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***Research Article***

# CHROMOSOME ASSOCIATIONS OF TWO FLOWERING GARLIC CLONES

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## ABSTRACT
The main goal of this study was to examine the meiotic chromosomes pairing particularly those with nucleolar regions for clarifying the frequencies and types of structural aberrations occurred during gametogenesis of two flowering garlic clones called Egaseed 2 and EGA3 cultivated in Egypt. Results revealed that all examined PMCs in both studied garlic clones couldn't exhibit normal or regular chromosome synapsis (primary association of the eight chromosome pairs). Data also showed that chromosome pairs 1, 2, 3 and 4 associated and paired altogether and/or with the remaining four chromosomes. Ring or chain octa- and multivalents were the dominant configurations. Chromosome pair no.5 partially associated, often in a chain form, with pair nos. 1, 2, 3, 4 and 8. In the same manner, chromosome no. 6, 7 and 8 paired with the four larger chromosomes (1, 2, 3 and 4) with different ratios in both two studied clones. The appearance of univalents was very rare. Thus, at least seven associations for each one of the larger chromosomes (nos. 1, 2, 3 and 4) could be calculated. Likewise, four associations become visible of each smaller one (pair nos. 5, 6, 7, 8). Few PMCs showed regular anaphase I while chromatid bridges and fragments were frequently observed. Two cell types were detected according to the number of nucleoli. About half examined cells, of the two studied clones, exhibited one nucleolus and the other showed two nucleoli. It was clearly observed that not only chromosome pair nos. 6 and 7 attached nucleoli but also some other chromosomes. The role of chromosome association in clarifying garlic origins and evolution was discussed.

***Keywords*:** *Allium Sativum, Chromosomes, Multivalent, Nucleolus and Meiosis*

## INTRODUCTION
Meiosis has unique cytological and biochemical features to facilitate reduction and to produce with a high degree of fidelity genetically distinct and balanced gametes (Jenkins and Okumus, 1992). One such feature and an integral part of meiosis in the majority of higher eukaryotes studied is the ability of homologous chromosomes to recognize one another and to synapse intimately along their lengths. Fertility and seed setting are the challenges that faced the classical hybridization and breeding of garlic (*Allium sativum*) clones long time. Therefore, rare commercial garlic varieties produce regular flowers or seeds. Taxonomical situation of those bolting, flowering and seed producing accessions is still more debatable (Kamenetsky and Rabinowitch, 2001; El-Mamlouk *et al.*, 2002; Ata, 2005; Osman *et al.*, 2007 and Ata and Osman, 2009). A number of garlic genotypes, mostly landraces from Central Asia, proved to be fertile (Etoh *et al.*, 1988; Pooler and Simon, 1993, 1994; Jenderek and Hannan, 2000; Kamenetsky *et al.*, 2005). Additionally, fundamental physiological and molecular studies enabled the induction of flowering and restoration of fertility by environmental manipulations (Kamenetsky *et al.*, 2004 and Shemesh-Mayer *et al.*, 2015).
To study meiosis in garlic, a few workers reported multivalent chromosomes or desynapsis in garlic (Takenaka, 1953; Gohil and Koul, 1971; Ata and Osman, 2009 and Mahmoud *et al.*, 2017). The formation of ring chromosomes caused occurrence of lagging chromosomes observable during the first anaphase in some flowering accessions (Konvička and Levan, 1972). In addition, some fertile clones were meiotically examined by Koul and Gohil (1970), Etoh and Ogura (1978) and Pooler and Simon (1994) who found that pollen mother cells (PMCs) exhibited multivalent chromosomes. The frequency of octavalent chromosomes was greater than that of any other multivalent chromosomes. In most of the diploid species the mitotic complement includes only one nucleolar chromosomes pair but there are some

cases with two pairs (Sharma, 1964; Ata, 2005; Osman *et al.,* 2007 and Ata and Osman, 2009). Garlic accessions collected from Egypt exhibited three nucleolar chromosome types with secondary constrictions, 4 followed by 3 and 2 (Ata, 2005; Osman *et al*., 2007; Ata and Osman, 2009 and Mahmoud *et al*., 2017). Thus, studying the meiotic pairing of garlic chromosomes particularly those with nucleolar regions for clarifying the types of structural aberrations occurred during gametogenesis of two flowering clones is the main goal of the present work.

## MATERIALS AND METHODS

### *Plant Material*

Two bolting Garlic (*Allium sativum* L.) clones namely Egaseed 2 and EGA3 were cultivated and the flowering budds were colected to study garlic chromosome paring and separation. The cloves of the two clones were kindly provided by the vegetable Branch, Horticulture Department, Faculty of Agriculture, Minia University.

### *Meiotic Preparation*

Young flowering buds of the flowering clones (Egaseed 2 and EGA3) were collected at 8-9 O'clock morning and immediately fixed with Farmer's solution (3 ethyl alcohol: 1 acetic acid) for 24 h at 10°C. Pollen mother cells (PMCs) were prepared from the fixed anthers and stained with aceto-carmine. The meiotic behavior of chromosomes in pollen mother cells (chromosome pairing) was examined (100 PMCs/ clone). Number of nucleolus and nuclear chromosomes was considered. Some PMCs were photographed using CCD camera (Olympus C-4040).

## RESULTS AND DISCUSSION

### *Results*

All examined PMCs, in both studied garlic clones (Egaseed 2 and EGA3), couldn't exhibit normal or regular synapsis (primary association of the eight chromosome pairs). Arrangement and whole size (about 150-160 microns) of chromosome bivalents was carried out according to chromosome sizes measured before (El-Mamlouk *et al*., 2002; Ata, 2005; Ata and Osman, 2009 and Anwar, 2011).

*Chromosome Pairing and Association*

Data in Table (1) showed that chromosome pairs 1, 2, 3 and 4 associated and paired altogether and/or with the remaining four chromosomes at 100% of examined PMCs in both two examined clones. Ring or chain octa- and multivalents were the dominant configurations representing those larger chromosomes at diakinesis and Metaphase I as shown in Figure 1. Chromosome pair no.5 partially associated, often in a chain form, with pair nos. 1, 2, 3 and 8 of 45% and with pair nos. 1, 2, 3 and 4 of 85% examined PMCs of Egaseed 2 and EGA 3 clones, respectively (Figure 2). In the same manner, chromosome no. 6, 7 and 8 paired with the four larger chromosomes (1, 2, 3 and 4) with different ratios in both two studied clones. The appearance of univalents was very rare. Thus, at least seven associations for each one of the larger chromosomes (nos. 1, 2, 3 and 4) could be calculated. Likewise, four associations become visible of each smaller one (pair nos. 5, 6, 7, 8). The total associations become around forty. Few PMCs showed regular anaphase I while chromatid bridges and fragments were frequently observed as well as lagging chromosomes and bivalent (Figure 3).

*Nucleolus and Nucleolar Chromosomes*

Two cell types were detected according to the number of nucleoli (Figure 3). About half examined cells, of the two studied clones, exhibited one nucleolus and the other showed two nucleoli (often different sized). It was clearly observed that not only chromosome pair nos. 6 and 7 attached nucleoli but also some other chromosomes as shown in Figure 4.

### *Discussion*

The diploid number and chromosomes of garlic (*Allium sativum*) were early described as 2n=16 (Levan, 1935; Mensinkai, 1939, Khoshoo *et al.,* 1960 and Battaglia, 1963). The karyotype of *A. sativum* varied greatly among individual clones. Thus, it was extremely difficult to analyze all the chromosomes into pairs (Sato *et al.,* 1980) particularly when compared with those of congeneric species (Mukherjee and

Roy, 2012 and Ramesh, 2015). To our knowledge there is no standard karyotype of common garlic so far. This may due to the accumulation of structural chromosomal aberrations throughout the frequent vegetative clonal propagation as a result of its apomictic nature. So, in the present work, studying of chromosome pairing and associations during meiosis is crucial for determining the frequencies and types of those aberrations. About forty associations in 160 total genome length (160 microns) as measured before (El-Mamlouk *et al.*, 2002; Ata, 2005; Ata and Osman, 2009 and Anwar, 2011) means that one association occurred per 3 microns. High frequency of associations may due to occurrence of different types of translocations. The appearance of bridges and fragments at anaphase I indicated to paracentric inversions and/or reverse duplications. Lagging chromosomes and bivalents may result from chromatin alterations and point gene mutations. These events resulted in more instable of garlic genome and interpreted the great variability of karyotypic configurations. For instance, in Italian garlic, Bozzini and De Luca, (1991) observed 6 acrocentric while Yüzbaşioğlu and Unal (2004) reported that except submetacentric pair No.5, all chromosomes were metacentrics in Turkish garlic. Different karyotypes were also suggested in different countries such as, in New Zealand (Sukias and Murray, 1990), in India (Mukherjee and Roy, 2012 and Ramesh, 2015) in Pakistan (Wajahatullah and Vahidy, 1990) and in Egypt (El-Mamlouk *et al.*, 2002; Ata, 2005; Osman *et al.*, 2007 and Mahmoud *et al.*, 2017).

**Table 1: Associations between Different Chromosome Pairs in Genomes of Two Flowering Garlic Clones**

| | Egaseed2 | | EGA3 | |
|---|---|---|---|---|
| **Pair No.** | **Association Degree** | **Association with Chromosome Number** | **Association Degree** | **Association with Chromosome Number** |
| I | 100 % | 2 or 3 or 4 (100 %) | 100 % | 2 or 3 or 4 (100 %) |
| II | 100 % | 1or 3 or 4 (100 %) | 100 % | 1 or 3 or 4 (100 %) |
| III | 100 % | 1 or 2 or 4 (100 %) | 100 % | 1 or 2 or 4 (100 %) |
| IV | 100 % | 1 or 2 or 3 (100 %) | 100 % | 1 or 2 or 3 (100 %) |
| V | 45 % | 1 or 2 or 3 (90 %) or 8 (10 %) | 85 % | 1 or 2 or 3 or 4 (100 %) |
| VI | 30 % | 1 or 2 or 3or 4 (100 %) | 20 % | 1 or 2 or 3 or 4 (100 %) |
| VII | 20 % | 1 or 2 or 3 or 4 (100 %) | 45 % | 1 or 2 or 3 or 4 (100 %) |
| VIII | 20 % | 1 or 2 or 3 or 4 (75 %) or 6 (25 %) | 30 % | 1 or 2 or 3 or 4 (100 %) |

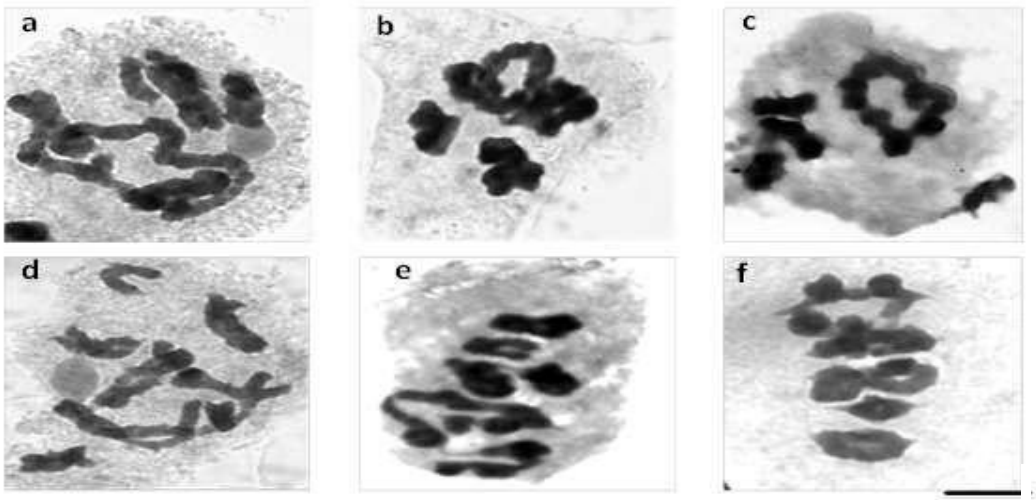


**Figure 1: Meiotic Paring and Association Showing Large Ring Multivalent (a) at Diakinesis, (b) at Metaphase I both were from Egaseed 2 Clone and (c) at Metaphase I from EGA 3 Clone, Chain Multivalent (d) at Diakinesis, (e) at Metaphase I both from Egaseed 2 Clone and (f) at Metaphase I from EGA 3; Scale bar= 10 Microns**

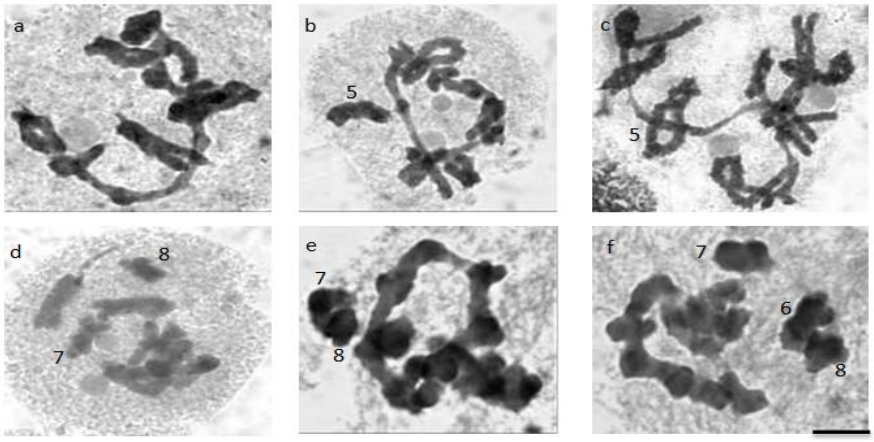


**Figure 2: Association of Pair Nos. 5, 6, 7 and 8 with Other Larger Chromosomes at PMCs of Egaseed 2 and EGA 3 Garlic Clones; (a) All Chromosome Pairs Associated in Large Chain and Small Rings, (b) and (c) All Chromosomes Except Pair No.5 Associated in Large Ring Including Two Nucleolar Chromosomes, (d, e and f) Showed Association of Pair No. 6, 7 and 8. Scale Bar = 10 Microns**

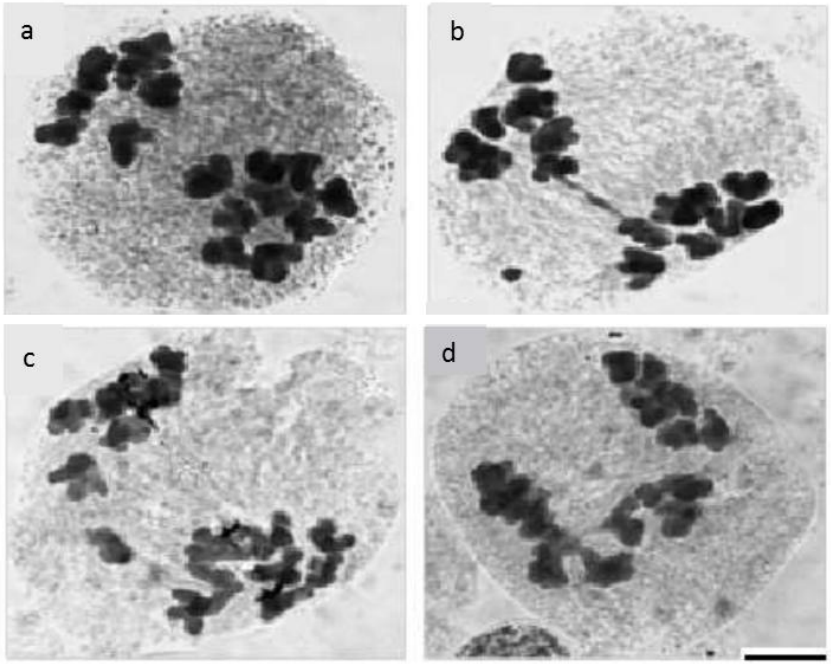


**Figure 3: PMCs at Anaphase I Showing Products of Chromosomal Aberrations in Two Garlic Clones (Egaseed 2 and EGA 3); a. Unequal Separation, b. Bridge and Fragment, c and d. Lagging Chromosome and Bivalent, Respectively; Scale Bar = 10 Microns**

In the present work, grown evidence is found to support an existence of preferential pairing between pairs nos. 1, 2, 3 and 4 in octavalent formula leading to think hybrid origin of garlic. This is in agreement with hypothesis of Takenaka (1953) who assumed that garlic is a hybrid originated from a natural or artificial cross between two related species whose chromosome complements have become differentiated by translocations, inversions or deficiencies in the course of evolution. Thus the karyological instability of garlic might be due to the accumulation of somatic mutations. At the same manner Etoh and Ogura (1977) stated that if garlic was originated from more than one line, each clone of garlic may have possessed their

original chromosome configurations at meiosis. The meiotic irregularity may result from hybridity, polyploidy and/or common structural chromosomal aberrations such as multiple translocations, which sometimes involving eight or even ten chromosomes (Etoh and Pank, 1996 and Friesen and Klaas, 1998). These genomic aberrations may cause the malformation of pollen grains during sporogenesis as revealed in the two examined flowering clones (Egaseed 2 and EGA 3).

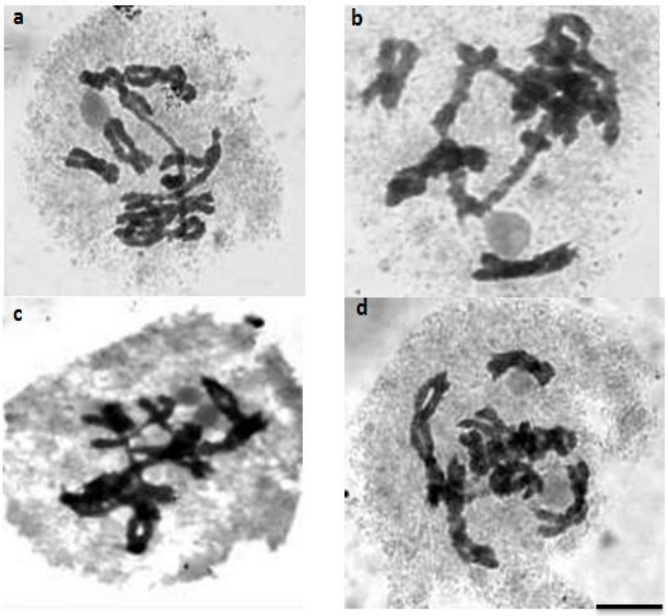


**Figure 4: PMCs of Egaseed 2 and EGA 3 Clones Showing Diakinesis with (a and b) One Nucleolus Attached Chromosome 7 and Other Larger and (c and d) Two Nucleoli Attached Three Chromosomes (Chromosome 7 Clearly Visible); Scale Bar = 10 Microns**

Number of nucleolar chromosomes (with constrictions also called SAT chromosomes or *Sativum* type) in garlic genome are still quiz. It has been reported that number of SAT chromosomes are different among clones or varieties. In the present material PMCs exhibited different nucleoli attached-chromosomes (pair nos. 5, 6, 7, 8 and sometimes, others).

Data reported herein are in agreement with those reported before (Sato *et al*., 1980; Osman *et al*., 2007 and Mahmoud *et al*., 2017). This may due to DNA rearrangements at NORs (Nucleolar Organizing Regions) that found on SAT chromosomes leading to change in their positions and chromosome numbers. In *Allium sativum* the satellite chromosomes were affected by structural deviations strikingly more often than the other chromosomes (Konvička and Levan, 1972 and Sato *et al*., 1980).

From cytogenetical point of few the fail of gametogenesis progress is due to genomic alterations on chromosomal and molecular level of PMCs themselves. Otherwise, a comparison of morpho-physiological and molecular traits of fertile and male-sterile garlic flowers suggests that respiratory restrictions and/or non-regulated programmed cell death of the tapetum can lead to energy deficiency and consequent pollen abortion (Shemesh-Mayer *et al*., 2015).

It could be concluded that garlic chromosome associations during meiosis clarified the frequencies and types of chromosomal alterations which share in studies of fertility and origin of garlic clones. Furthermore, molecular and cytogenetic studies particularly those interested in nucloelar attached chromosomes should be carried out.

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