PHILIPP AGRIC SCIENTIST
Vol. 103 No. 4, 362-369
December 2020

ISSN 0031-7454

Research Note

Cytogenetic Analysis of Eggplant (*Solanum melongena* L.) and Some of Its Wild Relatives Found in the Philippines

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Cytogenetics is known to offer relevant information useful in understanding phylogenetic relationships, genetic mapping and plant breeding studies. In particular, the knowledge of meiotic chromosomal behavior is highly vital in working out pathways for transferring desirable traits from related species to cultivated ones. Cytogenetic characterization of *Solanum aethiopicum, S. americanum, S. hirtellum, S. mammosum, S. melongena, S. pseudocapsicum* and *S. torvum* was done using iron-acetocarmine squash technique. *S. aethiopicum, S. americanum, S. hirtellum, S. melongena, S. pseudocapsicum* and *S. torvum* were found to be diploid species with chromosome number 2n=24 while *S. mammosum* had a chromosome number of 2n=22. Lagging chromosomes, bridge formation and asynchronous cell division were observed in insignificant frequencies (1.96–22.06%; 12.07–20.69%; and 1.59–18.18%, respectively). Literature on the basic and applied chromosome features of *Solanum* species is quite insufficient in the Philippines. Results of this study can be utilized as benchmark information for future interspecific hybridization programs in eggplant.

Key Words: cytogenetics, eggplant, iron-acetocarmine squash technique, *Solanum* species, wild relatives

INTRODUCTION

The genus *Solanum* belongs to the family *Solanaceae* in the order Sonales. This plant genus carries great importance for food security in most of the developing countries (Obute et al. 2005). The known *Solanum* species are mostly cultivated as vegetable crops (Kopp 1983), as pot herbs (Pursglove 1968) and even grown for pharmaceutical purposes (Simmonds and Choudhury 1976). Despite the economic importance of *Solanum*, there is a scarcity of information on the cytogenetics of *Solanum* species found in the Philippines. With this, initial steps to utilize the available species for interspecific hybridization programs are perpetually challenged. Through the years, insufficient information about their immediate uses in plant development has led to subsequent genetic erosion (Obute et al. 2005). Hence, the rapid depletion of these potentially useful plant resources calls for an urgent reappraisal of the available germplasm in the area through cytological analysis as one of the preliminary moves.

As far as cytogenetic studies of *Solanum* species in the Philippines are concerned, only two publications (Roxas et al. 1995; Callano et al. 2015) described the chromosomal behavior of *S. melongena* (cultivated eggplant) and *S. aethiopicum* (wild eggplant). Thereafter, no similar data were reported for the existing wild crop relatives of *Solanum* found in the country. Hence, there is a need to conduct cytological assessment on the said plant species to gain basic information for hybrid development in eggplant. The hybrids of eggplant have many advantages compared with open-pollinated cultivars in terms of yield and disease resistance (Callano et al. 2015). In 1975, Choudhuri reported several related wild crop relatives of *Solanum* that bear opportunity for interspecific transfer of disease, insect and nematode resistance. This particular breeding strategy in eggplant was introduced by Grubben et al. (1977) and has been carried out in the Philippines in the 1990s (Roxas et al. 1995).

To successfully exploit the genepool of existing germplasm collection, the cytogenetic relationship of the crop species and its wild relatives must be thoroughly explored. Adajar et al. (2017) stated that detailed information on the meiotic behavior of plant species is instrumental in producing fertile gametes, which is an imperative consideration in intraspecies and interspecies hybridization projects. Through this, the chromosome number, chromosome behavior, as well as cytogenetic

relationships between closely related species can be ascertained. Furthermore, these information can be utilized to identify pathways leading to the transfer of desirable traits from a closely related wild species of eggplant to its improved cultivated varieties.

This cytogenetic assessment study was conducted to determine the chromosome number and observe the chromosomal behavior of *S. melongena* and its crop wild relatives found in the Philippines.

MATERIALS AND METHODS

Sample Collection

Microsporocytes of seven *Solanum* species (Table 1) were collected from plant samples maintained in the experimental area at Cordillera St., Umali Subd., Brgy. Batong Malake, Los Baños, Laguna, Philippines. Time of microsporocyte collection was from 9:00 A.M. to 12:00 P.M. The samples were immediately preserved in freshly prepared Farmer's solution (3:1 95% ethanol:glacial acetic acid) (Roxas et al. 1995) for cell fixation. A drop of Ferric chloride was added to the solution as mordant. The samples were fixed for 3 d to allow the ferric chloride to be absorbed by the chromosomes. Afterwards, they were transferred into 70% ethanol and stored for cytogenetic analysis.

Cytogenetic Analysis

Cytogenetic analyses of the fixed samples were done using iron-acetocarmine squash technique (Smith 1947). The preparations were observed using Zeiss Axioskop Microscope (Carl Zeiss Jena GmbH, Jena, Germany) under an oil immersion objective (OIO). At least 50 cells per stage were observed during diakinesis, metaphase I and II, anaphase I and II, telophase I and II. Both normal and abnormal chromosomal behaviors were noted. Photomicrographs of cells representing the different stages of meiosis and observed chromosomal aberrations were taken using Canon EOS 550D (Canon Inc. Tokyo, Japan) camera.

Pollen fertility test was also conducted. Pollen grains that were freshly collected from an open flower were stained with Iodine potassium iodide (I2KI) to determine the pollen fertility status of the studied species. Three trials were conducted and at least 500 pollen grains per trial were scored for fertility test. Small, shrunken and unstained pollens were considered sterile. The percentage of pollen fertility was calculated using the formula:

% Fertility = [(No.of fertile pollen)/(Total no.of pollen observed)] x 100

Table 1. List of *Solanum* species used in the cytogenetic analysis.

Scientific Name	Collection Site	Type
S. aethiopicum L.	Brgy. Balindog, Kidapawan City	Wild
S. americanum Mill.	Los Baños, Laguna	Wild
S. hirtellum (Spreng) Hassl	Pob. Compostela, Compostela Valley	Wild
S. mammosum L.	Brgy. Katangawan, General Santos City	Wild
S. melongena L.	San Fernando, La Union	Landrace
S. pseudocapsicum L.	Pasar, Leyte	Wild
S. torvum Sw.	Brgy. Binoligan, Kidapawan City	Wild

RESULTS AND DISCUSSION

MEIOSIS I

Diakinesis. As far as has been determined, the species in the *Solanaceae* family have basic haploid chromosome number of 12. Out of the seven studied *Solanum* species, only *S. aethiopicum, S. americanum, S. hirtellum, S. melongena, S. pseudocapsicum* and *S. torvum* showed a chromosome number of 2n=24 (Fig. 1a, 1f, 1c, 1e, 1b, and 1f, respectively). These reports concur with the findings of Roxas et al. (1995), Olet et al. (2015), Verma et al. (2016), Rigor (2000), Federov (1969) and Singha et al. (2018), respectively. On the other hand, *S. mammosum* showed a chromosome number of 2n=22 (Fig. 1d), the same count as that of Heiser (1971) in plant samples found in Latin America. As observed in all studied species, the chromosomes become smaller and thicker throughout this

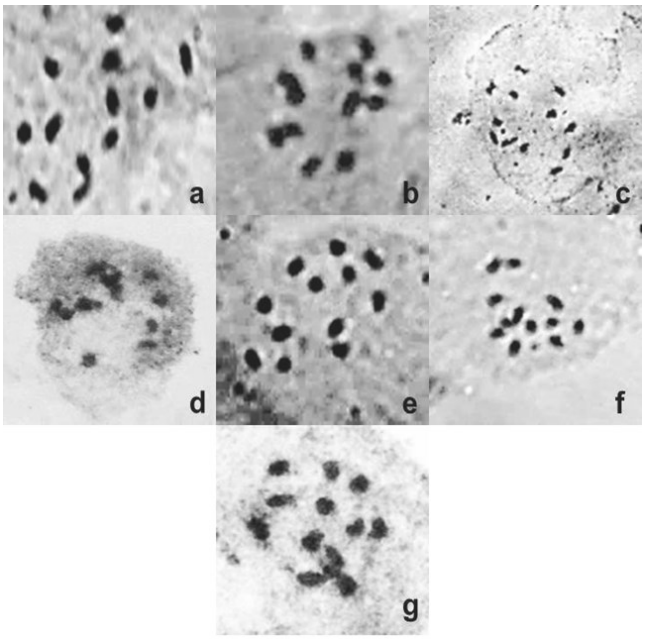

Fig. 1. Diakinesis (12II; 2n=24 and 11II; 2n=22) in (a) *Solanum aethiopicum* L. (2n=24), (b) *S. pseudocapsicum* L. (2n=24), (c) *S. hirtellum* (Spreng.) Hassl. (2n=24), (d) *S. mammosum* L. (2n=22), (e) *S. melongena* L. (2n=24), (f) *S. americanum* Mill. (2n=24), and (g) *S. torvum* Sw. (2n=24).

Table 2. Chromosome behavior of *Solanum aethiopicum* L., *S. americanum* Mill., *S. hirtellum* (Spreng.) Hassl., *S. mammosum* L., *S. melongena* L., *S. pseudocapsicum* L. and *S. torvum* Sw. at Meiosis I.

Stages	Frequency (%)						
	S. aethiopicum	*S. americanum*	*S. hirtellum*	*S. mammosum*	*S. melongena*	*S. pseudocapsicum*	*S. torvum*
Diakinesis							
Normal (12II & 11II)	100 (12II)	100 (12II)	100 (12II)	100 (12II)	100 (12II)	100 (12II)	100 (12II)
No. of cells	62	59	52	81	54	75	71
Metaphase I							
Normal	92.98	98.04	85.71	96.36	77.94	86.59	90.00
Laggard	7.02	1.96	14.29	3.64	22.06	13.41	10.00
No. of cells	57	51	63	55	68	82	60
Anaphase I							
Normal	79.31	95.95	100	79.31	87.30	76.67	98.28
Laggard	0	4.05	0	8.62	12.70	0	1.72
Bridge	20.69	0	0	12.07	0	23.3	0
No. of cells	58	74	67	58	63	60	58
Telophase I							
Normal	100	96.92	100	100	100	100	100
Laggard	0	3.08	0	0	0	0	0
No. of cells	51	65	58	69	64	55	54

substage. In addition, chromosomes tend to clump together as they change in length and diameter. This particular chromosomal behavior is seen to be common in most *Solanum* species (Roxas et al. 1995; Rigor 2000; Callano et al. 2015).

Table 2 shows the chromosomal configuration during diakinesis. All species exhibited high frequency of bivalent formation (12II and 11II). According to Choudhuri (1975), successful bivalent formation is enhanced by small sizes of chromosomes. This concept can be applied to the sample species since they have been seen to have relatively small chromosomes in general.

Metaphase I. In plant species, the alignment of the bivalents midway between the poles of the spindle apparatus characterizes metaphase I. Shorter bivalents usually lose their chiasmata before the longer chromosomes and the homologous chromosomes may proceed toward their respective pole while longer chromosomes still appear as bivalents. The chromosomes at this stage (Fig. 2) clumped together in the middle of the cells because they become very sticky (Casal 1994). During cell assessment, all studied *Solanum* species displayed relatively normal cells at metaphase I that ranges from 77.94% to 98.04% (Table 2). Notably, the highest percentage of normal cells was observed among the wild species (*S. americanum*) and the lowest percentage in the cultivated one (*S. melongena*). This behavior is due to the highest percentage of lagging chromosomes (22.06%) in *S. melongena* (Fig. 3e) throughout the cell analysis. There are two possible origins of laggards or non-congression of chromosomes. Tarar (1980) postulated that laggards could either be bivalents which arrived late at metaphase plate or could be univalents observed during diakinesis. In some instances, Garcia (1992) reported that laggards could have been due to a defect in the spindle formation and eventually leads to the failure of bivalents to reach the equatorial plate.

Anaphase I. The separation of the homologous chromosomes in the bivalents is usually synchronized. The loss of all chiasmata for one or more bivalents marks the onset of anaphase I. Relatively speaking, most of the cells at this stage were found to be normal (Fig. 4) with

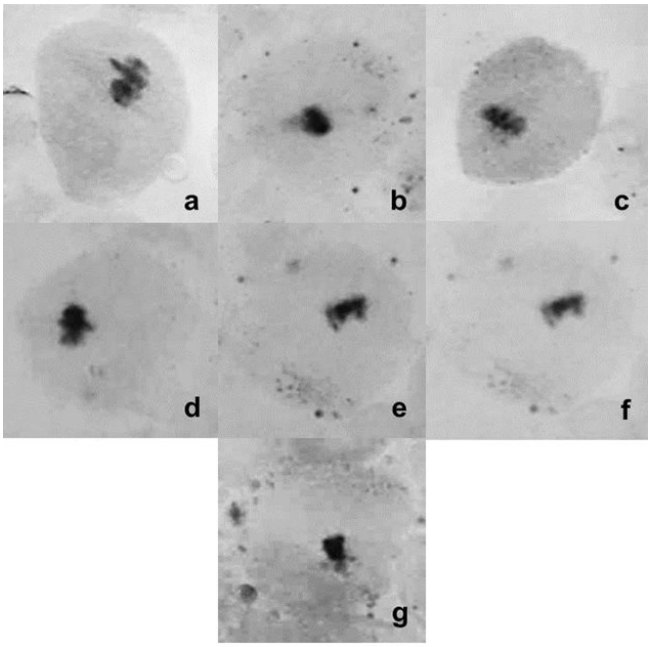

Fig. 2. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at metaphase I.

minor chromosomal aberrations. The high bivalent formation at diakinesis and high percentage of normal cells at metaphase I could be the possible reasons for the high incidence of normal chromosome segregation among the studied species. As noticed, the movement and the separation of chromosomes were not all the same due to some abnormalities. Lagging chromosomes (Fig. 3h-k) and bridge formation (Fig. 3l-n) were noted except in *S. hirtellum*. Lagging chromosomes at metaphase I persisted in the cells of *S. melongena* (12.70%), *S. mammosum* (8.62%), *S. americanum* (4.05%) and *S. torvum* (1.72%). As observed by Choudhuri (1975), bivalent chromosomes often show difficulty in separation and consequently lag behind.

Formation of bridges in the cells (Fig. 3l-n) was detected in *S. aethiopicum* (20.69), *S. pseudocapsicum* (23.30%) and *S. mammosum* (12.07%). Bridge formation in *S. aethiopicum* was also observed by Roxas et al. (1995), Rigor (2000) and Callano et al. (2015) in their cytogenetic analyses. Callano et al. (2015) assumed that bridges are formed on the basis of the position of chiasmata in relation to the centromere. Nonetheless, this chromosomal behavior could be due to a reunion at the end of the breakage of sister chromatids and to the stickiness of the terminal segments of the chromosomes (Rigor 2000).

Telophase I. Among the cytogenetically analyzed *Solanum* species, only *S. americanum* (96.92%) was not able to exhibit 100% normal cells at telophase I (Fig. 5). Lagging chromosomes (Fig. 3o) from anaphase I still persisted up to this stage but at a very insignificant rate (3.08%). Notably, laggards observed in anaphase I from *S. mammosum* and *S. torvum* were able to catch up with the rest and reached the opposite poles and eventually formed into normal cells.

MEIOSIS II

Metaphase II. When cell division reached this stage, the nuclear membrane disappears and the chromosomes are eventually aligned in a plane midway between the poles of the spindle apparatus. Cytogeneticists also believe that while the centromeres of homologous chromosomes in bivalents at metaphase I are associated with fibers from only one pole, the centromere of chromosomes at this stage are already associated with fibers from opposite poles (Adajar et al. 2017). At this particular cellular episode (Fig. 6), only *S. pseudocapsicum* and *S. torvum* were detected to show 100% normal cells (Table 3). Other *Solanum* species such as *S. hirtellum* (98.41%), *S. melongena* (97.06%), *S. aethiopicum* (88.89%), *S. americanum* (84.31%) and *S. mammosum* (81.25%) were found to express minor abnormalities (Fig. 7).

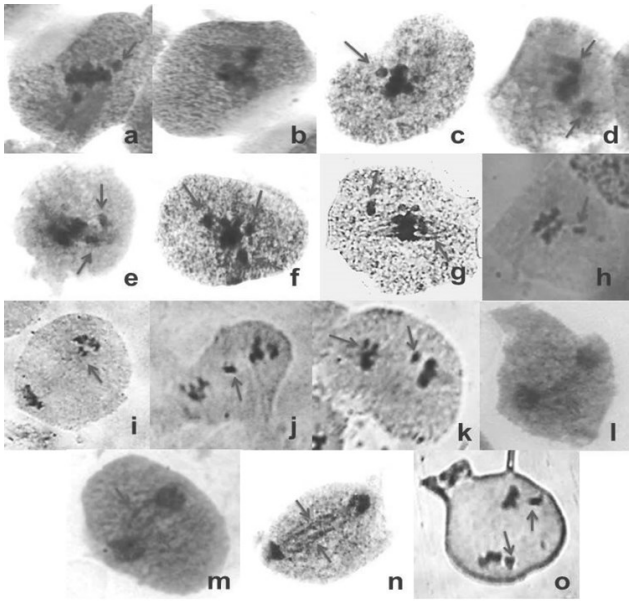

Fig. 3. Lagging chromosomes of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americamum* Mill., and (g) *S. torvum* Sw. at metaphase I; Lagging chromosomes of (h) *S. mammosum* L., (i) *S. melongena* L., (j) *S. americanum* Mill., and (k) *S. torvum* Sw. at anaphase I; Bridge formation of (l) *S. aethiopicum* L., (m) *S. pseudocapsicum* L. (Spreng.) Hassl., and (n) *S. mammosum* L. at anaphase I; Lagging chromosome of (o) *S. americanum* Mill. at telophase I.

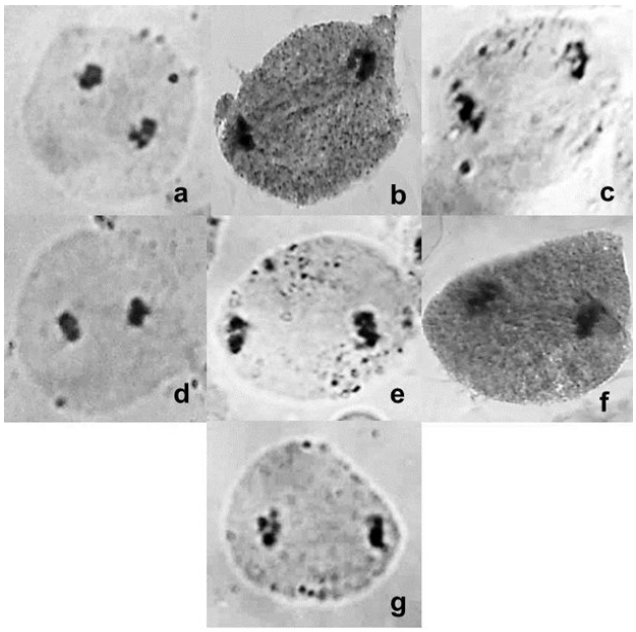

Fig. 4. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at anaphase I.

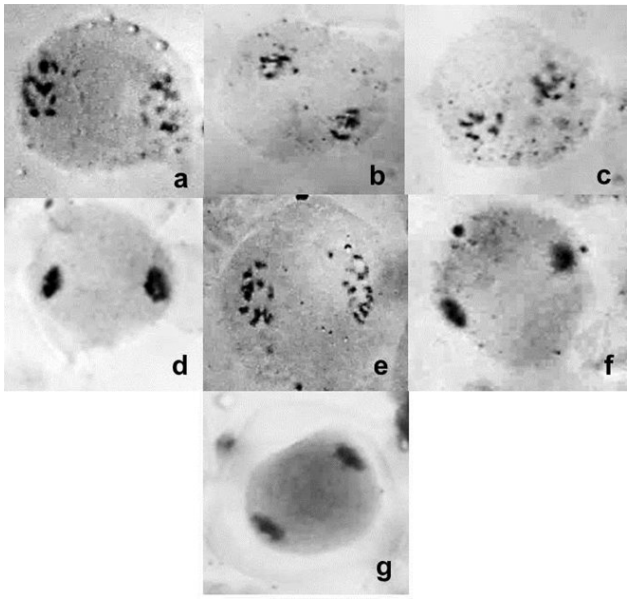

Fig. 5. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at telophase I.

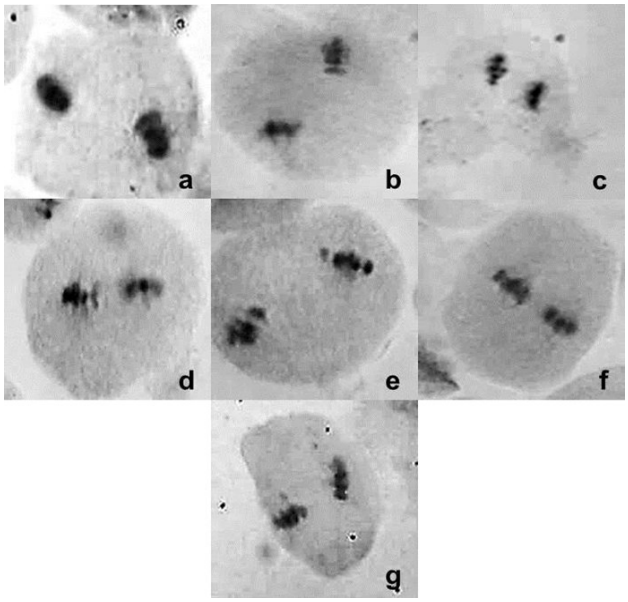

Fig. 6. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at metaphase II.

Lagging chromosomes and asynchronous division of cells were present in some cell samples. Consistently, lagging chromosomes from *S. aethiopicum* (Fig. 7a) persisted up to this stage but at a low number (7.02%), while other species showed asynchrony among their dividing cells. Asynchrony was only seen in the cells of *S. mammosum* (18.18%), *S. americanum* (15.69%), *S. aethiopicum* (8.77%), and *S. melongena* (2.94%) and *S. hirtellum* (1.59%) (Fig. 7d, 7f, 7b, 7e and 7c, respectively). Although cell division is perceived to be synchronous in the early stage of embryogenesis, asynchronous cell division can also occur in later development (Adajar et al. 2017). As observed, the chromosomes from the seven studied *Solanum* species were noticed to be very small and sticky. These chromosomes were even noted to clump together due to stickiness. The asynchronous cell division in *Solanum* species may have been caused by delayed separation of chromosomes from the previous phase. Jonsson et al. (2006) stated that cells are assumed to expand and divide at constant speed. But the cells tend to divide asynchronously because the chromosomes of the studied cell samples tend to clump together during division, and the length of time before the next division will already be different. In another perspective, this phenomenon can also be attributed to the fact that most of the plants under *Solanaceae* are considered to be vegetables. According to Taiz and Zeiger (2002), vegetable cells are usually surrounded by rigid cell walls that prevent on time cell division.

Anaphase II. The separation of the chromatids at the centromere signals the onset of this stage. Usually, each group with the haploid chromosome number proceeds

Table 3. Chromosome behavior of *Solanum aethiopicum* L., *S. americanum* Mill., *S. hirtellum* (Spreng.) Hassl., *S. mammosum* L., *S. melongena* L., *S. pseudocapsicum* L. and *S. torvum* Sw. at Meiosis II.

Stages	Frequency (%)						
	S. aethiopicum	*S. americanum*	*S. hirtellum*	*S. mammosum*	*S. melongena*	*S. pseudocapsicum*	*S. torvum*
Metaphase II							
Normal	88.89	84.31	98.41	81.82	97.06	100	100
Laggard	7.02	0	0	0	0	0	0
Asynchrony	8.77	15.69	1.59	18.18	2.94	0	0
No. of cells	57	51	63	55	68	82	60
Anaphase II							
Normal	92.06	83.78	83.58	100	96.83	100	89.66
Asynchrony	7.94	16.22	16.42	0	3.17	0	10.34
No. of cells	63	74	67	58	63	60	58
Telophase II							
Normal	100	100	100	100	100	100	100
No. of cells	54	58	64	69	65	55	59

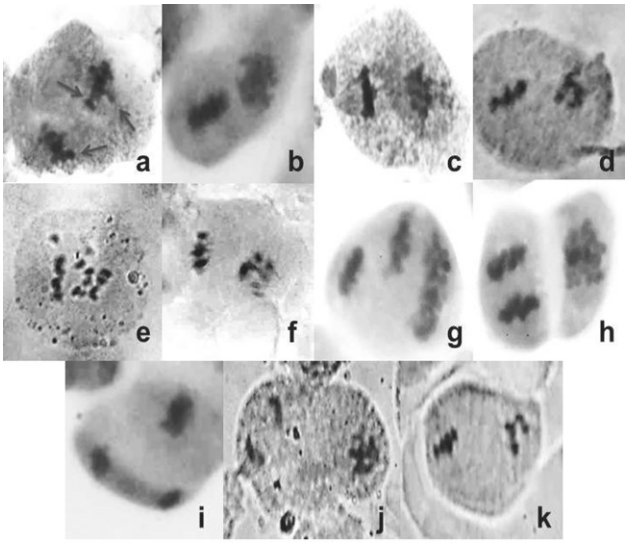

Fig. 7. Lagging chromosome of (a) *Solanum aethiopicum* L. at metaphase I; Asynchronous cell division of (b) *S. aethiopicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., and (f) *S. americanum* Mill. at metaphase II; Asynchronous cell division of (g) *S. aethiopicum* L., (h) *S. hirtellum* (Spreng.) Hassl., (i) *S. melongena* L., (j) *S. americanum* Mill., and (k) *S. torvum* Sw. at anaphase II.

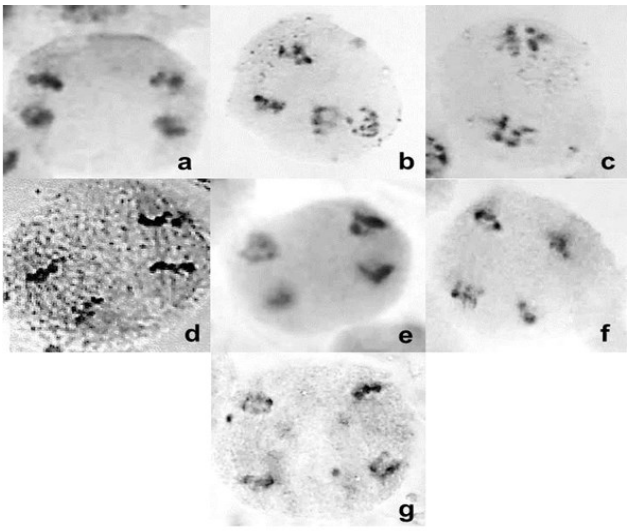

Fig. 8. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at anaphase II.

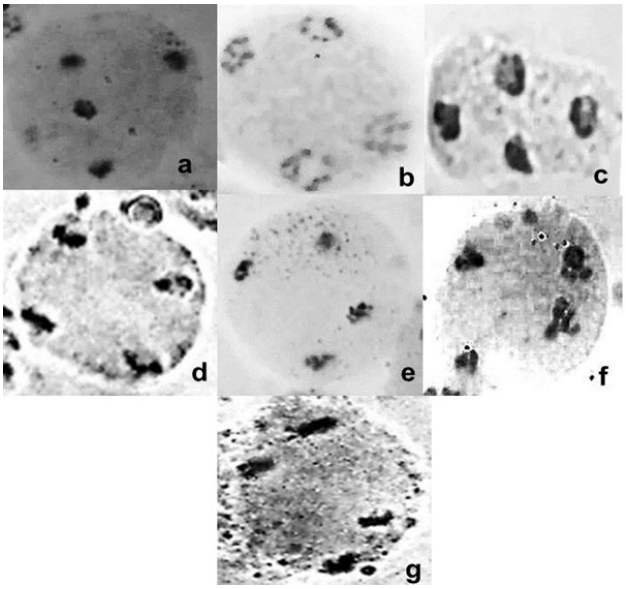

Fig. 9. Normal cells of (a) *Solanum aethiopicum* L., (b) *S. pseudocapsicum* L., (c) *S. hirtellum* (Spreng.) Hassl., (d) *S. mammosum* L., (e) *S. melongena* L., (f) *S. americanum* Mill., and (g) *S. torvum* Sw. at telophase II.

toward its respective poles (Fig. 8). However, it was recorded that *S. hirtellum* (16.42%) (Fig. 7h), *S. americanum* (16.22%) (Fig. 7j), *S. torvum* (10.34%) (Fig. 7k), *S. aethiopicum* (7.94%) (Fig. 7g) and *S. melongena* (3.17%) (Fig. 7i) showed asynchronous cell division at this stage.

Interestingly, the laggards seen in the cells of *S. aethiopicum* at metaphase II have reached their respective poles resulting in normal segregation of chromosomes.

Telophase II. At the last stage of meiosis, each group of chromosomes is enclosed within a nuclear membrane, and each spindle apparatus disappears. Since plants from *Solanum* species have single secondary meiocyte, cell division in two planes yields four cells, each with a haploid nucleus. Generally, all *Solanum* species examined during this stage revealed 100% normal cells (Fig. 9). The incidence of asynchronous cell division from metaphase II and anaphase II was insignificant (1.59%–18.18%) to cause abnormalities up to this stage.

Pollen Fertility

Pollen fertility test was done in the studied *Solanum* species to determine their potential as parent materials for interspecific hybridization (Table 4). Among these species, *S. torvum* (98.35%) had the highest recorded pollen fertility, followed by *S. americanum* (95.43%), *S. hirtellum* (92.71%), *S. mammosum* (90.58%) *S. melongena* (89.63%), *S. pseudocapsicum* (83.46%) and *S. aethiopicum* (65.24%). *S. aethiopicum's* present pollen fertility result nearly conforms to that of Callano et al. (2015) on the species' pollen fertility status (61.18%). This is contrary to the findings of Roxas et al. (1995) and Rigor (2000) where *S. aethiopicum* exhibited the highest percentage of fertility among other *Solanum* species used. It could be that the present *S. aethiopicum* accession used is the same accession used by Callano et al. (2015), while Roxas et al. (1995) and Rigor (2000) had possibly utilized a different accession in their studies. The current situation explains the variability of different accessions within a particular

Table 4. Pollen fertility of *Solanum aethiopicum* L., *S. americanum* Mill., *S. hirtellum L.*, *S. mammosum* L., *S. melongena* L., *S. pseudocapsicum* L. and *S. torvum* Sw.

Solanum Species	Fertility (%)
S. aethiopicum L.	65.24
S. americanum Mill.	95.43
S. hirtellum L.	92.71
S. mammosum L.	90.58
S. melongena L.	89.63
S. pseudocapsicum L.	83.46
S. torvum Sw.	98.35

species.

As far as pollen fertility test is concerned, all the studied *Solanum* species have the potential of becoming one of the putative parent materials for future interspecific hybridization programs in eggplant.

CONCLUSION

Cytogenetic analysis revealed that *S. aethiopicum*, *S. americanum*, *S. hirtellum*, *S. melongena*, *S. pseudocapsicum* and *S. torvum* are diploid species with a chromosome number of 2n=24 while *S. mammosum* was found to have a chromosome number of 2n=22. Chromosomal aberrations such as lagging chromosomes, bridge formation and even asynchronous division of cells were noted in insignificant frequencies. The pollen fertility of the studied *Solanum* species ranged from 65.24% to 98.35%.

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