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Cytological study of *Allium cepa* and *Allium sativum*

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Abstract

The large number of species in the Allium genus has necessitated comparative analysis of related species which has always been used in many cases to describe patterns and directions of chromosomal evolution within a group. This study was carried out to cytologically analyse two species of Allium- Allium cepa and Allium sativum. All the four stages of cell division were observed in both species, although well spread metaphase chromosomes could not be observed in A. sativum which limited the extent to which we could cytologically evaluate the species. Allium cepa in this study show cell with a complement of sixteen chromosomes. A total chromosome length of 577.5 µm was observed in the best c-metaphase spread with the longest chromosome being 43.4µm and the shortest 27.9 µm. Further studies are still required in the karyotype analysis of A. sativum from Nigeria, since we could not obtain cells with good colchicines-induced metaphase arrest for karyotype analysis.

Keywords: Karyotype; *Allium sativum; Allium cepa;* Garlic; Onions; Cytology.

Introduction

The genus *Allium* is the largest in the family Amaryllidaceae, and comprises more than 800 species of monocotyledonous perennial, mostly bulbous flowering plants (Fritsch *et al.*, 2010).

Although favourable cytological characteristics make species from the genus *Allium* attractive subjects for study, chromosome numbers are known for only about one-third of them and detailed cytological data are very limited (Ramesh, 2015). The chromosomes of *Allium* have been studied for decades (Sharma and Aiyangar, 1961; Koul and Gohil, 1970; Gohil and Kaul, 1980; Puizina and Papeš, 1996; Fritsch *et al.*, 2001; Cui *et al.*, 2008) for their diversity in size, structure and number. The most intriguing cytogenetic

features of the genus *Allium* are polyploidy and the frequent appearance of B chromosomes (Bs) and species differences in levels of ploidy. Vujošević *et al.,* (2013) enumerated 97 species in which Bs have been found and in ten of these species, Bs are present in polyploids as well. The variations in karyotypes are common both between and within species (Cui *et al.*, 2008). Vijayavalli and Mathew (1990) reported the existence of intraspecific polyploidy within several species of *Allium*. They have also reported that chromosomal difference is also associated with morphological difference in some cases.

Due to the large number of species in the *Allium* genus, comparing karyotypes of related species has been used in many cases to describe patterns and directions of chromosomal evolution within a group and to infer the evolutionary role of karyotype changes (Sharma and Sharma, 1959; Das *et al*., 1999; Vanzela *et al*., 2000; Shan *et al*., 2003). Karyotype analysis has been proved to be useful in many cases including *Borago* (Selvi *et al.*, 2006), *Sideritis* (Esra *et al.*, 2008); *Secale* (Masoud and Ali-Jarrahei, 2008); *Artemisia* (Naseri *et al*., 2009) and so on. Karyotype analysis has been successfully employed at the intraspecific level in several cases including the study of cultivars of *Agave tequilana* (Guadalupe *et al*., 2008); *Gossypium hirsutum* (Sheidai *et al.*, 2008) and populations of *Bidens pilosa* (Maria *et al.*, 2008).

In addition, the cytogenetic characteristics of *Allium* species have been found to vary with the geographic location of the plants. This has informed the several studies of *Allium* species, including *A. cepa* and *A. sativum* in different geographic and climatic conditions. Heterochromatin variation in *A. pulchellum* where higher altitude plants are found to have an increased heterochromatin content (Vosa, 1996). New species are still being documented from different parts of the world, creating the need for cytogenetic studies which have been performed on some of them (Oyuntsetseg*et al.,* 2013; Tzanoudakis and Trigas, 2015).

Materials and methods

Bulbs of *Allium cepa* and *Allium sativum* used in this study were obtained from the Oyingbo market in Lagos, Nigeria in July 2015. The onion bulbs were left for two weeks to dry properly, after which the loose scaly part of the onion bulbs was carefully peeled off and the dead roots at their base carefully scraped away without destroying the root primordial. Twenty five small bulbs (between 15-17g) were then placed on small bottles filled with distilled water so that only the base of the bulbs touched the distilled water for 48hours to induce root growth and also determine the viability of the onions. The set up was kept at room temperature for it to germinate. The level of the water was maintained in each bottle so that the root will always touch water. The set ups were kept in the light beside the window. After completing 48hours, only the onions with the best growing roots were used for the study.

For the *Allium sativum*, a series of five cloves were wrapped in tissue paper, water was then sprinkled on it to make it moist .They were allowed for 2 weeks for new roots to sprout at room temperature. The set-ups were kept moist all through the 2 weeks by constantly sprinkling water on them.

The slide preparation and karyotyping was performed according to the method of Mukherjee and Roy (2012).

Prepared slides were examined on a compound microscope under oil immersion lens (x 100). Photomicrographs were taken from the well spread preparations. All measurements were taken using the software Micromeasure 3.3 downloaded free from www.colostate.edu/Depts/Biology/Micromeasure.

The Total chromosome length (TCL), Average chromosome length (ACL) and the Arm ratio (AR) for each species was calculated according to Kutarekar and Wanjari (1983). The chromosomes having the arm ratio less than 0.51 were termed as subtelocentric (st), 0.51 to 0.75 as submetacentric (sm) and 0.76 to 1.0 as metacentric (m). The mean centromeric index (TF %) was calculated in each complement following Huziwara (1962). All computations were performed with the Micro-measure software. The chromosomes were cut using the Adobe Photoshop tools -select, cut and paste - and arranged according to their length, arm ratio and position of the secondary constrictions, if present.

Cells with chromosomes at different stages of mitosis were examined under the compound microscope and photomicrographs taken at a magnification of x100 for both species of *Allium*. The prophase, Metaphase, Anaphase and Telophase stages in both species are then compared on observable chromosome behaviour.

Results

The *Allium cepa* examined in the study exhibited all the four mitotic stages: prophase, metaphase, anaphase, and telophase.

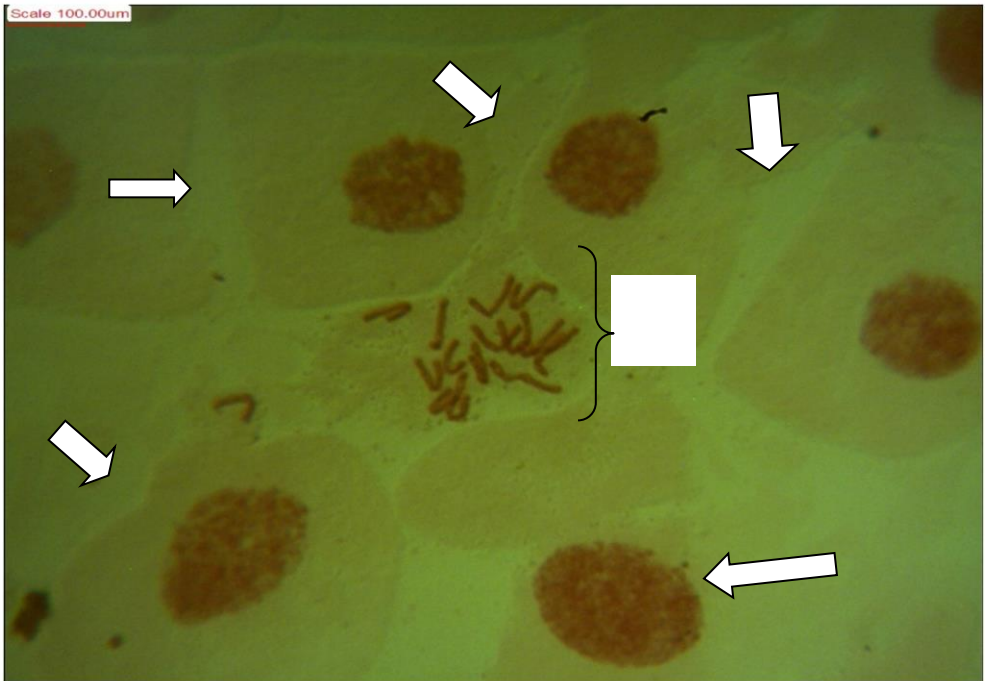

Figure 1: Plate showing five cells at interphase and a cell with colchicines-induced metaphase

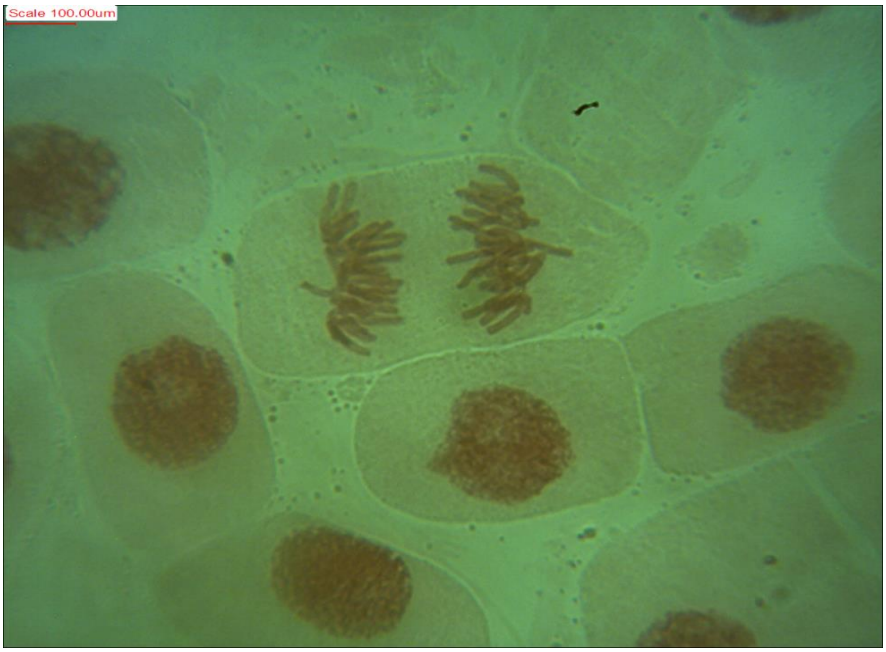

Figure 2: Cell with Anaphase surrounded by interphase cells

Allium cepa in this study shows cell with a complement of sixteen chromosomes. A total chromosome length of 577.5 µm was observed in the best c-metaphase spread with the longest chromosome being 43.4µm and the shortest 27.9 µm. The karyotype formula of the *Allium cepa* is fourteen metaphase and two subtelocentric chromosomes (14m0sm0st). (See Table 1 and Figure 2 below)

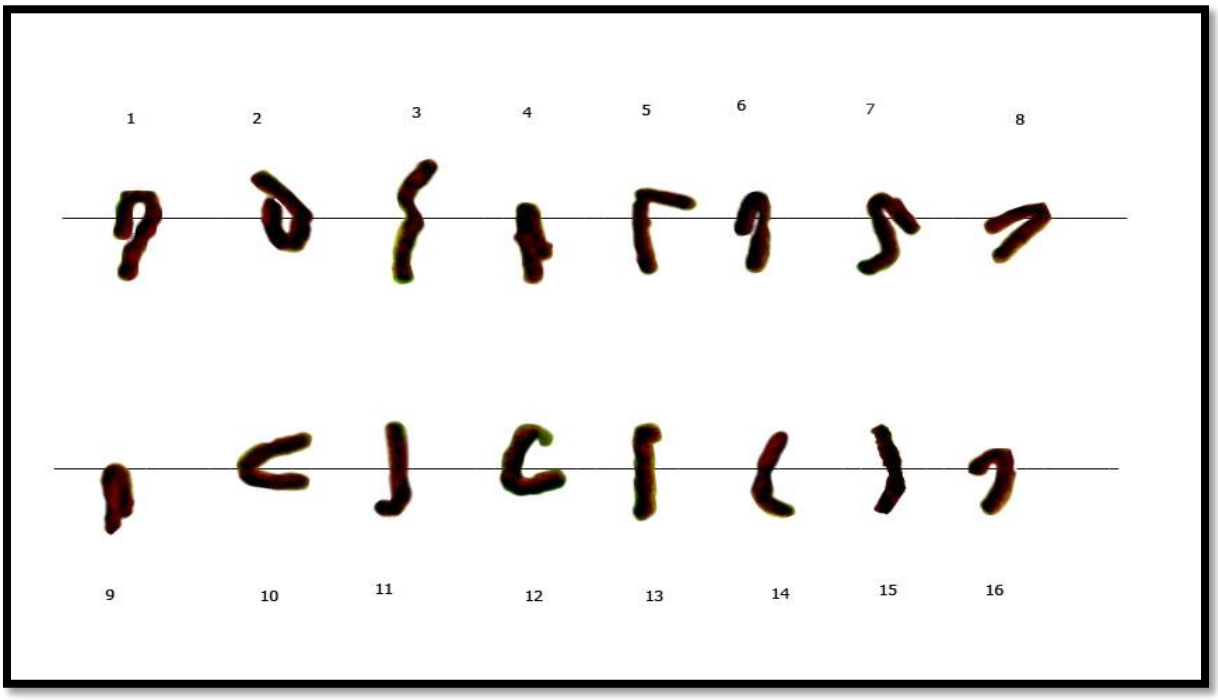

Figure 3: A karyogram of the sixteen *Allium cepa* chromosomes arranged by order of decreasing length. The line indicates the position of the centromere.

The A. sativum examined, showed cells to at different stages of mitotic division: prophase, metaphase, anaphase, telophase. However, the cells at metaphase didn't spread well as to allow close analysis.

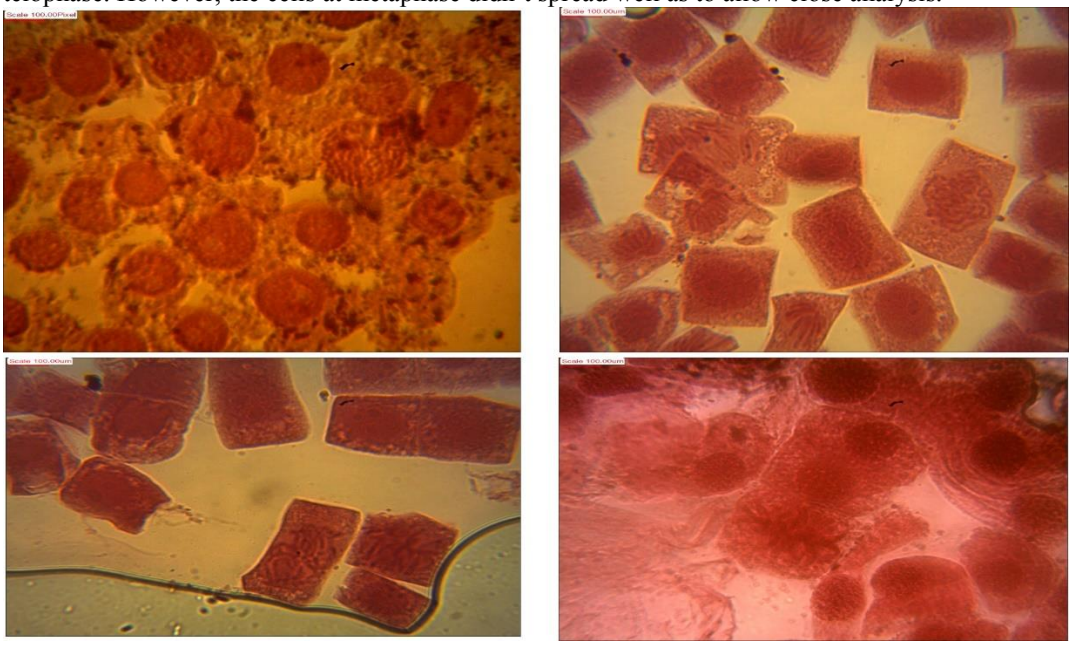

Fig. 4: *Allium sativum* micrograph showing cells at different stages of division. Most clearly observed in this figures are cells in prophase stages.

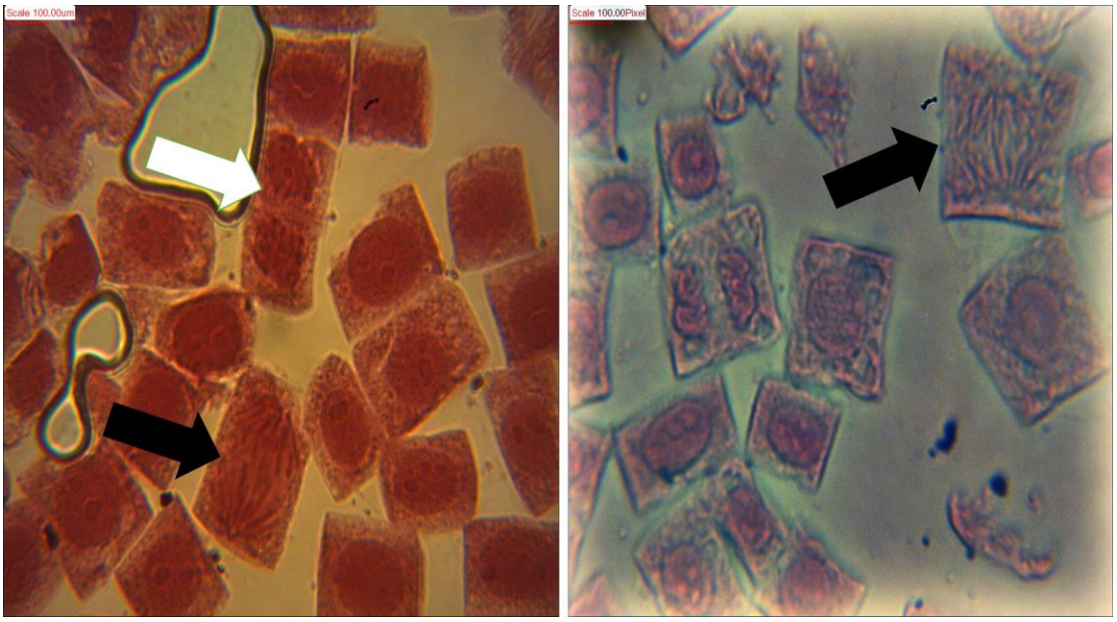

Fig 5: Photpmicrograph from *Allium sativum* showing some cells at anaphase (Black arrows) and telophase (White arrow) stages of cell divisions. Cells of other division stages are also present in the figures.

Table 1: Different chromosomal indices of all the investigated *Allium cepa.*

Rank	**Chromosome Length (CL)** (µm)	**% of total chromosome length**	**Length of Long arm (L) (µm)**	**Length of Short arm (S) (µm)**	**Arm Ratio (L/S)**	**Cent. Index (TF) (S/(L+S))**	**Centromere position**
1	43.39322	7.5138%	23.75811	19.63511	1.209981	0.452492511	M
2	42.29425	7.3235%	25.12788	17.16636	1.463786	0.405879266	M
3	40.78857	7.0628%	23.75549	17.03306	1.39467	0.417593958	M
4	40.42579	7.0000%	21.79165	18.63414	1.169448	0.460946855	M
5	39.53119	6.8450%	22.39391	17.13727	1.306738	0.433512569	M
6	39.27911	6.8014%	24.12983	15.14929	1.592802	0.385683139	M
7	38.58155	6.6806%	20.9931	17.58843	1.193574	0.455876847	M
8	37.80603	6.5463%	29.63296	8.173066	3.625684	0.216184177	ST
9	37.22971	6.4465%	20.55854	16.67116	1.23318	0.447791847	M
10	36.77738	6.3682%	20.41866	16.35872	1.248182	0.444803703	M
11	33.11463	5.7340%	17.38724	15.72739	1.105539	0.474937731	M
12	31.98211	5.5379%	17.06639	14.91571	1.144189	0.466376757	M
13	30.13793	5.2185%	17.98971	12.14821	1.480852	0.403087288	M
14	29.35787	5.0835%	15.38452	13.97335	1.10099	0.475966164	M
15	28.9391	5.0110%	15.21743	13.72167	1.109007	0.474156848	M
16	27.87699	4.8271%	20.45647	7.420515	2.756746	0.266187784	ST
Totals for set:	577.5154						

TCL: Total chromosome length; CL: Average chromosome length; Arm Length; Arm ratio; TF: mean centromeric index value; M metacentric chromosomes, submetacentric chromosome, ST: subtelocentric chromosome

Discussions

Several cytogenetic studies have been done, particularly chromosome number and morphology at mitotic division, and chromosomal association and behaviour during meiotic division, in *A. cepa*. The *Allium cepa* cells in this study undergo identifiable four stages of cell division: prophase, metaphase, anaphase and telophase. At interphase, the cells have amorphous nuclei that are stained in darker shade of pink, magenta or purple compared to the cytoplasm. At prophase chromatin threads begin to appear creating the appearance of white patches in the hitherto amorphous homogenously stained nuclei. The chromosomes of the cell become clearly visible at metaphase and were evaluated to have a complement of sixteen (16) chromosomes. These chromosomes duplicate and separate equally at anaphase before completing the cycle with a telophase.

The *Allium cepa* in this study obtained in Lagos, Nigeria has a karyotypic formula of 14m0Sm2St with n=8 in all the cells examined. The same karyotype formula has been reported in *Allium cepa* L. var *cepa* Helm., *Allium cepa* L. var. *aggregatum* in India by Mukherjee and Roy (2012). A large proportion of *A. cepa* chromosomes are usually metacentric as was also seen in a study of ten populations in Iran (Paknia and Karimzadeh, 2010). Two of the Iranian populations had 16m0sm0st chromosomes, eight populations had 14m2sm0st and two populations had 12m4sm0st showing slight variations in the number of submetacentric and subtelocentric chromosomes (Paknia and Karimzadeh, 2010). The Indian varieties of *Allium cepa (Nasik, White and Kashmiri)* examined by Ramesh, (2015) all had a chromosome complement of 2n=16 (8M+6Sm+2St).

The chromosomes in the Lagos *A. cepa* had a total length (TCL) of 577.51 µm, which is far higher than in any reported study. Ramesh *et al.* (2015) presented average chromosome lengths 45.0 µm 61.6 µm and 76.5 µm for the Nasik, White and Kashmiri varieties respectively. *Allium cepa* L. var *cepa* Helm. and *Allium cepa* L. var. *aggregatum* G. Don have been reported to have mean TCL of 167.70um and 175. 50um respectively. Total chromosome length of 189 µm have been reported in Turkey (Okumus and Lutful, 2000). The large deviation in TCL from this study compared to that document in literature may either be due the cells being in an early stage of metaphase (Okumus and Lutful, 2000), the length of treatment in colchicine which is known to affect chromosome length if prolonged (Sharma and Sharma, 1980) or to errors of measurement caused by using micrometers on microscope objectives or inapproprioiately scaled printed copy of chromosomes during measurement (Okumus and Lutful, 2000).

The longest chromosome in this study is metacentric similar to the longest chromosome in all other reported studies of *A. cepa*. The centromeric index (TF) of 0.45 is comparable to that reported for the Indian *Kashmiri* variety (0.47) and close to that in the *Nasik* and *White* variety both with a TF of 0.50 (Ramesh, 2015).. The chromosome however surprisingly contributed 7.5% of the total length of the chromosome set compared to 16.6%, 14.2% and 15.3% reported for the Nasik, White and Kashmiri *A. cepa* varieties respectively. The percentage lengths are rather almost evenly distributed with only one chromosome having a length contribution below 5.0%. The centomeric indices and arm ratio for the other chromosomes in the complement is similar to what was obtained in the literature (Paknia and Karimzadeh, 2011; Mukherjee and Roy, 2012; Ramesh, 2015).

The difference in karyotype among members of the *A. cepa* species characterized by the differences in relative length of haploid complement of chromosomes, arm ratio, centromeric index and chromosome type, may arise by dramatic chromosomal rearrangements, such as translocation, deletion and inversion (Mukherjee *et al.* 2012). The karyotype can also be changed through inter-specific hybridization. In the present study, the variation in the number and morphology of chromosomes may be due to the mutations in the natural populations. The role of structural alteration of chromosomes in the evolution of races is evidenced by detailed analysis of karyotype. The constancy of the karyotype within the population indicates certain adaptability to the micro environmental condition to which they are subjected.

The *Allium sativum* in this study had cells at different stages of mitosis. The prophase, metaphase, anaphase and telophase stages of the mitotic cycle were clearly identifiable. However, cells with colchicine-induced metaphase arrest were rare and the few observed had poorly spread chromosomes. It was therefore difficult to perform karyotype analysis of the *Allium sativum* in the study. The problem was aggravated by the repeated failure of most of the garlic to sprout. Few plants and roots were therefore available for the cytogenetic study. Several studies had reported difficulties in karyotype analysis of *A. sativum*. According to Osman *et al.*, (2007), frequent chromosomal breaks can be responsible for the inability to make karyotypes of *Allium Sativum*. There have been reports of other factor causes such as; high percentages of large fragments that misleads the karyotype making; the presence of one or more chromosomal constrictions or chromosomal breaks which are very similar to the centromere in their appearance and the great variation in satellite number and size among the studied genotypes in the failed attempts to karyotype *A. sativum* (Takenaka, 1931; Krivenko, 1938; Gohil and

Koul, 1971; Konvicka and Levan, 1972; Verma and Mittal, 1976; Etoh, 1979; Etoh, 1985).

The result of this studies proves more studies are still needed especially on the cytology of *A. sativum*. This is required in order to identify the right conditions for obtaining cells with well spread metaphase chromosomes for karyotype studies of garlic in Nigeria.

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