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Full Length Research Paper

Karyomorphotypic variation in *Eriospermum abyssinicum* Baker

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The karyomorphology of the cotton seed lily, *Eriospermum abyssinicum* Baker (family Eriospermaceae) was investigated through mitotic and meiotic studies. The chromosome complement was karyotypically analysed based on chromosome arm ratio and centromeric indices. The somatic chromosome complement of 2n = 24 recorded at metaphase I and the 12 bivalents at prophase I of meiosis suggested x = 6 basic chromosome number and paleopolyploidization process in the evolution of this species. There was no evidence of B chromosome or nucleolar-organizer in the complements. Variation in position of centromere ranged from the median to sub-median and sub-terminal. We found slight variation between some homologues in terms of lengths which suggest hybrid origin (allopolyploidy) of the genome. The microspore mother cell had normal meiosis with subsequent formation of 4 daughter nuclei and normal spores suggesting that the changes in chromosome behaviour occurred at very low frequency and these changes were transient with no evidence of phenotypic and genomic instability (aneuploidy) consequences. It was therefore inferred that the population of *E. abyssinicum* studied might have evolved through changes in chromosome structure or through natural hybridisation between closely related populations.

Key words: *Eriospermum abyssinicum*, lily, chromosome, karyotype.

INTRODUCTION

Eriospermum (Baker) (Perry) formerly part of the monocotyledonous *Liliaceae* is now placed in its own family *Eriospermaceae* (Vosa and Perry, 1999; Leistner, 2000; Opel, 2002). The family comprises a single genus with 102 species (Perry, 1994). As a genus, *Eriospermum* has been neglected in the past. The revision by Perry (1994) was the first attempt to study the genus since the accounts by Baker (1898). Though little known beyond specialists in the biology of deciduos geophytes, the genus is well distributed in the central, south and eastern parts of Africa. In Nigeria, it has a very wide distribution, being found on rock outcrops with humous topsoil from around Ilorin (guinea savannah – west-central Nigeria) up to Yola (sudan savannah - north-eastern Nigeria). *E. abyssinicum* has both medicinal and aphrodisiac properties (Mwafongo et al., 2010). It is used as an appetizer; as cure for swollen spleen, and for managing heart palpitations as well as love portion in some parts of Malawi in central Africa (Mwafongo et al., 2010).

Botanically, *Eriospermaceae* is well noted as a very isolated and advanced family. *E. abyssinicum* plants do not show morphological variations in spite of the wide distribution across varied geoecological zones. Leaf forms of *Eriospermum* vary from species to species. Leaves may be simple, heart shaped, or covered with protrusions called “enations”. *E. abyssinicum* however,

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possesses long-petiolate leaves which are linear-lanceolate and acute at each end. They are about 15 cm long and 1 to 1.3 cm broad. The root - stalk is a corm. It has slender raceme about 25 cm in length. Typical of all species, the subterranean potato-like tuber or corm produce leaves and flowers above-ground during the growing season, this die down when the plant enters dormancy, thus the plants survive unfavourable periods by retreating underground.

The ecology, systematics, and morphology of *Eriospermum* are poorly understood (Opel, 2002). In West Africa and Nigeria in particular, only a few species of the family *Eriospermaceae* have been subjected to analytical studies probably due to lack of appreciable representatives in the sub-region (Adeyemi, 1981); and thus accounts for the paucity of information on the genus.

Cytological studies have been extensively utilised to elucidate information on the taxonomy of various taxa of angiosperms (Oyewole, 1988; Clive, 2000; Tabur, 2012). Variations in size and form of the chromosome between genera or related species, and sometimes within populations of the same species have been of importance in classification and for certain evolutionary considerations. Such variations are known to represent different karyotypes. This study therefore investigated *E. abyssinicum* cytologically; using samples obtained from selected sites in Ilorin, west central Nigeria with a view to establish number of chromosome and understand karyomorphology and evolutionary relationship with other species within the genus.

MATERIALS AND METHODS

Plant materials

The *E. abyssinicum* plant populations used for this study were collected from wild populations growing across the 50 ha campus of the University of Ilorin, Nigeria. The sampling area is located in the southern guinea savannah agro-ecosystem on Lat. 8° 36'N and Long. 4° 36'E. Rainfall (1200 mm) is bimodal with peaks in June and September. Elevation above sea level is 375 m and sunshine is about 6.5 to 7.7 h daily from November to May (Ajadi et al., 2011).

Mitotic chromosome preparation

The root stalk of *E. abyssinicum* were transplanted in newly prepared pots filled with mixture of sand and saw dust and watered daily for 4 weeks to facilitate root and shoot development. The young root tips (1-2cm) were excised with a pair of scissors between 07.00 and 10.00 hours (local time). These were washed with water and pre-treated in 1, 4 – paradichlorobenzene for one hour at room temperature. Thereafter, the root tips were removed and washed in two changes of tap water and fixed in freshly prepared 1:3 glacial acetic alcohol. The specimen was labelled and stored in the refrigerator at 4°C for further analysis. Root tips for mitotic studies were removed from fixative, washed in two changes of tap water and hydrolysed in 5N HCL for 8-10 minutes at room temperature. Each root tip was placed on a clean microscope glass slide and the root cap teased away with a pair of clean forceps. Hydrolysed meristematic region of the root tip (about 1-2mm to the apex) was quashed and stained in few drops of 2% aceto-carmine stain on microscope glass slide. The chromosomes spread counts and measurements were carried out during metaphase at x400 magnification using Olympus SZ ^{R} optical research microscope and micrographs were made on a FOMAPAN DX 100, ASA 135 24 x 36mm for idiographs. At least 10 good spreads were measured for each plant sample per populations.

Mitotic chromosome counts and measurement

Chromosome counts of at least ten spreads were recorded for each sample made at x400 magnification. Measurement of chromosome size was done at x400 using a previously calibrated eyepiece micrometer graticle, on which one single unit of the micrometer eyepiece graticle is an equivalent of 2.5 and 1.0 µm at the 400 and x1000 magnifications, respectively. To minimize bias, ten spreads for chromosome counts were used for chromosome size measurements and an average was taken as the relative chromosome length. The centromeric position was identified as a position that separated the long arm (l) from the short arm (s) of each chromosome and the total chromosome length (c) were also recorded for each chromosome. Chromosome pair was identified based on the length and position of the centromere using an r-value centromeric index (the ratio of the log arm to the short arm) (Love and Love, 1974). The morphology of each member of homologous chromosome pair was examined for structural differences and similarities with regard to thickness, length and centromeric position. Idiograms of homologous chromosomes were constructed according to their relative length from the measurements made, to show the length of both the long and short arms, as well as the relative position of the centromere. These were then classified beginning with the longest to the shortest as adopted by Levan et al. (1964) and Oyewole (1975).

Meiotic chromosome studies

Fixed flower buds of E. *abyssinicum* (collected at the pre-anthesis stage) growing in the natural environments with the sample area (University of Ilorin, Ilorin) were used for meiotic chromosome studies. One flower bud was cut out and placed in a staining dish. The perianth segments were removed and the anthers released using a pair of mounted needles. One anther at a time was transferred onto a clean glass slide. The anther was thoroughly squashed and stained in few drops of 2% aceto-carmine stain and the microscope glass slide was made permanent with a clean cover slip smeared with glycerine albumen and flamed dry on a spirit lamp. Observations were made at x400 and cells at right stage with well spread chromosomes were studied for chromosome counts and behaviour at meiosis. The number of bivalents formed per complement was recorded. The number of chromosome bodies at metaphase I was counted for comparison with mitotic spread. Male meiotic products, mostly tetrads were also recorded at x400 magnification. Micrographs of meiosis at prophase I to spore tetrads were made using Carlzeiss photomicroscope with film FOMAPAN DX 100, ASA 135 24 x 36 mm.

RESULTS

The karyotype nomenclature and classification data of *E. abyssinicum* obtained from Ilorin, a southern guinea savannah agro-ecological zone of Nigeria is presented in Table 1. The Karyotype analysis was based on the absolute length of the chromosome and ratio of

Table 1. Karyotype nomenclature and classification data of *E.abyssinicum* (Ilorin population) (Homologous length in µm).

Karyotype Classification	Homologous length (µm)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
c	13	12	12	11	10	9	9	8	7	6	5	4
l	7.5	7.5	9.5	7.5	7	7.5	7	7	5	5	3	3
s	5.5	4.5	2.5	3.5	3	1.5	2	1	2	1	2	1
r	1.3	1.6	3.8	2.1	2.3	5	3.5	7	2.5	5	1.5	3
cl	m	m	st	sm	sm	st	st	st	sm	st	m	sm

c=individual chromosome length, l=long chromosome arm length, s=short chromosome arm length, r=chromosome arm index, cl=chromosome classification. Total chromosome complements length=106µm.

Table 2. Summary of centromeric positions and arm index (r-value) of *E. abyssinicum.*

Centromeric position	symbol	r- value	Calculated r-value	Number of chromosome per haploid complement
Median position	M	1.0	-	None
Median region	m	1.1 - 1.7	1.3 - 1.6	3
Sub-median	sm	1.8 - 3.0	2.1 - 3	4
Sub-terminal	st	3.1 - 7.0	3.5 - 5	5

Nomenclature and classification scheme adapted from Levan et al. (1964) and Oyewole (1975). Karyotype formula = 6m (3*2) + 8sm (4*2) + 10st (5*2).

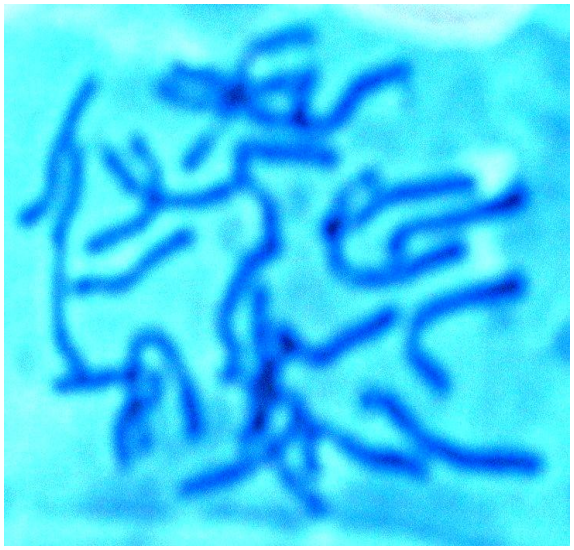

Figure 1. Somatic metaphase complement with twenty-four mitotic spread chromosomes from root tips of *E. abyssinicum* (Mag.x400).

chromosome short arms to long arms (centromeric location) (Levan et al., 1964; Oyewole, 1975). Total somatic chromosome counts of 2n = 24 was obtained for all the cells sampled at the metaphase stage of mitotic cell division (Figure 1). The chromosomes were morphologically grouped into 12 homologous pairs (Figure 2), whose length varied; ranging from 13 µm for the longest to 4 µm for the shortest chromosome (Table 1). Three of the twelve pairs of chromosome complement have centromere in the median region (r = 1.3 - 1.6), four in the sub-median region (r = 2.1 - 3), and five in the sub-terminal region (r = 3.5 - 5) (Table 2). The first pair of chromosomes has its centromere in the median region but it members show variation in the length of their long and short arms as well as in size (Figure 2). The second pair of chromosomes had slightly sub-equal arms with their centromeres located in the median region (Figure 2). The third pair of chromosomes had its centromere in the sub-terminal region, with a secondary constriction at the median region on the long chromosome arms. The fourth pair of chromosomes has a distended centric region in the sub-terminal position although there is a slight difference in the lengths of the long arms of the two chromosomes. The fifth pair of chromosomes is morphologically similar with sub- medially locate centromere. The sixth, seventh and eight pairs of chromosomes have their centromeres in the sub-terminal region, but the long arms of each pair are not equal. The centromere of the ninth pair of chromosome was located in the sub-median region but each member differed greatly in the length of its long and short arms. Similarly, the centromere of the tenth pair of chromosomes was in the sub-terminal region and each member also differed in the length of its long arms. The eleventh pair of chromosomes has their centromere in the median region, but differs slightly in the length of their long arms. The twelfth pair has the centromere in the sub-median position, the short arms are slightly sub-equal, but are

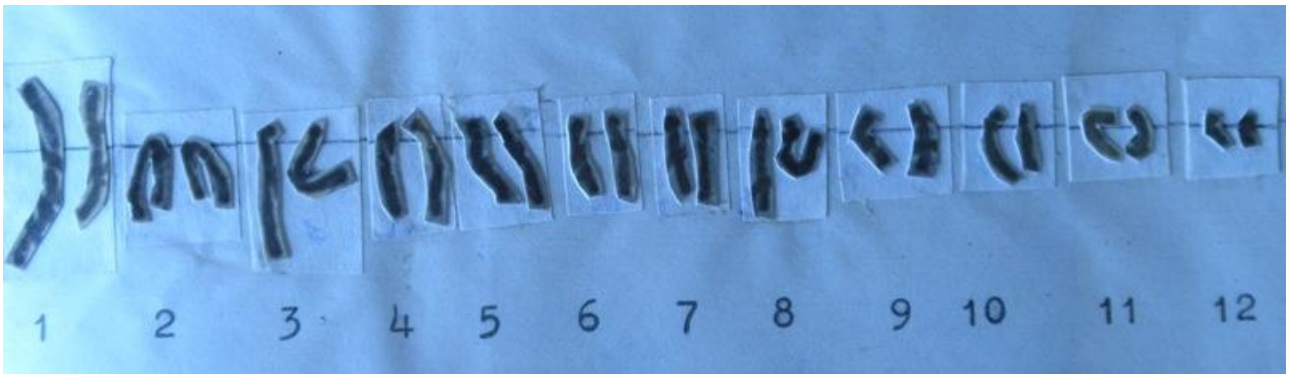

Figure 2. Twenty four somatic chromosomes (2n = 24) of *E. abyssinicum* showing each pair (homologues) in descending of size.

Figure 3. Twelve bivalents at prophase I of meiotic spread from flower buds of *E. abyssinicum* (Mag. x400).

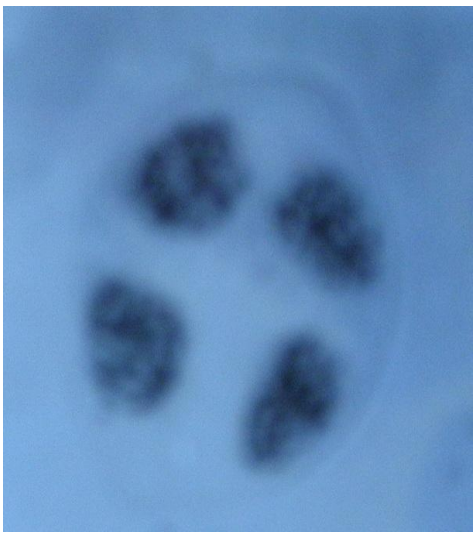

Figure 4. Telophase II of meiosis in *E. abyssinicum* showing 4 daughter nuclei (tetrads formation) (Mag.x200).

however more or less morphologically similar. In all, nine chromosome–pair members (1, 2, 4, 6, 7, 8, 9, 10, 11) showed morphological differences of unequalness of chromosome arms of varying degrees, while 3 pairs (3, 5 and 12) are more or less morphologically similar (Figure 2). The chromosomes were all median (m), sub-median (sm) and sub-terminal (st) according to the classification system of Levin et al. (1964) and Oyewole (1975). Thus, the population of *E. abyssinicum* studied has 2n = 24 chromosome complements with karyotype formula of 6 m + 8 sm + 10 st (Table 2).

Meiotic behaviour of *E. abyssinicum* with regard to pairing and segregation of chromosomes was normal. At pachytene stage in early prophase, 12 pairs of bivalents were observed (Figure 3). There were also 12 pairs of chromosomes at metaphase I stage of meiotic division, confirming the chromosome number established in the mitotic studies (Figures 1 and 2). Telophase II was normal, leading to formation of 4 daughter cells (tetrads) with haploid sets of the chromosomes for reduced pollen (male gamete) production (Figure 4).

DISCUSSION

The chromosome is a unique, definite and stable entity of any living organism and its form, number, and size have been used along with morphological and ecological differences to circumscribe populations of plants (Böcher et al., 1953; Harding et al., 1991; Greilhuber, 1995; Aliyu and Awopetu, 2007). In this study, it has been shown that *E. abyssinicum* possessed 24 somatic chromosomes (2n = 24) which were morphologically grouped into 12 homologous pairs and confirmed by meiotic pairing. However, slight variation recorded in the karyomorphology of each pachytene pair of chromosomes could help in understanding the process of chromosome evolution not only in this plant but other importance close relatives like *Aloe species*. When such significant differences occurred, it pointed unmistakably to the existence of more than two genomic contributions

to the complement (Winge, 1917; Oyewole, 1982). Due to the poor development of the pachytene chromosome photographs, the pachytene chromosome could not be studied effectively. However the individual somatic chromosome resolved into twelve pairs shows morphological differences such as unequalness of chromosome arms which is most evident in pairs 1, 4 and 10 and less evident in pairs 3, 5 and 12. These morphological differences suggest the existence of multiple genomic contributions to the complement. Furthermore, the morphological difference alone may not totally explain the multiple genomic complementation since morphologically different chromosomes could have uniform and homologous euchromatic material (Oyewole, 1988). Even then, such morphological differences denote differences of origin of the chromosomes. It is therefore probable that the population of *E. abyssinicum* studied originated by natural hybridisation.

The evolution of chromosome of genus *Eriospermum* is complex, with a proposition that x = 6 basic chromosome number of *E. abyssinicum* must have evolved through chromosomal fusion and reduction in haploid set from x = 7 to x = 6 (Vosa and Perry, 1999). Our data here corroborated this x = 6 basic chromosome number of this plant species. The 2n = 24 recorded from the somatic tissues suggest that modern *E. abyssinicum* is polyploid arising from genome duplication (chromosome doubling) consequence of induction by genomic shock arising from either genomic hybridization or environmental factors or both (Comai et al., 2003). While the focus of this study was not targeted at the evolution of chromosome of *E. abyssinicum*, the 2n = 24 chromosomes consistently recorded from the somatic tissues and slight variation between homologues pointed towards the idea that evolution of *E. abyssinicum* chromosome could have arisen through hybridization of closely related species with x = 7 and x =5 basic chromosome to form an unstable hybrid with 2n = 12 and the hybrid undergoes allopolyploidization to form stable “allotetradiploid” 2n = 24 through paleopolyploidization to restore diploidy. The co-occurrence of many closely related species such as *E. fragile, E. ciliatum*, *E. arenosum* etc. with x = 7 and occurrence of *E. aphyllum*, *E. spirale*, *E. flavum* with x = 5 basic haploid chromosome sets favours hydridization and polyploidization theory for evolution of *E. abyssinicum* chromosome more than chromosomal loss theory (Vosa and Perry, 1999). In actual fact, there are footprints of “tetraploid set” in the chromosome compliment (Figure 2) that tend to support the allopolyploidization theory. Examples of hybrid species that contain two or more diploid sets of parental genomes are common in nature (Soltis and Soltis, 1993; Leitch and Bennett, 1997; Rieseberg, 2001; Ma and Gustafson, 2005). Few among these ‘diploid hybrid’ species that display the unmistakable footprint of a diploidized allotetraploid genomes are maize (Gaut et al., 2000), buffelgrass (*Pennisetum ciliare* L.) (Jessup et al., 2003), and soybean (Lee and Verma, 1984; Nielsen et al., 1989). Allohexaploidy has been reported in Sweet potato (*Ipomea batatas* L.) of subgenus *Eriospermum*, a closely related genus of *E. abyssinicum* (Austin, 1988). Therefore, allopolyploidy has been described as an important mechanism of reticulate evolution and Ma and Gustafson (2005) remarked that many plants traditionally considered to be diploid are actually stabilized diploid-like paleopolyploids.

The morphological differences in the chromosome homologues observed in this study could also have resulted from changes in the chromosome structure. It is known that changes in chromosome structure occur in nature quite frequently and have been instrumental in the evolution of many species of plants and animal (Sinha and Sinha, 1980). Rapid genomic sequence elimination is known to play significant role in the diploidization process of allopolyploids (Ma and Gustafson, 2005). Also, the changes in chromosome structure could have occurred through breakage of chromosome in which the broken piece attaches to one end of a non-homologous chromosome (Vosa and Perry, 1999). The absence of nucleolar organiser synonymous with secondary constriction (Berns and Cheng, 1971) in contrast to Vosa and Perry (1999) further advances the chromosomal structural changes during evolution of modern *E. abyssinicum*. Such nucleolar organizers must have been lost during polyploid evolution (Vaughan et al., 1993). Normal pairing of the chromosome homologues in microspore mother cell however indicates that such changes have not affected meiotic recombination significantly to produce any change in the genetic system.

This study had revealed also that *E. abyssinicum* showed normal meiosis. This normal pairing of the microspore mother cell chromosomes which resulted in regular formation of four daughter nuclei and subsequent formation of spore tetrads is an indication of the potential high fertility of the pollen grain (Aliyu and Awopetu, 2007) and a stable genetic system. This suggests that changes in chromosome behaviour may have occurred at a very low frequency such that these changes are transient and of no adaptive importance.

CONCLUSION AND RECOMMENDATIONS

In this study, our data have shown evidence that *E. abyssinicum* (Ilorin population) could have evolved through changes in the chromosome structure or through natural hybridisation between closely related populations. This implies that *E. abyssinicum* might consist of a complex species with related individuals capable of sexual reproduction. Nonetheless, such structural changes have not affected the normal meiotic recombination process that lead to stable genetic system. Further study on individuals/populations adapted to

different geo-ecological conditions would through more light in the direction of evolution and genetic diversity of this important plant species. High resolution technique like fluorescence in situ hybridization (FISH) in addition to molecular marker technologies will further elucidate the evidence of their genome evolution and provide reliable physical map for this plant species.

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