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Chromosomes Behavior at Meiosis in *Chlorophytum stenopetalum* Bak at Pachytene, Diakinesis and Diplotene

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ABSTRACT: Meiotic prophase is classically subdivided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. The objective of this paper is to evaluate the behaviours of Chromosomes in *Chlorophytum stenopetalum* Bak.at pachytene /diakinesis and metaphase. The flower buds at right age were harvested, fixed in Cornoy's solution (3 part of absolute alcohol and 1 part of acetic alcohol) and preserved in a refrigerator at -4°*C* for at least thirty minutes. The flower buds were then hydrolyzed in 10% HCl for 3-5 minutes. Prepared slides were viewed using an Armscope microscope equipped with digital automatic camera. At diakinesis seven bivalents (7 II) were predominantly observed (74.2%). Chromosomal stickiness is quite evidence. In addition, cross configuration and its resultant, ring formation at metaphase indicate the presence of translocation heterozgosity in the chromosomes of the species investigated. These abnormalities are likely to affect microsporogenesis and pollen viability.

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Chlorophytum Ker-Gawl is a genus in the family Asperagaceace, sub-family Agavoideae of monocotyledonous perennial flowering plant, order Asparagales (Angiosperm Phylogeny Group IV, 2016). This position stated in Angiosperm Phylogenic Group III, 2009 is maintained the new classification (Steven, 2007). The genus comprises about 234 species distributed in tropical and subtropical regions (Gudadhe, Nathar and Dhoran, 2012). Its probable centre of origin and diversification lie in the tropical and sub-tropical Africa and Asia, where 85% of the species are found (Bordia *et al.*, 1995). A large number of species of *Chlorophytum* are found on the forest floor in West Africa while several others occur in the guinea savanna regions (Poulsen and Nordal, 2005; Meerts and Bjora, 2012). *Chlorophytum stenopetalum* is found in the guinea savanna region in Nigeria. The species is a tuberous herb, with rosette of leaves close to the ground. Leaves are linear-lanceolate and leave margin is wavy. Inflorescence is about one fifth as long as leaf, bearing congested fruit. One to three inflorescences grow simultaneously from the centre of the leaves rosette, with the prime inflorescence at the middle. Roots are long bearing tubers at the median position. Though, *Chlorophytum stenopetalum* is sometimes confused with *Chlorophytum macrophyllum*, Omokanye *et al*. (2020), separated the two based on their leaf epidermal morphology, especially stomata index. The genus *Chlorophytum* is characterized by polyploid evolution having (x=7 or 8) chromosomes (cf. Baldwin and Speese 1951, Darlington and Wylie 1955, Sharma and Chatterjee 1958, Sharma and Raju 1967). Thus, some of the species belong to 7-basic series while others belong to 8-basic series. Previous cytological works on species of *Chlorophytum stenopetalum* indicate a diploid chromosome number of 2n=14 (Adeyemi ,1974; Ngwa, 1979 and Omokanye 2016). Meiotic chromosomes behavious in *Chlorophytum stenopetalum* is investigated with the view of verifying the existing diploid number and also to unravel processes that may hinder normal microspore formation hence affecting pollen fertility.

MATERIALS AND METHODS

Samples of species of *Chlorophytum stenopetalum* used for these studies were collected at the onset of the rain in the month of March, 2013, from a village called Shika dam, about 35km south of Ahmadu Bello University, Zaria. These materials were raised in pots containing garden soil, in the Department of Plant Biology of University of Ilorin. Most of the plants produced flowers approximately a mouth after vegetative growth has been established, while others produce flowers much later.

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The flower buds at different stages of development were harvested and fixed immediately in freshly prepared Cornoy's solution (3 part of absolute alcohol and 1 part of acetic alcohol) and preserved in a refrigerator at -4°C for at least thirty minutes, to allow for proper fixing of the cells and the removal of mucilage from the anther. The flower buds were then hydrolyzed in 10% HCl for 3-5 minutes so as to allow for easy removal of the perianth segments. Anther from hydrolyzed flower bud was squashed on a clean slide on which a drop of acetic orcein stain had been placed. Prepared slides were viewed using an armscope microscope equipped digital automatic camera.

Plate 1. *Chlorophytum stenopetalum* at maturity stage

RESULTS AND DISCUSSION

The meiotic cell division was studied from pachytene/diakinesis to full meiotic metaphase by observing microsporocytes. A total of 863 microsporocytes were studied. In diakinesis, the pollen mother cells (PMCs) revealed n=7 (bivalents) for the taxa. These results thus complement the mitotic results reported earlier. Apart from the bivalents, the taxon presented different chromosomal associations, with varying frequencies of quadrivalent and hexavalents as shown in each of the plates 3. Table 1 shows frequency of occurrence of the pairing patterns in *C. stenopetalum.*

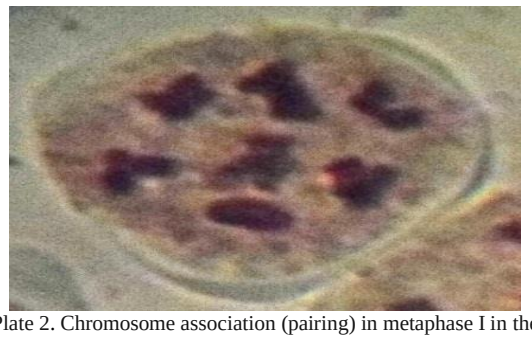

Plate 2. Chromosome association (pairing) in metaphase I in the *C,stenopetallum*, showing diakinesis with seven bivalents (7 II).

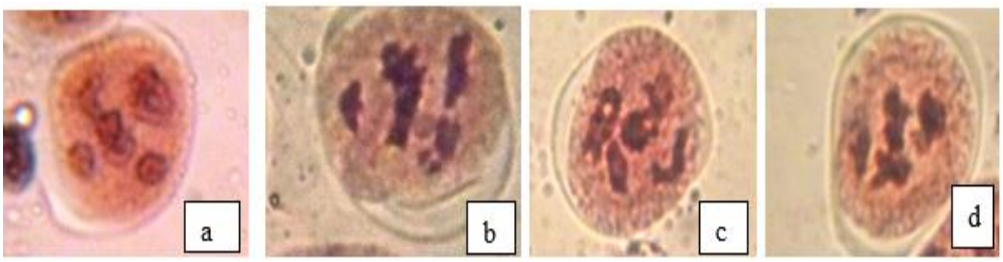

Plate 3a-d): Chromosome association (pairing) in metaphase I in the taxon. a) Metaphase with 1IV+5II. b) Metaphase showing 2IV+3II c) Metaphase with 1VI+4II. d) Metaphase with 3IV+1II. Other meiotic irregularities observed include; chromosome clumping on the spindle fibers, cross shaped configuration at the pachytene stage of meiosis and twisted ring and open ring configuration at meiotic metaphase.

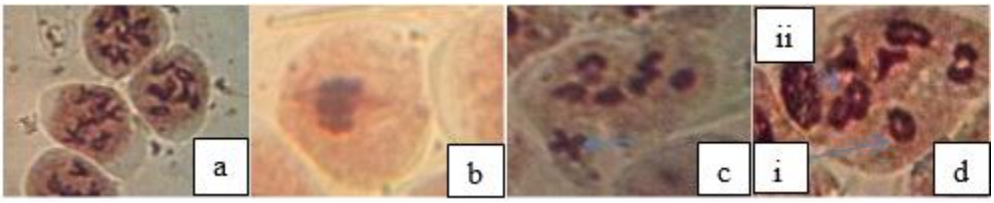

Plate 4; Meiotic irregularities: a, Chromosome pairing at pachytene stage b, first meiotic metaphase with spindle apparatus and chromosome clumping c, Cross shaped configuration at pachytene, di, Open ring configuration at meiotic metaphase ii, Twisted ring configuration at meiotic metaphase

Table 1. Chromosome pairing configurations (%) at diakinesis in Chlorophytum *stenopetalum* (I = univalents; II = bivalents; IV = tetravalents)

Taxon	Chromosome number		Pairing	Configurations (%)		
	2n=14	7II	5II+1IV	4II+1VI	3II+2IV	1II+3IV

C.stenopetalum	74.2	12.8	8.3	3.3	1.4

Table 2. Chromosome behavior at pachytene/diakinesis and metaphase I in Chlorophytum *stenopetalum.* Percentage (%) of observed configurations

Taxon	Observed formations at each of the stages			
	Cross configuration at pachytene	% of PMC with twisted ring	% of PMC with open ring	% Chromosome clumping
C.stenopetalum	25.3	12.1	21.3	40.4

The present study constitutes the first report on meiotic chromosome abnormalities and behaviour in *Chlorophytum stenopetalum* which revealed a high incidence of translocation heterozygosis with a remarkable variety of meiotic chromosome behaviour of a wild population of the genus *Chlorophytum* in Nigeria. During the meiotic analysis seven bivalents were observed at diakinesis confirming that the species exhibits 2n=14 chromosomes. This number (2n= 14) is in accordance with the previous reports for the species (Adeyemi, 1981; Ngwa, 1979 and Omokanye, 2016). The present work thus indicates *C.stenopetalum* belongs to x=7 series of the genus. Though most of the pollen mother cells at diakinesis had regular meiotic behaviour showing seven bivalents (74.2%), some of the homologous chromosomes paired up forming multivalent. In most cases, chromosomes intermesh losing their individual structure, forming a compact mass on the spindle fiber (Plate 4b), an abnormally generally referred to as chromosomal stickiness. Chromosome stickiness constitutes the highest frequency (40.4%) of abnormality observed at metaphase I. According to Pagliarini *et al.*(1993) and Muniyamma and Kameshwari (1996) the meiotic abnormalities such as tetravalents, precocious movement of chromosome, lagging chromosomes and chromatin bridges are found frequently in most of the species of *Chlorophytum*. Chromosome stickiness is caused due to genetic and environmental factors and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000). Gaulden (1987), reported that stickiness may result from defective functioning of one or more types of specific non histone protein involved in chromosome organization, which are needed for chromosome separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them (hereditary stickiness) or by the direct action of mutagens (induced stickiness). Olorode (1972) however, attributed meiotic irregularity such as multivalents, univalents laggard and non-disjunction in some Nigerian plants to structural heterozygosity or Polyploidization. Evidence of translocation heterozygosity is easily detectable as cross configurations which are observed at the pachytene stage (plate 4c). This can result from segmental translocation. At metaphase, the translocation heterozygotes have problem in pairing up hence the formation of open ring configuration at meiotic metaphase (Plate 4di) and twisted ring configuration at meiotic metaphase (Plate 4dii). However, the percentage of cross configuration observed (25.3%) is outstretched by the sum of total of twisted and open ring formation (33.4%), the presence of the two configurations in reasonable number establish the presence of translocation heterozygosity in the species. The spartial orientation of meiotic chromosomes of translocation heterozygotes in relation to the poles of the cell division influences the proper distribution of chromosome complements into daughter nuclei during meiosis I, and subsequent viability of spore formed by meiosis (Oyewole, 1987). The haploid number (n=7) reported in this work for *Chlorophytum* stenopetalum indicates that the species belongs to n = 7 series in the genus, thus confirming the existing diploid number (2n=14) reported for the species. Also, meiotic irregularities concerning chromosomes pairing (at metaphase I) is established. These anomalies may affect normal microspore formation, thereby reducing pollen viability and fertility of the species.

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