Gametogenesis of two garlic clones relevant to Egyptian origin.

Conference Paper · September 2009

2 authors:


Abdeltawab Ata
Minia University


Sayed Abdel-Maksoud Osman
Minia University

African Crop Science Conference Proceedings, Vol. 9. pp. 483 - 487
Printed in Uganda. All rights reserved
ISSN 1023-070X/2009 $ 4.00

Gametogenesis of Two Garlic Clones Selected from Egyptian Indigenous Forms

M. ABDEL-TAWAB ATA & A.M. SAYED OSMAN
Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Egypt

Abstract Garlic (*Allium sativum* L.) is one of the ancient condiment vegetables. In order to determine the main cause of sterility of garlic some morphological and cytological criteria were studied. Morphological traits (i.e. inflorescence morphology, mean number of white and pink flowers per plant, anther number and morphology, morphology of fertilized eggs, morphology of locules and pollen grain stainability) and chromosome behavior during meiosis were studied on two soft necked garlic clones (called Egaseed-2 and 3) that were selected from the Egyptian origin. The differentiated inflorescences have nearly 5 to 10 bulblets and little number of white (0-8) and pink (0-15) flowers in both garlic clones. Data showed that each flower contains six petals, six anthers and three locules (each locule has 2 ovules). The percentages of stained pollen grains were 23.7 and 4.67 in Egaseed 2 and 3, respectively. Concerning the chromosome pairing during meiosis, the regular PMCs were scarce and exhibited high frequencies of chromosomal abnormalities at all investigated stages. The sterility in these clones may be attributed to the existence of these irregularities and abnormalities.

Keywords: Abnormalities, *Allium sativum*, clones, inflorescence,

Introduction

Garlic (*Allium sativum* L.) is one of the ancient condiment vegetables (Simon and Jenderek 2003). The sativum variety, or common garlic, produces a weak flower stalk, if it bolts, and has a bulb with many pure white or pink-blushed bulblets (Pooler and Simon 1993). It is only asexually propagated by the planting of the cloves or the bulbils (Osman *et al*., 2007). Clonal lineages within this species show a remarkably high degree of phenotypic diversity. New genotypes have not been obtained through hybridization, but through the selection of spontaneous mutations expressing traits of horticultural interest. More than 200 named garlic clones are commercially available in the United States (Ata 2005; Volk *et al*., 2004). The karyotypic analyses of various clones have been tried by many workers (Katayama 1936; Krivenko 1938 and Elmamlouk *et al*., 2002), but the meiotic chromosome behavior of garlic was investigated by only a few workers (Koul and Gohil 1970 and Etoh 1982). This may be due to the fact that in some clones no seed stalks develop, or that few flowers are formed amongst the bulbils in the spathes (Flaishman and Kamenetsky 2006). Aberrations in floral initiation and differentiation may lead to modifications in inflorescence and flower structure, which may reduce the decorative value of the plant. In some cases, however, deviations in florogenesis may give rise to new and unusual forms of flower, which, being carried through to future generations, may encourage breeding of new varieties of ornamental crops (Stevens and Bougourd 1991). Garlic is one of the completely sterile plants, and the pollens degenerate at the infantile stage of microspores. This is the reason why the chromosome pairing at meiosis in garlic breeding programs should be carefully observed. Garlic is a diploid species (2n = 2X = 16) of obligated apomixes, therefore its reproduction is vegetative (McCollum 1987; Figliuolo *et al*., 2001; Ipek *et al*., 2003; Ipek *et al* 2006 and Mario 2007). Several workers reported the occurrence of eight bivalents of 16 chromosomes in some cases in garlic (Konvicka 1972; Konvicka and Levan 1972; Novak 1972; Konvicka 1973; Pooler and Simon 1994 and Jenderek 1998). In order to determine the main cause of sterility of Egyptian garlic, some morphological and meiotic chromosome features were studied on two soft necked garlic genotypes (EGA 2 and EGA 3) That were selected from the Egyptian indigenous forms.

Materials and Methods

Plant Materials. Two garlic clones called Egaseed 2 (EGA 2) and Egaseed 3 (EGA 3) which were kindly offered by the Vegetable Branch, Horticulture Department, Faculty of Agriculture, Minia University, El-Minia, Egypt were used in this study. The bulbs of the two accessions were planted at Minia University Farm. The total numbers of the examined flower stalk plants were 35 and 42 of EGA 2 and EGA 3, respectively.

Cytological preparations and meiotic analysis. The flowering buds were picked and the pollen grains were stained with acetocarmin. The viable and unviable pollen grains were estimated.

The chromosome behavior during meiotic stages in the respective clones was investigated microscopically in anthers fixed in farmer's fluid. The anthers were smeared in iron-acetocarmine solution, and the meiotic behavior of the chromosomes in about 879 pollen mother cells (PMCs) was examined at first and second meiosis. The appropriate meiotic spreads were photographed using SIS computer program with OLYMPUS camera 4040. Cytological studies were carried out in Genetics Department, Faculty of Agriculture, Minia University, El-Minia, Egypt.

Results

In the present work, some morphological traits (e.g. inflorescence morphology, mean number of white and pink flowers per plant, number and morphology of anthers, morphology of fertilized eggs, morphology of locules and pollen grain stainability) as well as chromosome behavior during meiosis were studied on two soft necked garlic (*Allium sativum* L.) genotypes (EGA 2 and EGA 3) that were selected from the Egyptian indigenous forms.

Inflorescences morphology. The general morphology of the inflorescences of these plants is shown in Fig. (1). Mean numbers of pink and white flowers per plant in the present material are shown in Table (1). The Garlic inflorescences have a combination of flowers and bulbils with variable number of pink and white flowers. The mean number of pink flowers per inflorescence is 2.43 in EGA 2 and 4.69 in EGA 3. On the other hand, the number of white flowers ranged from 0.0 to 5 with mean value of 2.66 in EGA 2 and from 0.0 to 8 with mean value of 2.83 in EGA 3.

Table 1 mean numbers of pink and white flowers per plant in EGA 2 and EGA 3.

Clones	No of plants examined	Mean numbers of pink flowers/plant	Mean numbers of white flowers/plant
EGA 2	35	2.43	2.66
EGA 3	42	4.69	2.83

Figure 1. Garlic inflorescences with a combination of flowers and bulbils.

Flower morphology. Like other alliums, garlic flowers are perfect, with 6 petals, 6 anthers, and 3 locules consisting of 2 ovules each as shown in Fig. 2.

Pollen grain stainability. Regarding, the determination pollen grain stainability (viability), the percentage of viable grain in EGA 2 and EGA 3 were 23.70% and 4.67% respectively as shown in Table (2).

Table 2 Number and percentage (%) of the examined viable and unviable pollen grains of EGA 2 and EGA 3 garlic clones.

Clones	Total No of examined grains	Viable grain	Unviable grain
EGA 2	3739	886 (23.70%)	2853 (76.30%)
EGA 3	3918	183 (4.67%)	3735 (95.33%)

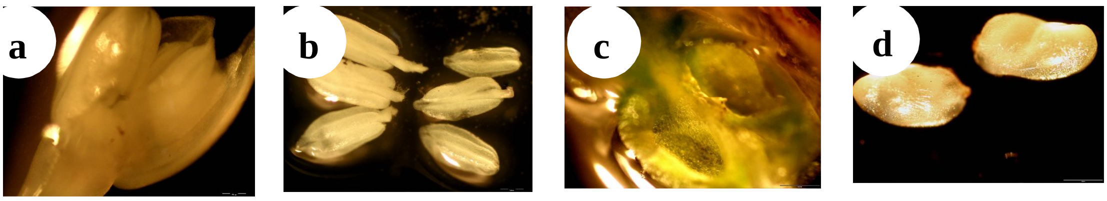

Figure 2 a- Garlic flower with six anthers, b- Six anthers separated from one garlic flower, c- locules of Garlic flower, d- Two Embryos of Garlic flower

Meiotic behavior of the chromosomes. In the present work, the behavior of Chromosome pairing during the first meiotic division was studied in the two examined genotypes as shown in Tables (3 and 4), and Figs. (3 and 4). The total number of examined cells was 354 and 523 for EGA 2 and EGA 3 respectively. It was noticed that most of the chromosomes in the examined cells were in multivalent associations while the formation of bivalent association was restricted to few chromosome pairs in these cells. The percentage of normal cells exhibiting 8 bivalents was 1.98 % in EGA 2 and no cells i.e. 0 % in EGA 3.

Meiotic irregularities. The occurrence of meiotic aberrations were examined at metaphaseI, ana-telophase I, ana-telophase II, tetrad and interphase stages. These abnormalities (as shown in table 4 and Fig.4) included chromatid breaks, univalents, laggareds, bridges and micronuclei. The total percentages of abnormal PMCs showing these aberrations reached 3.95% in EGA 2 and 2.48% in EGA 3. It should be noted that most of scored aberrations here are not numerical chromosomal changes.

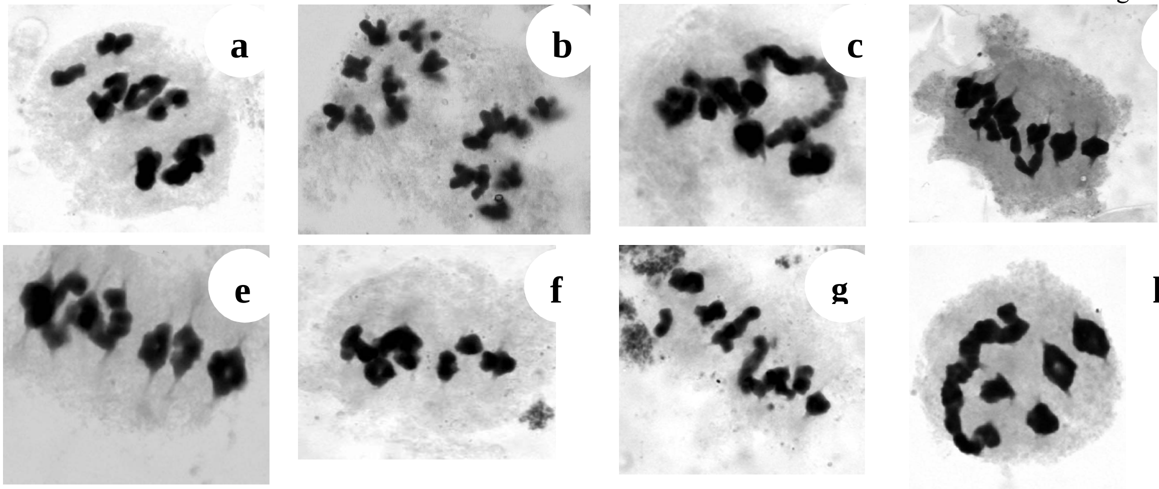

Figure 3 Meiotic chromosome behaviour of two garlic clones showing: a- metaphase with univalent, b- normal anaphase I, c- metaphase with only one bivalent, d- two bivalents, e- three bivalents, f- three bivalents, g- three bivalents, one trivalents and one univalents, and h- four bivalents. The reminder chromosomes at c, d, e, f, and g were in chain configuration. Scale bar = 10μ

Table 3 Numbers and percentages of PMCs containing different categories of chromosome pairing of EGA 2 and EGA 3 garlic clones.

Clones	No. of examined PMCs cells	1 II + multivalents	2 II + multivalents	3 II + multivalents	4 II + multivalents	5 II + multivalents	6 II + multivalents	All II
EGA 2	354	49	67	75	131	18	7	7
%		13.84%	18.93%	21.19%	37.01%	5.08%	1.98%	1.98%
EGA 3	525	202	194	86	36	5	2	0
%		38.48%	36.95%	16.38%	6.86%	0.95%	0.38%	0.00%

Table 4 Numbers and percentages of EGA 2 and EGA 3 PMCs showing meiotic aberrations at different stages of meiosis.

Clone	Total No. of PMCs	Univalents	Chromatid breaks	Lag	Bridge	Micronuclei	Total
EGA 2	354	0.0	0.0	3	3	8	14
%		0.0 %	0.0 %	0.84%	0.84%	2.26%	3.95%
EGA 3	525	2	1	1	1	8	13
%		0.38 %	0.19 %	0.19 %	0.19 %	1.52 %	2.48%

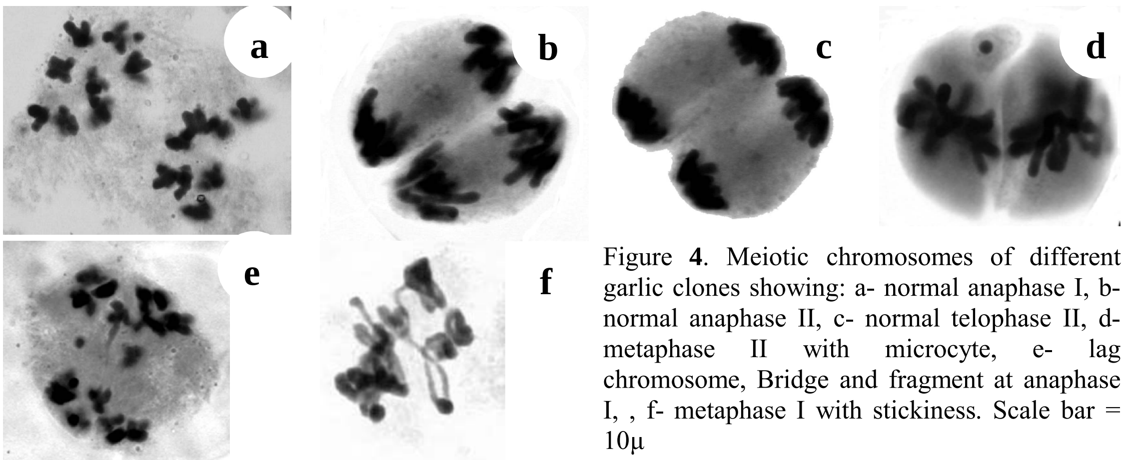

Figure **4**. Meiotic chromosomes of different garlic clones showing: a- normal anaphase I, b- normal anaphase II, c- normal telophase II, d- metaphase II with microcyte, e- lag chromosome, Bridge and fragment at anaphase I, , f- metaphase I with stickiness. Scale bar = 10μ

Discussion

Garlic (*Allium sativum* L.) is vegetatively propagated. Hence the study of its meiosis and the seed production is very rare (Katayama 1936; Stevens and Bougourd 1991; Takenaka 1931 and Takenaka 1953). It is well known that Egyptian garlic is secondary rooted from the garlic ancestor. The present material exhibited soft necked plants under the Egyptian conditions and developed onto flowering ones. Indeed, these flowers couldn't produce complete fertile seeds. Garlic flowers are smaller than onion. In the present material the studied morphological traits showed that the development of flowers and anthers could not reach the proper stage of fertilization and seed sitting.

The causes of garlic floral abortion and sterility are not well understood. An accumulation of pathogens, chromosome aberrations, postmeiotic tapetal degeneration, and unsuccessful competition of floral development with bulbils have been implicated as contributing to the failure of flowers to develop (Etoh 1985). One of the most famous reasons of these phenomena in garlic is the disturbance of meiotic behavior either in ovum or anther. Several authors observed quadrivalents, hexavalents, octavalents, and decavalents in microsporogenesis of numerous diverse bolting Eastern garlic clones that were all male sterile, while only a small number of Eastern clones, notably those from India, had all bivalents in the first meiotic division. These Eastern clones with regular bivalents may represent the subtropical group noted by Maaß, and Klaas (1995) to differ significantly from other Eastern garlic based on molecular "fingerprinting." The multivalent associations in Eastern garlic north of India indicate a clear basis for their male sterility, since gametes that result will have unbalanced chromosome sets. In contrast, Central and Western garlic clones usually form only bivalents, with multivalents rarely noted, although desynapsis has been noted in some clones. Yet Western garlic clones are almost always male sterile, while Central clones are often fertile. Post-meiotic tapetal degeneration is observed in those Western clones closely analyzed (Novak 1972; Gori and Ferri 1982 and Gori 1983), as well as in sterile Eastern clones (Etoh 1982), but the cause of degeneration is not known. Genetic and cytoplasmic male sterility in seed-propagated crops is only beginning to be understood after much effort, and a similar mechanism accounting for male sterility in Western garlic may be uncovered. The meiotic chromosome pairing data revealed that normal PMCs in both studied clones are very poor leading to produce irregular pollen grain and ova. The existence of high percentage of different chromosomal aberrations especially those of structural models are also leading to_believe that the produced gametes are not completely viable (Etoh and Ogura 1978; Etoh 1979). In order to assure this conclusion, the pollen grain stainability was estimated. The obtained cytological data on the first and second meiotic division confirmed that most pollen grains are unviable. There is colinearity between meiotic data and pollen stainability. This may help to understand the main cause of sterility in these clones that originated from the Egyptian indigenous forms.

5. Conclusions

Garlic flowers of two selected clones called EGA-2 and EGA-3 are perfect, with 6 petals, 6 anthers, and 3 locules consisting of 2 ovules each. The determination of pollen grain viability was 23.70 % and 4.67 % in EGA 2 and EGA 3 respectively. It was noticed that most of the meiotic chromosomes in the examined PMCs (pollen mother cells) were in multivalent associations while the formation of bivalent association was restricted to few chromosome pairs in these cells. The percentage of normal cells exhibiting 8 bivalents was very rare in both examined clones. The total percentages of abnormal PMCs showing these aberrations reached 3.95% in EGA 2 while it was 2.48% in EGA 3. The most scored aberrations here are not numerical changes. The meiotic chromosome pairing data revealed that normal PMCs in both studied clones are very poor leading to produce irregular pollen grain and ova. The existence of high percentage of different chromosomal aberrations especially those of structural models are also leading to_believe that the produced gametes are not completely viable. The obtained cytological data confirmed that most pollen grains are unviable.

References

Ata, A. M. 2005. Constitutive heterochromatin diversification of two Allium species cultivated in Egypt. Proc. Afr. crop sci. conf., 7, 225-231.

Elmamlouk, E. A.k. H., Mahmoud, M. A.H., Ata, A. M., Foly, H. M. H. & Allam, H. Z. 2002. Cytological features and isozymes profile of some Allium Sativum Genotypes (Garlic) Cultivated In Egypt. Proc. Minia Conf. 1, 1429 – 1440.

Etoh, T. & Ogura, H. 1978. Multivalent chromosomes in garlic, *Allium sativum* L. Mem. Fac. Agr. Kagoshima Univ., **14,** 53–59.

Etoh, T. 1979. Variation of chromosome pairings in various clones of garlic, *Allium sativum* L. Mem. Fac. Agr. Kagoshima Univ. **15,** 63–72.

Etoh, T. 1982. Development and degeneration of the tapetum in garlic, *Allium sativum* L. Mem. Fac. Agr. Kagoshima Univ. **18,** 75–84.

Etoh, T. 1985. Studies on the sterility on garlic, *Allium sativum* L. Mem. Fac. Agr. Kagoshima Univ**. 21,** 77–132.

Figliuolo, G. V., candido, V., Logozzo, G., M.ccoli, V. & Spagnoletti-Zeuli, P. L. 2001. Genetic evaluation of cultivated garlic germplasm (Allium sativum L. and A. ampeloprasum L.). Euphytica **121,** 325 – 334.

Flaishman M. A. & Kamenetsky, R. 2006. Florogenesis in Flower Bulbs: Classical and Molecular Approaches. Floriculture, Ornamental and Plant Biotechnology. I Global Science Books 33-43.

Gori, O. 1983. Ultrastructural changes in the wall of the sporogenous cells in *Allium sativum*, clone Piemonte during microsporogenesis. Ann. Bot. **51,** 139–143.

Gori, O., & S. Ferri. 1982. Ultrastructural study of the microspore development in *Allium sativum* clone 'Piemonte'. J. Ultrastruct. Res. **79,** 341–349.

Ipek, M., Ipek A., & Simon, P. 2006. Sequence homology of polymorphic AFLP markers in garlic (Allium sativum L.). Genome **49,** 1246-1235.

Ipek, M., Ipek, A. & Simon, P. 2003. Comparison of AFLPs, RAPD markers and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. J. Amer. Soc.Hort. Sci. **128,** 246 – 252.

Jenderek, M. M. 1998. Generative reproduction of garlic (*Allium sativum*) (in Polish). Zeszyty Naukowe Akademii Rolniczej im. H. Kollataja w Krakowie **57,** 141–145.

Katayama, Y.1936. Chromosome studies in some Alliums. J. coll. Agr. Imp. Univ., Tokyo. **13,** 431-441.

Konvicka, O. 1972. Cytotaxonomische Studien von vier sterilen Arten der Gattung *Allium*. Biol. Plant. (Praha), **14,** 62–70.

Konvicka, O. 1973. Die Ursachen der Sterilitaet von *Allium sativum* L. Biol. Plant. (Praha), **15,** 144–149.

Konvicka, O. and Levan A. 1972. Chromosome studies in *Allium sativum*. Hereditas. **72,** 129–148.

Koul, A. K. & Gohil, R. N. 1970. Causes averting sexual reproduction in *Allium sativum* Linn. Cytologia, **35**, 197-202.

Krivenko, A. A. 1938. A cytological study of garlic (*Allium sativum* L.) (in Russian). Biol. Zhurnal, **7,** 47-68.

Maaß, H. I., & M. Klaas. 1995. Infraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. Theor. Appl. Genet. **91,** 89–97.

Mario, P. C., Viviana, B. V. & Maria, G. A. 2007. Low genetic Diversity among Garlic (Allium sativum L.)accessions detected using random amplified polymorphic DNA (RAPD). Chilean J. Agric. Research **68,** 3-12.

McCollum, G. D. 1987. Onion and allies P. 186-190. In N. W. simmonds (ed.) Evolution of crop plants. Longman S. & T., London, England.

Novak, F. J. 1972. Tapetal development in the anthers of *Allium sativum L.* and *Allium Longicuspis* Regel. Experientia, **28,** 363-364.

Osman, S.A.M. A., M. Ata & Gad El-Hak, S. H. 2007. Morphological, Germination, Bolting and Cytogenetical Characteristics of Fourteen Promising Garlic Genotypes. Proc. Afr. Crop Sci. Conf., **8,** 2005-2012.

Pooler M. R. & Simon, P. W. 1993. Characterization and classification of isozyme and morphological variation in a diverse collection of garlic clones. Euphytica, **68,** 121-130.

Pooler, M. R., & P. W. Simon. 1994. True seed production in garlic. Sexual Plant Reprod. **7,** 282–286.

Simon, P. & Jenderek, M. 2003. Flowering, seed production, and genesis of garlic breeding. Plant Breeding Reviews **23,** 211- 244.

Stevens J. P. & Bougourd,M. 1991. The frequency and meiotic behaviour of structural chromosome variants in natural populations of Alliums choenoprasum L. (wild chives) in Europe. Heredity **66,** 391 - 401.

Takenaka, Y. 1931. Further reports of the cytological investigations on the sterile plants. J. Chosen Natural Hist. Soc. (in Japanese with English summary), **12,** 25-41.

Takenaka, Y. 1953. Ring formation in the meiosis of *Allium scorodoprasum var. viviparum*. Ann. Rep. Nat. Inst. Genet. **4,** 41-42.

Volk, G. M., Henk, A. D. & Richards,C. M. 2004. Genetic Diversity among U.S. Garlic clones as detected using AFLP methods. J. Amer. Soc. Hort. Sci. **129,** 559 – 569.