

Short Communication

Cytogenetic studies on some Nigerian species

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Cytogenetic studies to determine the chromosome number, structure and behaviour of some species of *Solanum* in Nigeria was carried out. Attempt was also made to induce polyploidy in the species. Comparative analysis of the cytological behaviour of the diploid and polyploid cytotypes was made. The studies show that the somatic chromosome number in the genus *Solanum* is $2n = 24$. Colchiploidisation produced tetraploids with $2n = 48$ and aneuploids with $2n = 69$. The karyotype analysis indicates that the chromosomes were generally very small. There were also slight variations in centromeric positions and arm lengths. Meiotic behaviour of the chromosomes involved a combination of bivalent and multivalent associations especially at the polyploid levels. The significance of this work in the understanding of cytogenetic behaviour of plants and crop improvement efforts are discussed.

Key words: Cytogenetics, *Solanum*, colchiploidisation, crop improvement.

INTRODUCTION

The genus *Solanum* L belongs to the family Solanaceae in the order Sonales. It is one plant genus of great importance for food security in most countries of the developing world. Some of its members are cultivated mostly as vegetable crops (Kochlar, 1981), while the active principles such as solinidine and other steroids extracted from the roots and leaves of some species are useful pharmaceuticals (Simmonds and Choudhury, 1976). Purseglove (1968) noted that many *Solanum* species are used as potherbs or ornamentals in the tropics. However the lack of immediate known utilities for some members of this group has led to their neglect and subsequent genetic erosion. Rapid depletion of such potentially useful plant resources because of skewed selection pressure calls for an urgent reappraisal of germplasm exploitation and conservation in our area (Obute and Omotayo, 1999; Okujagu, 2005). Despite the importance of *Solanum* species, there is still paucity of

information on the karyotypes of the members of the taxa in Nigeria.

Studies on cytogenetics, chromosome structure, behaviour and manipulation in plants are well documented (Karpenchenko, 1925; Sarbhoy, 1977; Okoli and Olorode, 1983; Obute, 2001). The usefulness of information from such studies in the understanding of phylogenetic relationships, genetic mapping and breeding studies has been very significant (Okoli, 1993; Heslop-Harrison and Schwarzacher, 1993; Hartwell et al., 2000; Kurata et al., 2002). Available literature on the basic and applied chromosome features of *Solanum* species is rather scanty. This is quite precarious given the urgent need for the documentation of the basic chromosome structure and behaviour of important crop species as an initial step towards manipulating these chromosomes for specific purposes in crop improvement. It is against this realization that the present attempt at describing the chromosome features in some Nigerian species of *Solanum* L. in relation to their morphology, distribution of chromatin, karyotype features as well as targeted doubling of chromosomes for production of gigas characters is embarked upon.

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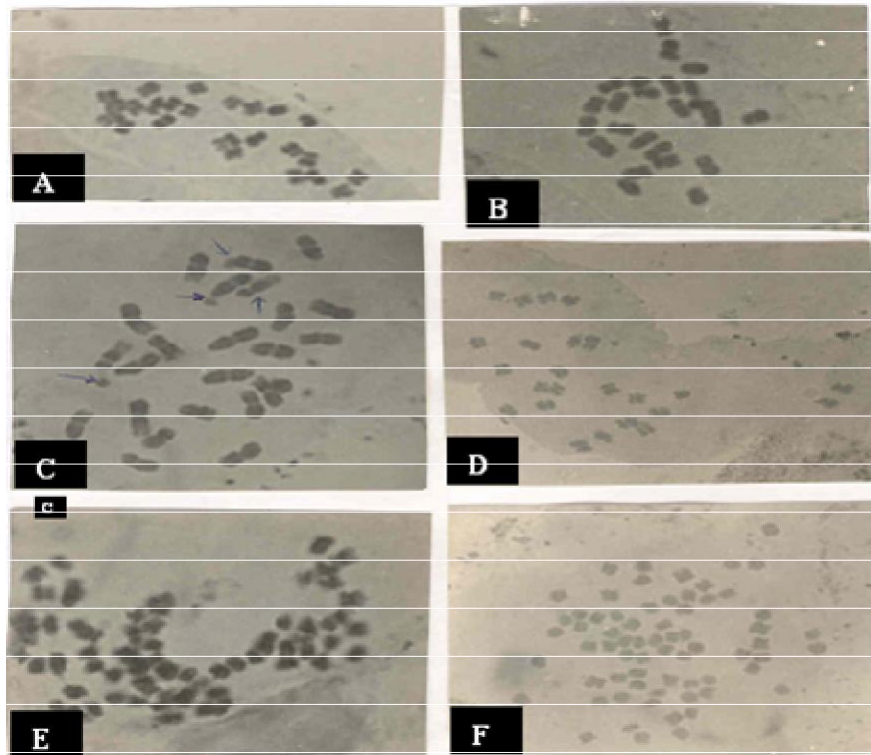


Figure 1. Mitotic chromosomes of *Solanum* L. (A) $2n = 24$ in *S. indicum*, (B) $2n = 24$ in *S. torvum*, (C) $2n = 24$ in *S. melongena* showing satellite chromosomes (arrow - indicated), (D) $2n = 24$ of *S. aethiopicum* (notice light heterochromatin), (E) an autotetraploid in *S. aethiopicum* $2n = 48$ and (F) an aneuploid cell in *S. indicum* with $2n = 69$.

MATERIALS AND METHODS

Collection

Fruits of *Solanum* species were collected during field trips to different parts of southern Nigeria. Seeds extracted from mature fruits of the various species; *S. torvum*, *S. aethiopicum*, *S. melongena* and *S. indicum*, were grown to the F_3 generation in the green house of the Botanic Garden of the University of Port Harcourt before using them to generate roots. The root tips for mitotic studies were obtained from healthy seedlings.

Mitotic studies

F_3 seeds were germinated on moistened absorbent paper in Petri dishes kept in the dark at temperatures of 28 - 30°C. Primary roots measuring about 5 to 10 mm long were harvested during late afternoons for slide preparation. Seedlings of *S. torvum* and *S. indicum* could not yield enough roots for this protocol. To enhance root production, therefore, their root systems were completely excised just below the soil level and the stems dipped in 10 ppm indole butyric acid for up to 6 h. They were later transferred to dilute nutrient solution for about 168 h to yield a good crop of roots. Pretreatment in all the species was with 0.002 M aqueous solution of 8-hydroxyquinoline (w/v) for at least 3 h. The root tips were then fixed in 1 part glacial acetic acid and 3 parts ethanol (v/v) for a minimum of 24 h. The roots that were not required immediately for slide preparation were stored in 70% ethanol in a refrigerator. The

root tips were hydrolyzed in 9% hydrochloric acid for a minimum of 5 min and squashed in formic-lactic-propionic acid-orcein (FLP-orcein) stain. Chromosomes were examined at x400 magnification using Leitz Labolux microscope fitted with photographic equipment. Good plates with well spread chromosomes were photomicrographed while measurement of chromosomes was done with an ocular micrometer.

Colchiploidisation

Chromosome doubling was carried out using the cotton-plug method. This involved assaulting growing shoot tips in nursery boxes with cotton wool soaked with 0.2% w/v colchicine for at least four days before transplanting the seedlings to the field. The treatment was carried out for 10 stands of each of the species under study while untreated specimens served as controls. Treated specimens were monitored till maturity and their fruits collected along side those of the controls. Seeds from such putative colchiploid fruits were germinated and squashes made from roots collected from these following the above protocols of pretreatment, fixation, hydrolysis, staining and squashing. Chromosome counts were made to confirm doubling of chromosomes.

Meiotic chromosome behaviour

Immature flower buds were collected from flowering plants of both the diploids and colchiploid during late afternoons and immediately



Figure 2. Responses to colchicine treatment in species of *Solanum* L. (A) Gigas nature of first formed leaves following colchicine treatment in *S. aethiopicum* and (B) suppression of shoot growth in *S. indicum* following treatment with colchicine.

fixed in Carnoy's fluid for a minimum of 3 h. Anthers were dissected out from these buds and fixed in FLP-orcein. The stages of meiosis were monitored and the associations during diakinesis stage were assessed in both the diploid and colchiploid material, to ascertain chromosomal affinity. Staining pollen grains from immature flower buds with a drop of cotton blue in lactophenol was used to assess pollen grain characteristics. After staining these were left to stand for at least 48 h to ensure proper turgidity of the pollen grains for easier assessment. Deeply stained grains were adjudged fertile while poorly stained, unstained and malformed pollen grains were taken to be sterile.

RESULTS

Mitotic chromosome counts

Mitotic chromosome counts showed that the $2n = 24$ was the diploid number for *S. indicum*, *S. torvum*, *S. aethiopicum* and *S. melongena*. The chromosomes were generally small in size and differed slightly in their centromeric positions. The centromeres stained lightly compared to the arms in all the species studied. A high level of heterochromatinization was noticeable in *S. indicum*, *S. torvum*, *S. melongena* and the colchiploid of *S. indicum* than in the diploid and colchiploid of *S. aethiopicum* (Figure 1). Chromosomes of *S. indicum* (Figure 1a) were more or less identical in sizes and their centromeric positions were all metacentric. No secondary constriction was observed in any of these chromosomes. *S. torvum* chromosomes were comparatively of the same length; eight of the chromosomes or four pairs displayed submetacentric morphology while the rest were metacentric (Figure 1b). The chromosomes of *S. melongena* (Figure 1c) were fairly similar in size, four pairs showed sub-median centromeres and two pairs out of these had secondary constrictions (arrow-indicated).

For *S. aethiopicum* the chromosomes were about the same size as well, there were no secondary constrictions and the primary constrictions were metacentric in location (Figure 1d).

Cochiplodisation

The species responded differently to the treatment with colchicine. While there was increase in the number of chromosomes in two species (*S. indicum* and *S. aethiopicum*) this was not observed in *S. melongena* and *S. torvum*. There were variations in the manner of increase in chromosome number as an exact addition of two genomes was observed in *S. aethiopicum* giving $2n = 2x = 48$ (Figure 1e) while aneusomy was observed in *S. indicum* with $2n = 2x = 69$ (Figure 1f). Furthermore, the colchicine treatment affected the plants morphologically. It produced the first formed leaves after treatment as giant leaves in *S. aethiopicum* (Figure 2a) but suppressed growth in the shoot of *S. indicum* (Figure 2b).

Meiotic chromosomes

There was regular bivalent formation in the diploid cytotypes at diakinesis leading to production of pollen grains that were highly fertile. Trivalent, quadrivalent and multivalent associations were encountered though with minimal occurrence. However, colchiploids showed considerable multivalent associations of chromosomes at diplotene and diakinesis. Pollen stainability was low in the colchiploid cytotypes than in the diploids. Malformed pollen grains were generally preponderant in the colchiploid cytotypes.

DISCUSSION

The basic chromosome number of $x = 12$ reported by Kochhar (1981) was confirmed in these species. All the diploid species showed chromosome number $2n = 2x = 24$ but the sizes differed among the species. Such variations among diploid conspecifics arise because of differences in the repetitive DNA sequences, which eventually make up the genome size (Schmidt et al., 1998). Quicke (1997) recorded the possibility of having different chromatin and heterochromatin densities along chromosome arms. This observation was similar to those made in the present studies. In other words, genome evolution may have been directed in the genus by changes in chromosomal organization brought about by different families of repetitive DNA. Anamthawat-Jonsson and Heslop-Harrison (1993) reported the presence of different intercalary tandem repeat units of DNA as responsible for the variation in *Hordeum*, *Aegilops*, and *Triticum* genera. In the case here, the variations in the spread or dispersion of heterochromatin probably mark the location of such families of satellite DNA in the *Solanum* species. Confirmation of this is only possible with molecular cytogenetic procedures, which can discriminate between the repeat units based on size. The morphology of the chromosomes was mainly of the metacentric to submetacentric types. This symmetry in morphology is a reflection of relatively primitive karyotypes of the members of this genus. Even so, Davis and Heywood (1963) asserted that this view might not be universal since many highly evolved species are also known to show karyotype symmetry. The observed size differences in the species karyotypes could be attributed to cryptic chromosomal structural changes that have brought breeding barriers thus separating the species into the entities seen today. Hartwell et al. (2000) listed chromosomal inversions and translocations as relevant in such cryptic changes. Earlier on Zarco (1986) had noted the extra difficulty encountered in the karyotype analysis of such small-sized chromosomes.

Colchiplodisation did not elicit a universal effect on the species indicating variations in the chromosomal apparatus of these species. Reasons could not easily be provided for this outcome, but the production of an autotetraploid in *S. aethiopicum* shows that at least the chromosomes of members are amenable to manipulations. Of more importance though, is the array of aneuploids generated with this treatment in *S. indicum*. The leaves of some of these species are edible hence the gigas feature so formed in *S. aethiopicum* is a welcome outcome to *Solanum* vegetable growers and consumers alike. Several crop species have been thus improved. The suppression of growth by colchicine as was observed here is not strange, as it has been reported in another dicotyledonous plant, *Vigna unguiculata* (Ugborogho and Obute, 1994). The treatment was an assault to the genetic and cytokinetic integrity of the plant

at the apical shoot region thus disturbing the natural rate of cell cycle and by implication of overall growth.

Although meiotic chromosome behaviour showed regular bivalent formation at diplotene and diakinesis in the diploid species occasionally, multivalents were also observed. This is indicative of the chromosomal rearrangements that might have driven evolution in this plant genus despite similar diploid number in the species. The occurrence of multivalent associations in the colchiplod cytotypes was expected in that homologous chromosomes will tend to pair up in autopolyploids during meiosis.

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