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Research

Karyotypic analysis and meiotic chromosomes in eight taxa of *Solanum* species (Solanaceae)

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Abstract

Chromosome counts revealed n=12, 24 and 2n=24, 48 for the diploid and tetraploid species respectively. Chromosomes were symmetrical in Solanum erianthum, S. torvum and S. indicum var. distichum while the varieties of S. melongena and S. macrocarpon showed few asymmetric chromosomes. In these varieties, meiotic abnormalities revealed clumps, univalents, multivalents and bridges. From homomorphic pairing, chromosomes were all long in the varieties of S. melongena. They were 7 long and 5 short in S. macrocarpon population I and, 5 long and 7 short in populations II and III. In S. erianthum and S. gilo, chromosomes were 6 long and 6 short and, 8 long and 4 short respectively. Homomorphic pairing revealed structural details of the chromosomes and their possible origin. Meiotic chromosomes suggest past contact with foreign genes through hybridization which explains the rationale for the emergence of different adaptive features in the species. The evolution of the different chromosome races is discussed.

Keywords: Karyotype, chromosome, *Solanum*, anthesis, taxonomy

Introduction

The genus *Solanum* is of considerable economic importance in Nigeria and across West African sub-region. Several species are domesticated for food, medicinal and horticultural purposes. Few species grow among cultivated crops as weeds and are responsible for several crop failures over the years. The wild also serves as reservoir for many yet identified species.

The most frequently encountered chromosome number reports are 2n=24; 48 among the West African Species. The few variations that had been reported included 2n=58, 60 (Okoli, 1988); n=18, 36 (Bir and Neelam, 1984), established aneuploids, 2n= 54, 66 (Govindarajan and Vijayakumar, 1986). These latter reports raise questions as to the exact ploidy level of species and their relationships and evolutionary statuses.

Several chromosome reports described both the somatic and gametic counts but their structural details. Ugborogho and Oyelana (1999) and Omidiji (1983) opined that the dearth of information on the emerging structural differences in species' genomes may have been responsible for the many hybridization failures recorded among few species within the genus.

Consequently, the lack of sufficient information about the nature and extent of structural changes in chromosomes make this study imperative. This study highlights detailed structural peculiarities and behavior of chromosomes at meiosis. This is with a view to elucidating the origin of the different chromosomes races earlier reported and suggest species' relationship and evolutionary statuses within the genus.

Material and methods

Mitotic chromosomes

Young root tips obtained directly from seeds soaked

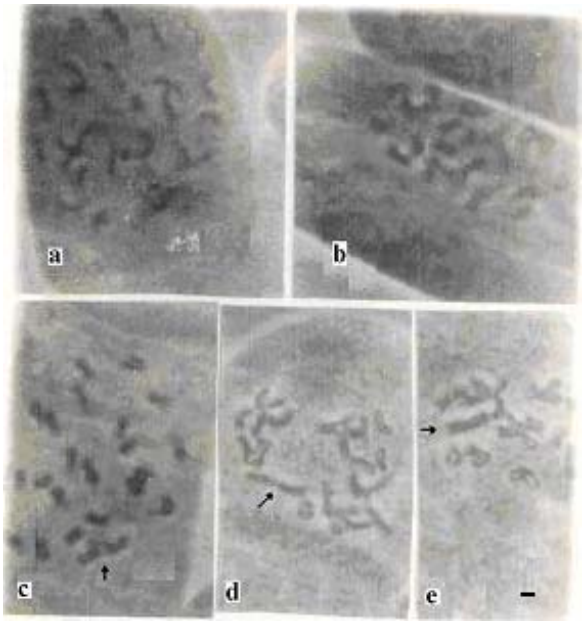

Fig. 1: Mitotic chromosomes a, b. *S. melogena* a. var. *inerme*, b. var. *bomo* c-e. *S. macrocarpon* c. population I, d. pop. II, e. pop. III, a-e. 2n=24, arrowed 2, Scale bar = 1µm

ed in Petri-dishes were fixed in saturated paradichlorobenzene for two hours, washed in distilled water and immediately transferred into 1:3 acetic alcohol for 24 hours. Hydrolysis was carried out in IN HCL for five minutes at 60°C. The hydrolysed roots were later stained in 2% acetocarmine and squashed.

Meiotic chromosomes

Young flower buds were fixed in 1:3 acetic alcohol for 24 hours. Anthers were teased out on slides, stained with drops of acetocarmine and squashed gently to tease out the pollen mother cells. Slides were observed under a Wild compound microscope for detail analysis of chromosome counts, morphology and meiotic configurations.

Measurement of chromosomes

The sizes of chromosomes were measured with an eye piece graticule at x40 objective. Chromosomes of 1.50-4.50µm and, 0.49-1.49µm were regarded as long and short respectively.

Results

The two subspecies of *Solanum nigrum* were tetraploids (2n=48) while the varieties of *S. melongena*, populations of *S. macrocarpon*, *S. gilo*, *S. erianthum*, *S. indicum* var. *distichum*, *S. torvum* and *S. aethiopicum* were diploids (2n=24) (Figs. 1-3). Table 1 shows details of chromosome counts for the past and present reports. Aneuploids were observed in few cells of *S. macrocarpon* population II (2n=20, 22) and *S. nigrum*

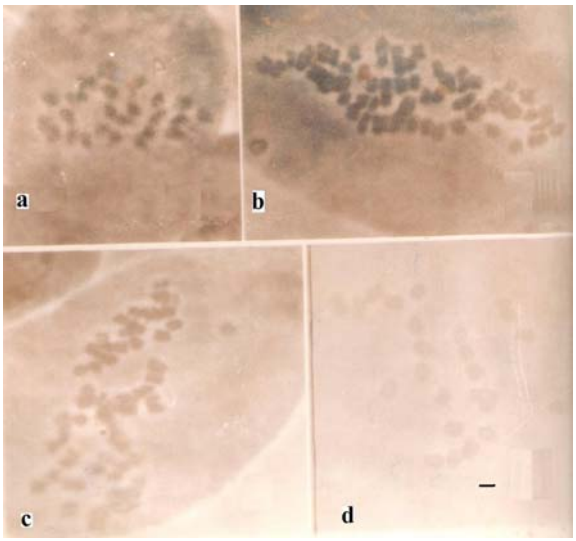

Fig. 2: a. *S. torvum* 2n=24, b-c. *S. nigrum* b. ssp. *erectum*, c. ssp. *nigrum* 2n=48, d. *S. erianthum* 2n=24, Scale bar = 2µm

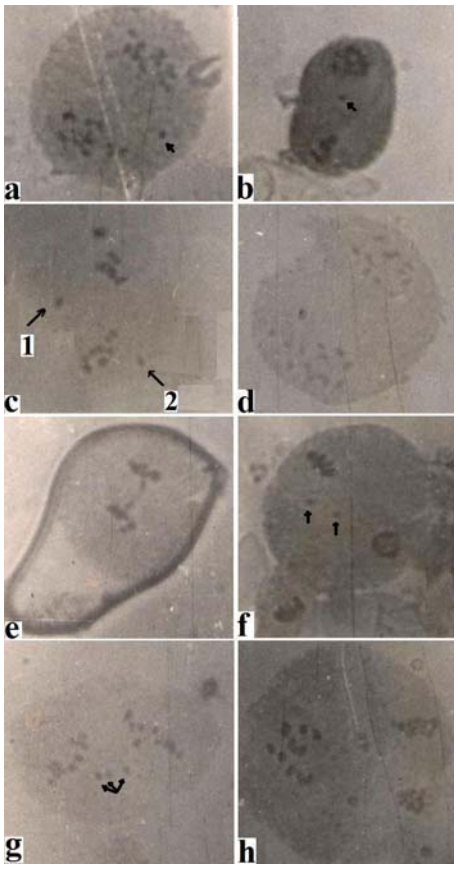

Fig. 3: Meiotic chromosome behaviour a-d. anaphase-1, a, b. S. melogena vars. inerme & bomo, c. S. aethiopicum, d. S. macrocarpon pop. I, e. anaphase bridge, S. gilo, f. telophase 1 & II, S. melongena var. bomo, g. metaphase II, h. anaphase II, g, h. S. aethiopicum; a, c2, g. eliminated bivalents/fragmented chromosomes (arrow), b, c1, f, h. lagging bivalents (arrow), d, h. unequal chromosome distribution.

Table 1: Past and present chromosomes reports

Species	Past report			Present report				
	Chromosome no.		Authority	Taxa	Chr. no			X
	n	2n			n	2n	(n)	(2n)
S melongena L.	12		Bir & Neelam, 1980, Choudhuri, 1975;	*S. melongena*				
		24	Rao *et al*., 1976, Choudhuri, 1975	var. *inerme*	12	24	-	-
				var. *bomo*	12	24	-	-
S. macrocarpon	12		Omidiji, 1983;	*S. macrocarpon*				
L.		24	Rao *et al*., 1976	pop. I	12	24	-	-
				pop. II	12	24	13	20, 22
				pop. III	12	24	-	-
S. indicum L.	12	24	Kirti & Rao, 1978; Krishnappa &	*S. indicum*	12	24	10,	22
			Chennavaeraiah, 1975	var. *distichum*			13	
S. aethiopicum L.	12	24	Okoli, 1988	*S. aethiopicum*	12	24	-	-
S. gilo Raddi	12	24	Rao *et al*., 1976	*S. gilo*	12	24	-	-
S. torvum SW.	12,24		Vasudevan, 1975, Randell&Symon, 1976;	*S. torvum*	12	24	-	-
		24	Rao *et al*., 1976					
S. nigrum L.	12,18,		Vasudevan 1975; Aninuddin *et al*., 1985,	*S. nigrum*				
	24,36			ssp. *erectum*	24	48	-	54,58
		48,54,	Ceschmedjiev, 1976, Labadie 1976;	*S. nigrum*				
		56, 72	Crompton & Bassett, 1976, Leslie, 1978	ssp. *nigrum*	24	48	-	-
S. erianthum Don	12		Bir *et al*., 1978, Vasudevan, 1975;	*S. erianthum*	12	24	-	-
		24, 66	Gill, 1975, Mehra, 1976					

X = variations in haploid and diploid numbers observed

Table 2: Somatic chromosome size of *solanum*

Taxa	Range µm		x± SD µm	Total genomic complement
	Min	Max		
S. melongena				
var. *inerme*	2.70	4.12	3.44±0.39	82.54
var. *bomo*	1.99	4.23	3.52±0.57	84.52
S. macrocarpon				
pop. I	1.08	3.65	2.33±0.95	56.06
pop. II	1.46	3.99	2.72±1.0	65.17
pop. III	1.18	3.69	2.38±0.93	57.14
S. indicum	1.06	1.41	1.23+0.12	24.53
var. *distichum*				
S aethiopicum	1.06	1.29	1.15+0.07	21.25
S. gilo	0.96	1.88	1.51+0.24	36.22
S. torvum	0.94	1.29	1.02+0.14	15.77
S. nigrum				
ssp. *erectum*	1.18	1.65	1.41+0.15	67.85
ssp *nigrum*	1.18	1.65	1.41+0.18	67.79
S. erianthum	1.06	1.88	1.46+0.23	35.16

subsp. *erectum* (2n=54, 58). Length of chro-mosomes varied between 0.94µm and 4.23 µm (Table 2). The homomorphic chromosome pairs were all long in the two varieties of *S. melongena*; 5 long and 7 short in *S. macrocarpon* population I and 7 long and 5 short for the other two populations (II and III). Homomorphic pairs were 6 long and 6 short; 8 long and 4 short in *S. erianthum* and *S. gilo* respectively while the 24 pairs were all short in the two subspecies of *S. nigrum* (Figs. 4-7). Chromosomes were generally symmetrical and the types based on the relative position of centromere and arm ratio (Figs. 4-7). Chromosomes were very small in *S. aethiopicum*, *S. torvum* and *S. indicum* var. *distichum* and the types based on the position of centromere could not be ascertained. The total genomic complement and mean sizes are given in Table 2. Haploid sets, n=24, in the tetraploid subspecies of S. *nigrum* and, n=12, in the diploid species were observed. In the tetraploids and few diploids such as S. *erianthum*, *S. torvum*, *S. indicum* var. *distichum*, bivalents were regularly paired while occurrence of clumps and multivalents were evident in the varieties and populations of *S. melongena* and *S. macrocarpon* (Fig. 3e & g). The univalents appeared as laggards or isolated chromosomes (Fig. 3a, b, e, f, & i). Delayed anaphic movement caused unequal chromosomes distribution in cells of few of the diploids viz: *S. macrocarpon* pops. II & III, *S. melongena* var. *inerme* and *S. aethiopicum* (Fig. 3d & f).

Chromatid bridges were encountered in S. *gilo* and varieties of *S. melongena*. The second meiotic division was generally regular as almost all the abnormalities earlier observed were successfully eliminated.

Discussion

The chromosome counts of n=12, 24; 2n=24, 48 in the respective diploid and tetraploid species confirm some of the earlier reports (Omidiji, 1983; Bir & Neelam, 1980). Somatic instability was evident in all the domesticated species viz: *S. aethiopicum* and the taxa of *S. macrocarpon* and *S. melongena*. This might have been responsible for the few aneuploid cells encountered in this report. Preponderance of aneuploid races and its implication to phylogeny and taxonomy of species among several genera in the family

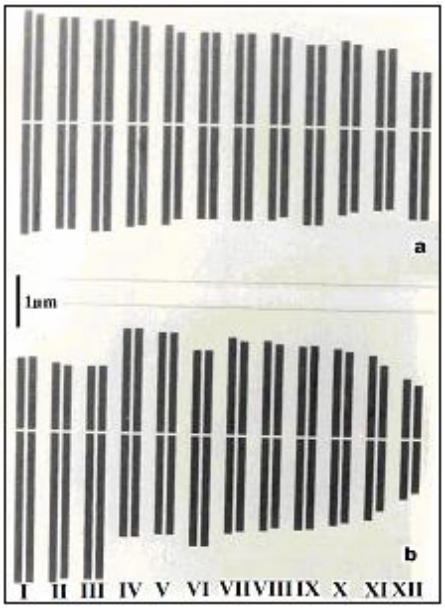

Fig. 4: Karyotype of S. melongena, a. var. inerme, chromosomes I-XI metacentric, XII submetacentric b. var. bomo, I-III submetacentric, IV-XII metacentric

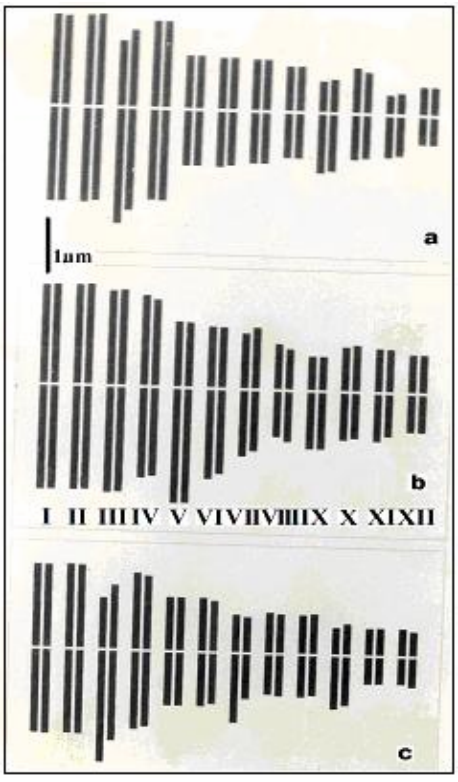

Fig. 5: Karyotype of S. macrocarpon a. population I, chrom. I, II, IV-VIII, X, XII metacentric, III & IX sub-metacentric, XI subtelocentric, b. pop. II, chrom. I-IV, X, XI, XII metacentric, V-VIII submetacentric, IX subtelocentric, c. pop. III, chrom. I, II, IV-VI, VIII, IX, XI, XII metacentric, III, VII submetacentric, X subtelocentric.

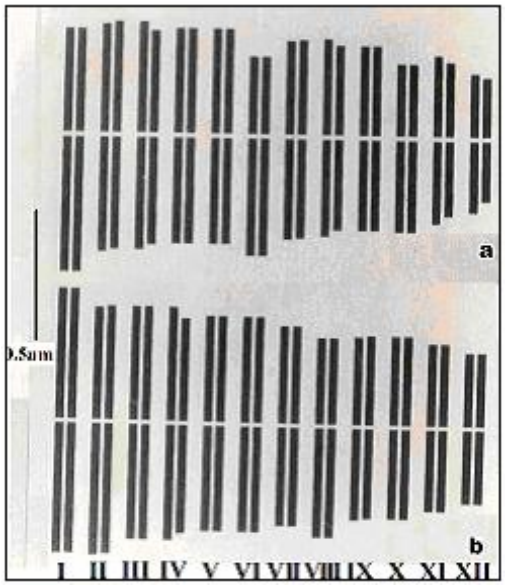

Fig. 6: Karyotype a. S. gilo, chromosomes I-V, VII- IX, XI, XIII metacentric, VI, X submetacentric. b. S. erianthum, all chromosomes are metacentric

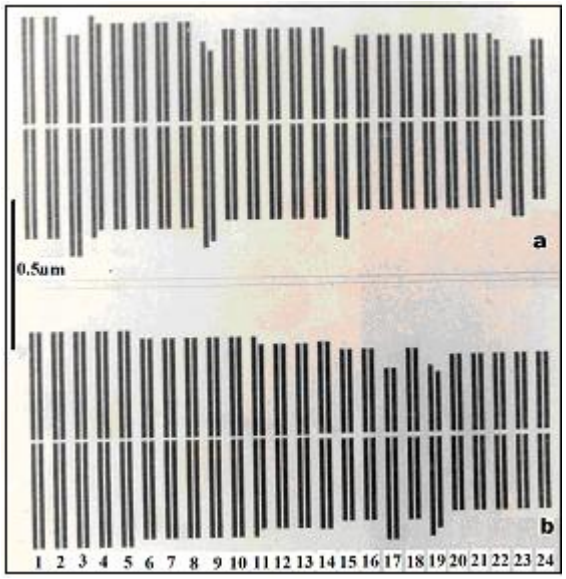

Fig. 7: Karyotype a. S. nigrum subsp. erectum chromosomes I, II, IV-VIII, X-XII, XIII, XIV, XVI-XX, XXI, XXII, XXIV metacentric, III submetacentric, IX, XV, XXIII subtelocentric. b. Karyotype of S. nigrum subsp. nigrum, all chromosomes metacentric except XVII, XIX which are submetacentric.

Araliaceae was extensively discussed by Ti *et al.* (2004). They equally implicated genomic instability in these species and underscored its importance in the evolution of the few aneuploids observed among related species within the family. The meiotic chromosomes of *S. aethiopicum*, *S. melongena* and *S. macrocarpon* were unstable. They were characterized by

clumps, univalents, multivalents and bridges in over 30% of the cells. Luceno and Adeo (1994) encountered similar irregularities in the meiotic chromosome configurations in Iberian species of *Carex* and subsequently employed this in identifying existing hybrids and the origins of other related species. The occurrence of lagging and isolated chromosomes which led to unequal distribution of chromosomes at anaphase I and II confirm unstable genomes in these groups of species and suggest probable introduction of foreign genes from past hybridization efforts.

Patterns of karyotype are increasingly being used in assessing phylogenetic relationships among different species (Pringle & Murray, 1991; Pandit & Babu, 1993; Diosdado & Pastor, 1993 and Brandhan, 1983). Basically, the karyotype of these species consisted mostly metacentric and submetacentric chromosomes. However, few subtelocentric chromosomes were encountered. The taxa of *S. melongena* and *S. macrocarpon* had the largest chromosomes while *S. torvum* had the smallest (Table 2). *S. torvum* was characterized by symmetrical chromosomes and regular bivalents at meiosis. This was contrary to the views of Pringle and Murray (1993) who observed a significant complement between large chromosome size and symmetry of chromosome arms. They equally observed this as a distinct trend in chromosomes of many genera of the Solanaceae, including few species of *Solanum*. Asymmetrical chromosomes were clearly evident in the species (*S. melongena* and *S macrocarpon*), as homomorphic pairs revealed chromosomes with unequal arms (Figs. 4-7). This might have been the result of shift in the position of centromere due to breaks and the subsequent rearrangement of chromosome arms. From the report of Hanson *et al*. (2003), asymmetry and non-homology of chromosomes have been linked to species' evolutionary divergence. Therefore, the emergence of several chromosome types in varieties and populations of *S. melongena* and *S. macrocarpon* appear to have been accompanied by changes in chromosome sizes. It is probable that genomic evolution among the species of *Solanum* resulted from structural chromosomal changes as evident in *S. melongena* and *S. macrocarpon* as well as changes in base chromosome number or ploidy in the few aneuploid cells encountered in the study. The latter confirms the origin of the few aneuploid species earlier reported for the genus (Okoli, 1988). The few chromosome fragments and laggards observed in some cells of *S. melongena*, *S. macrocarpon* and *S. aethiopicum* equally affirm this assertion. Consequently, in this genus, the evolution of metacentric chromosomes was likely a primitive feature while the submetacentric and subtelocentric chromosomes were advanced. Evidence could be seen in the occurrence of chromosome fragments and unequal pairing of bivalents in few species (Fig. 3). These are indicative of changes in chromosome structures and dissimilar chromosome complements among species in the genus. Interspecific hybridization equally may have introduced a number of foreign genes, leading to unstable genomes. Ugborogho and Oyelana (1999) and Omidiji (1983) shared the same view. As a consequence, the unreduced gametes that may have arisen from irregular meiosis gave rise to series of aneuploids, and hence, evolution of new chromosome races. This was achieved as unreduced pollen became successfully incorporated into any of the regular diploids through hybridization. Brysting *et al*. (2004) equally attributed the emergence of different chromosome races in the genus *Dupontia* to fertilization of regular diploids by unreduced gametes from one or more of the several ploidy levels. Several workers including Ugborogho and Oyelana (1992) have employed various chromosome configurations at meiosis to establish relationship among related species of angiosperms. It is therefore suggested that the varieties of *S. melongena* and populations of *S. macrocarpon* are likely hybrids from natural hybridization of closely related diploid species. Knapp (1991) had earlier identified a number of hybrid swamps and concluded that this is a common occurrence in the genus. It is pertinent to draw attention to the several ongoing attempts at improving the agronomic qualities of few domesticated species through intraspecific hybridization. This practice will continue to bring about changes in chromosome forms and unstable genomes in the genus.

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