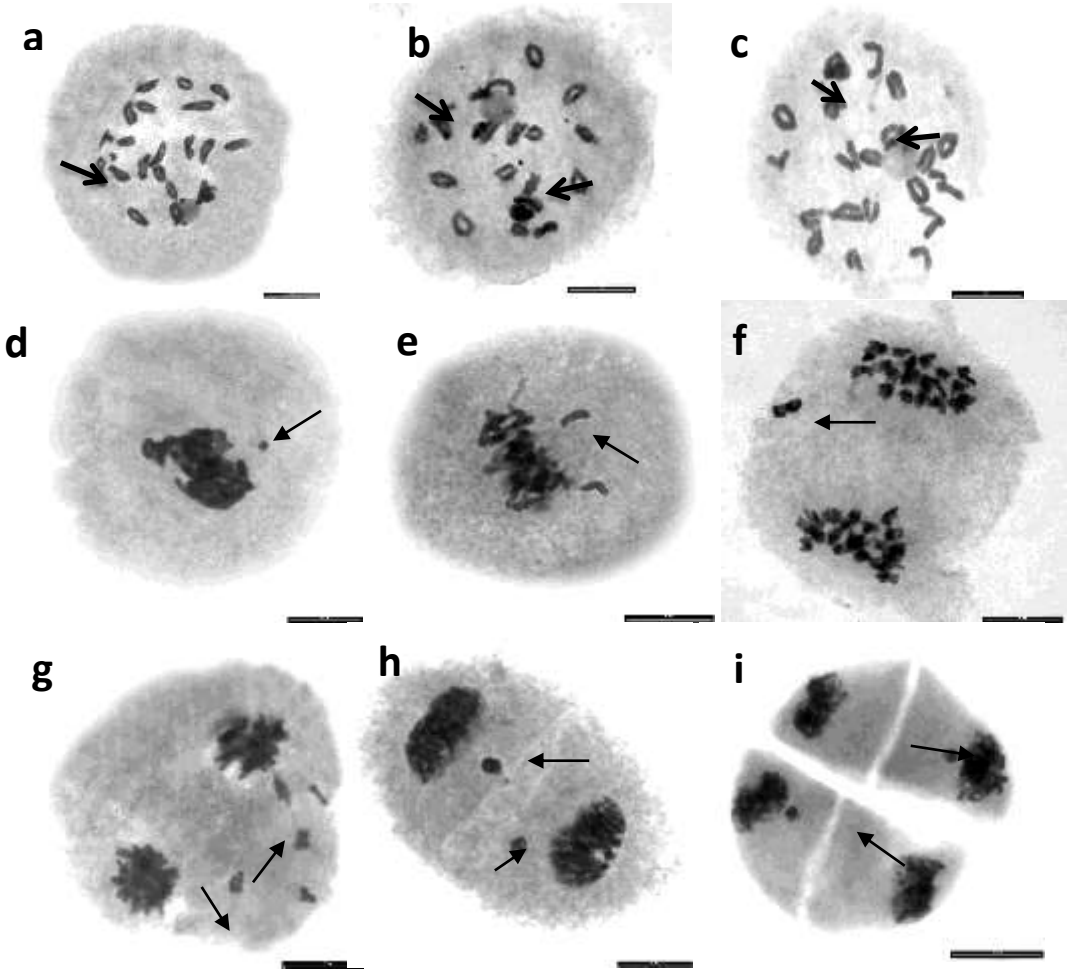

Fig. (1): Pollen mother cells (PMCs) of different wheat (*Triticum aestivum* L.) genotypes of 6 Egyptian cultivars and their 16 derived gametoclones showing different meiotic configuration: (a, b and c) diakinesis with regular 21 bivalents with 2 satellite chromosomes (arrowed), one of them is nucleolus-attached. Some of scored chromosomal abnormalities such as (d): sticky metaphase I with fragment (arrowed), (e): metaphase I with two outside univalent chromosome (arrowed), (f): anaphase I with lagging chromosomes (arrowed), (g): telophase I with four lagging chromosomes and chromatids (arrowed), (h): telophase I with two micronuclei (arrowed) and (i): telophase II with two micronuclei (arrowed). Scale bar = 10 microns.

Table (3): Mean percentage of abnormal PMCs in six hexaploid spring Egyptian wheat cultivars (*Triticum aestivum* L.) and it is derived 16 gametoclones.

Wheat genotypes	Total examined cells	Lagging chrom.	Chrom. bridge	Chrom. Fragment	Stickiness	Micro-nuclei	Micro-cyte	Outside chrom.	Total abnormalities
G139	192	4.69	2.09	2.60	-	-	-	-	9.37
G139-1	127	-	-	-	18.48	-	23.35	-	41.82
G139-2	217	5.49	4.53	1.41	8.82	6.96	-	6.09	33.30
G163	144	-	1.11	-	2.44	2.11	-	1.39	7.06
G163-4	276	20.60	-	-	-	9.36	-	2.19	32.16
G163-8	202	6.74	2.03	-	-	12.63	-	16.24	37.63
G163-13	176	4.09	0.53	-	-	-	1.78	10.40	16.80
G164	320	-	1.11	-	2.44	2.11	-	1.39	7.06
G164-23	184	20.60	-	-	-	9.36	-	2.19	32.16
G164-24	219	6.74	2.03	-	-	12.63	-	16.24	37.63
G164-25	249	30.50	1.94	1.25	0.53	6.58	-	13.00	53.79
G165	169	2.98	-	-	-	6.03	3.05	2.76	14.81
G165-26	657	2.66	1.19	0.78	3.59	-	-	4.11	12.33
G165-27	300	3.56	2.56	-	2.85	-	-	11.21	20.18
G165-28	701	1.55	2.45	-	3.62	-	-	5.42	13.04
Sids2	365	1.07	0.67	0.87	1.22	1.74	-	1.51	7.08
Sids2-29	413	16.59	7.56	6.41	1.16	4.81	4.44	4.00	44.95
Sids2-30	469	21.59	1.02	1.70	-	2.56	5.26	2.42	34.55
Sids2-32	541	3.13	6.69	2.84	4.52	-	0.15	2.84	20.17
Sids5	607	5.83	1.44	0.65	3.01	2.82	0.18	6.20	20.14
Sids5-39	581	0.92	2.07	0.55	3.27	5.14	1.51	0.73	14.19
Sids5-41	264	15.28	4.57	3.47	0.26	14.41	1.77	2.43	42.19

Cytological data of six wheat cultivars and 16 R_2 derived gametoclones confirmed that diploid number were (2n = 6X = 42) for all tested genotypes which possessed 21 chromosome bivalent units. Numerical changes were not observed in the present materials of wheat (R_2 plants), although Ahmed *et al*. (1999) recorded some of these aberrations at callus stage and somatic cells of the regenerated R_0 plants as result of *in vitro* anther culture. This stability may arise from the recovery processes occurred during the regeneration of intact plants (Karp, 1989; Ata *et al.*, 1998). It is well known that hexaploid wheat had contained three different genomes, A, B and D. The observed two satellite chromosome bivalents at PMCs of the studied R_2 gametoclones and their parent plants are normal and like those reported by Feldman and Levy (2012), who indicated that satellite chromosomes were 1B and 6B.

Chiasma frequencies per cell and per chromosome were significantly different depending upon the studied wheat varieties and gametoclones. Data of Sids5 variety and its two studied gametoclones represented a good example for pointing to significant differences of chiasma frequency values (per cell and per chromosome) between

parent plants and their derived gametoclones. Data reported herein are also in agreement with that of Stefanowska (1988). A clear example also was given by Chrzastek (2003), who observed significant 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; Attia and Lelley, 1987). In contrast, Dörgemüller and Lelley (1984) reserved the hypothesis that the number of chiasmata per chromosome depends on the size of blocks of constitutive heterochromatin. However, they showed that the number of chiasmata in bivalents depends on chromosome arm length.

In the present materials, considerable high percentages of abnormal cells were observed in PMCs of R_2 gametoclones when compared with those of parent varieties. These abnormalities were often due to structural aberrations (chromosome bridge, fragments…..etc). Lagging and outside chromosomes were also observed. Data reported herein were in agreement with those of Ibrahim (2016) who found significant differences in total abnormal PMCs between parent plants and their derivative gametoclones. Genetic changes induced during course of anther culture may be inherited throughout two successive generations depending on the cell repair mechanisms (Karp, 1989, 1994). These changes may include wide range from point mutations to chromosome aberrations and epigenetic (i.e. inheritable variation). It has been shown however that gametoclonal and somaclonal variation could be induced by chromosomal changes and/or point mutations (D'Amato, 1977; Pring *et al.*, 1981; Karp and Bright, 1985; Lee and Phillips, 1988; Karp, 1989; Logue, 1996). The observed lagging chromosomes might 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; Patil and Bahat, 1992; Ata *et al.*, 1998), The bridges may result from chromosome stickiness (Abraham and Koshy, 1979; 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 and from characteristic anaphase bridges (Tomkins and Grant, 1972; Grant, 1978; Ata *et al.*, 2017). 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; 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, the chromosome segments of broken bridges also form micronuclei (Masoumeh *et al.,* 2010).

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الملخص العربى

الثبات السيتولوجى لنباتات الجيل الثانى الجاميطى لقمح الخبز المصرى (*Triticum aestivum* L.)

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تهدف هذه الدراسة إلى تحليل التباينات الجاميطية على المستوى السيتولوجى (الميوزى) فى نباتات الجيل الثانى لعدد ستةعشر (16) سلالة ناتجة من زراعة المتوك لستة أصناف من قمح الخبز المصرية (جيزة 139 – جيزة 163 – جيزة 164 – جيزة 165 – سدس 2 – سدس 5) مقارنة بنباتات الأباء. ولقد أوضحت النتائج أن جميع النباتات التى تم فحصها ميوزيا (فى الخلايا الأمية لحبوب اللقاح) سواء من نباتات الأباء أو من نباتات الجيل الثانى للسلالات الجاميطية فى هذه الأصناف الستة كانت تحتوى على العدد الثنائى الكروموسومى (42=6X=2N)؛ وأظهرت جميع الخلايا وجود 21 وحدة كروموسومية ثنائية. ولقد ظهرت معظم هذه الوحدات فى شكل حلقى فى مرحلة الدور التشتتى الميوزى، ولقد أمكن تمييز وحدتين ثنائيتين تحتويان على توابع كروموسومية. أوضحت نتائج التحليل الإحصائى باستخدام قيم الLSD أن هناك تباينات معنوية فى تكرارات الكيازما فى الخلية وفى كل كروموسوم بين نباتات الجيل الثانى الجاميطى والنباتات الأبوية. ولقد أظهرت نباتات الجيل الثانى الجاميطى فى السلالة سدس5–39 أعلى تكرار للكيازما (54.7 و2.6) فى الخلية والكروموسوم تباعا بين كافة التراكيب الوراثية التى تم فحصها، سواء الاصناف الابوية او السلالات المستولدة منها، بينما امتلكت خلايا وكروموسومات نباتات صنف الأب سدس5 (35 و 1.67) تكرارا، وهى الأقل أيضا على الإطلاق. ولقد كان واضحا من خلال نتائج فحص التغيرات الكروموسومية الميوزية أن نباتات الجيل الثانى الجاميطى فى السلالة سدس5–39 (S5-39)، انها لم تصل بعد للثبات السيتولوجى، بينما كانت السلالة G163-12 اقل السلالات الجاميطية المفحوصة احتواءا على الشذوذات الكروموسومية الميوزية (**37.7**) مما يؤكد انها أكثر ثباتا من الناحية السيتولوجية. وكان من أكثر الشذوذات الكروموسومية التى شوهدت فى الخلايا الأمية لحبوب اللقاح هى الكروموسومات المتلكأة والكبارى الكروموسومية والشظايا والكروموسومات التى تكون خارج الصفيحة الإستوائية وكذلك الأنوية الصغيرة والخلايا الصغيرة، ولقد ناقشت الدراسة مدى استمرارية الثبات السيتولوجى خلال الأجيال الجاميطية المتقدمة قى القمح.