Comparative Taxonomic Studies on *Solanum aethiopicum* Linn. and *Solanum nigrum* Linn. (Solanaceae)

Chika Wahua¹, Sele Mercy Olaleye²*

¹Department of Plant Science and Biotechnology, University of Port Harcourt, Choba.  
²Department of Forestry and Wildlife Management, University of Port Harcourt, Choba.

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*Corresponding Author*  
Sele Mercy Olaleye  
E-mail: sele.olaleye@yahoo.com

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**ABSTRACT**

The study presented the comparative micro- and macro-morphological, anatomical and cytological properties of *Solanum aethiopicum* Linn. and *Solanum nigrum* Linn. Predominantly found in the wild in the Niger Delta Tropics, Nigeria. They are used as wild vegetable and medicine. Their habits are annual sub-woody plants which attain up to 120cm and 59cm or more in height respectively. The leaves are petiolate and simple, ovate measuring up to 10±5 cm in length and 7±3 cm wide for *S. aethiopicum* while *S. nigrum* is 7±3 cm in length and 4±1.5 cm broad with alternate phyllotaxy. Stalked solitary axillary flowers are present in both. The petals are yellowish and sepals greenish. The greenish berry fruits measure up to 1.2±0.4 cm in length for both species, when ripe is reddish and blue-black respectively. The epidermal studies reveal anisocytic stomata whereas the trichomes are simple uniseriate form and stellate type respectively. The anatomy of mid-ribs and petioles showed bicolateral vascular systems. There are 3 vascular traces and node is unilacunar in each species, the stems have 5 to 6 vascular bundles. The cytological studies showed a diploid chromosome number of 2n=24 for *S. aethiopicum* while *S. nigrum* is a polyploidy (tetraploid) with 2n=4x=48.
INTRODUCTION

The genus *Solanum* Linn. is one of the largest angiosperm genera with approximately 1,500 species distributed worldwide (Boh, 2005). The species in this genus are erect or climbing herbs, shrubs or rarely small shrub globally (Purseglove, 1968). Known cultivated species of economic importance are: *S. muricatun* Alton, Naranjilla (S. quitoense Lam.), Cocona (S. sessiliiforum Danal.), and tree tomato (*S. bataceum Cav.*) (Bohs, 2005). Some species of the genus *Solanum* Linn. are hairy, some are spiny and others are glabrous (Hutchinson and Dalziel, 1958; Purseglove, 1968). The following species of *Solanum* Linn. are known in West Africa: *S. incanum* Hutch. and Dalzi., *S. terminale* Forsk., *S. giganteum* Jacq., *S. mauritianum* Scop., *S. verbascifolium* Linn., *S. seaforthianum* Andr., *S. aethiopicum* Linn., *S. gilo* Raddii., *S. incanum* Linn., *S. melongena* Linn., *S. aculeastrum* Dunal., *S. albicaule* Kotschyan ex Dunal., *S. torvum* Sw., *S. cerasiferum* Dunal., *S. indicum* Linn., *S. dasyphyllum* Schum. and Thonn., *S. macrocarpon* Linn. Other cultivated species are *S. wrightii* Benth., *S. hispidum* Pers., the “European” Potato (*S. tuberosum* Linn.) and the tomato, *S. lycopersicum* Linn. (Hutchinson and Dalziel, 1958). In Nigeria however, some of the species of *Solanum* Linn. are known, these are: *S. clerodendrodes* Hutch. and Dalzi., *S. terminale* Forsk., *S. seaforthianum* Andr., *S. anomalum* Thonn., *S. verbascifolium* Linn., *S. gilo* Raddii., *S. incanum* Linn., *S. melongena* Linn., *S. indicum* Linn., *S. dasyphyllum* Schum. and Thonn., *S. macrocarpon* Linn. and *S. nigrum* Linn. are reportedly found in both Southern and Northern Nigeria respectively, whereas *S. tuberosum* Linn. and *S. cerasiferum* Dunal. are found in Northern part of Nigeria (Hutchinson and Dalziel, 1958). Twenty- two species of *Solanum* Linn. are known in Nigeria and most parts of West Africa. They are either wild, weedy or cultivated (Heine, 1963; Gbile, 1979; Omidiji, 1985). *S. lycopersicum* Linn. (tomato), was formerly known as *Lycopersicon esculentum*. At present, there has not been much interest in the cytogenetics of its Nigeria representatives (Okoli and Osuji, 2008). The basic chromosome number of *Solanum* Linn. is n=12 (Purseglove, 1968). *S. nigrum* Linn. was found to be tetraploid and hexaploid (Okoli, 1988). *S. macrocarpon* Linn., *S. aethiopicum* Linn. and *S. gilo* Raddii. Have diploid number of chromosomes 2n=2x=24 (Omidiji, 1985). In the Niger Delta, *Solanum* species common in the wild are *S. torvum* Sw., *S. nigrum* Linn., *S. aethiopicum* Linn., *S. verbascifolium* Linn., whereas *S. lycopersicum* Linn., *S. macrocarpon* Linn., *S. melongena* Linn., *S. anomalum* Thonn. and *S. erianthum* D. Don. are domesticated.

The relevance of the study is to improve on the information on the existing literature and taxonomic characteristics of *Solanum* species; this is due to the fact that they are wild economic plants. Thus the objective of the study is therefore aimed at considering: the comparative morphological, anatomical and cytological investigations of *S. aethiopicum* Linn. and *S. nigrum* Linn.

MATERIALS AND METHODS

The materials used for this study were collected from the wild and raised from seeds from the fruit. The geographic location of the parent plants studied were 04°52'34.31"N and 00°55'12.38'E at 18m altitude for *S. aethiopicum* Linn. and 04°52'34.31"N and 00°54'87.51"E at 20m altitude for *S. nigrum* Linn. A study of the macro-morphological features of the species was made using a 30cm ruler. The plants parts measured included: leaf length, leaf width, petiole length, stamen length, style length, fruit length and width and average plant height. The presence or absence of trichomes was observed painstakingly under a light microscope, and microphotographs were taken while relevant.

Floral biology: The opening and closing time of the flowers of the species were studied. The arrangement patterns of the petals and sepals (that is the aestivation type) were observed and the insect pollinators noted.

Epidermal Studies: Fresh materials (leaves and stem epidermal peels) were collected for this study; the fresh leaves were peeled and bleached using sodium hypochlorite for about 2 minutes following the method of Cutler (1978). The clear epidermal layers obtained were then washed in several changes of distilled water and stained with Alcian blue or safranin and temporarily mounted in aqueous glycerol solution (Cutler, 1978). Photomicrographs were taken from good preparations. Stomatal studies (Stomatal indices) were done from the cleared leaves. The length and width of the guard cells were measured using a calibrated eye piece graticule following the method of Arnold (1973). The stomata observed were viewed with the light microscope and were calculated in unit area using the stomatal index [S.I.] formula as shown below:

\[
S.I. = \frac{S}{E+S} \times \frac{100}{1}
\]

where S and E mean numbers of stomata and epidermal cells within the particular area under investigation. The same formula was applicable for the calculation of trichome indices (T.I.), in this case, trichomes (T) were used instead of stomata: T.I. = \[
T \div \frac{E+T}{1} \times \frac{100}{1}
\]

Comparative Anatomical Studies: Seeds of the plant materials were planted out in petri dishes containing wetted 110mm Whatman filter paper and the germination tests were calculated using similar formula as applied to stomatal indices but based on the percentage of the number that germinated divided by total number of seeds plated. Three days to two weeks after growth had occurred, stem and root systems studied were fixed, alongside with mature leaves, flowers, fruits and petioles harvested from mature plants, in FAA in the ratio of 1:1:18.
of 40% formaldehyde, acetic acid and 70% alcohol for at least 48 hours following the method of Johansen (1940) with some modifications of the leaves, petioles, fruits, flowers, stems and roots.

Free hand sectioning using a systematic arrangement of 5 razor blades, with 2 sets (nacet and tiger blades) crossed and a central vertical one (nacet) lying in between the 2 sets crossed. The blades were adjusted until the holes in them synchronized. The plant part to be sectioned was placed in the hole and using the first two fingers of the left hand to hold the vertical blade sets, while pressing down the 2 crossed sets with the first two fingers of the right hand to make a transverse section of about 20 to 25 µm thick. The sections made were passed through alcohol solutions in the order: 30%, 50%, 70%, 95% and absolute alcohol, allowing them for 5 minutes in each solution. The dehydrated materials were cleared of their natural wax by passing them through different proportions of alcohol and chloroform series in the following ratios (3:1: 1:1: 1:3) v/v for 10 minutes in each, and as the chloroform gradually replaced the alcohol, the process was repeated from the pure chloroform and down the series again within same time interval. These were rehydrated in alcohol solution starting with absolute then 95%, 70%, 50%, 30% and stained with 1% Alcian blue for 2 minutes, washed off with water before counter-staining with 1% safranin for 2 minutes. The stain was washed off and placed on clean glass slide with a drop of glycerol and a clean cover slip placed on it (Wahua et al., 2013). The slides so prepared are as good as those of microtomy and are near permanent ones. These slides were viewed with the light microscope and microphotographs taken from good preparations after proper examination.

**Cytological Study:** Healthy root tips for mitotic study were obtained from seeds of *S. aethiopicum* Linn and *S. nigrum* Linn grown in a petri dish containing 110mm Whatman filter paper wetted with water for a period of three days to one week. The early germinated roots were transferred to solution of 0.002M of 8-hydroxyquinoline for 3 hours specifically to suspend the spindle fibres or to transfer to solution of 0.002M of 8-hydroxyquinoline for 3 hours specifically to suspend the spindle fibres or to accumulate chromosomes at metaphase between 9 and 10 a.m. to be precise. The roots were treated with Clarke’s fluid (3:1 ethanol/acetic acid v/v) for 12 to 24 hours aimed at killing the cells. The roots were then preserved in 70% alcohol and kept in the refrigerator until when needed or used immediately by hydrolyzing in 9% HCl for 8 minutes and passing them through 70% ethanol for 10 minutes. 1mm of the root tip studied was excised from the apex and squashed in a drop of FLP-orcein stain (2g of orcein dissolved in 100ml of a solution of equal parts of formic acid, lactic acid, propanoic acid and water) under a coverslip, flattened out and examined under a light microscope, following the method of Okoli (1983). Photomicrographs of the chromosomes were taken from good temporary slides, using a sony digital camera (7.2 mega pixels). For the meiotic chromosomes, immature flower buds were used. These were treated with carnoy's fluid for 24 hours and preserved in 70% alcohol and kept in the refrigerator or used immediately as already described for mitotic chromosomes above following the method of Okoli (1983).

**RESULTS AND DISCUSSION**

**Morphological Characteristics:** The opening and closing times of the flowers was studied. It was revealed that the flowers commenced opening at 5:45 a.m. and opened completely at 7:36 a.m. while the closing time started at 6:30 p.m. and closed completely at 10:00 p.m. for *S. aethiopicum* Linn. while *S. nigrum* Linn. flowers started opening at 6:00 a.m. opened completely at 7:35 a.m. and commenced closing at 3:25 p.m and closed completely at 9:00 p.m. This feature is of taxonomic relevance as it aids supply information patterning the breeding status of the plants. The germination test conducted was 25% for *S. aethiopicum* Linn and 30% for *S. nigrum* Linn. The distributional pattern of the species has been recorded by Hutchinson and Dalziel (1958). These plants are wild erect annual sub-woods. *S. aethiopicum* Linn Plate 1 and *S. nigrum* Linn Plate 2. The leaves are simple, petiolate, ovate, lobed and glabrous, measured up to 1.2 cm in diameter. The petals are whitish up to 0.6 to 0.8 cm in length and 0.3 to 0.4 cm wide, the sepals are dark green in colour, measuring up to 0.6 to 0.8 cm in length and 0.3 to 0.4 cm wide, fused at base. The stamens have yellow tip and measuring up to 0.3 to 0.5 cm in length. The central structure in the midst of the stamens is the style, a stout rod of 0.4 to 0.5 cm in length. The fruit is a berry, dark green when unripe, red when ripe, oval in shape and measuring up to 1.2 cm in diameter. The seeds are quite numerous per fruit, spherical and measuring up to 0.2 cm in diameter. While *S. nigrum* Linn flower inflorescence has 2 to 7 flowers together, structured in small umbellate cyme on flower pedicels arising directly from the peduncle. The petals are white measuring up to 0.5 cm in length and 0.3 cm in width, fused at base. The sepals are green in colour, 0.4 cm long and 0.3 cm broad, also fused at base. The stamen has a yellowish tip up to 0.3 cm in length and style is up to 0.4 cm. The berry fruit has a transparent epicarp which at maturity reveals seeds inside it and when unripe is green but blue-black when ripe measuring up to 1.2 cm in diameter; the seeds are numerous and up to 0.2 cm in diameter. Aestivation type for the species studied is valvate. Insect pollinators are ants, spiders, house flies, bees and caterpillars. Pollinators started appearing at 7:00 a.m and were not seen at 2:20 p.m, and sometimes resurfaced later in the day.
Epidermal Studies: *S. aethiopicum* Linn. foliar epidermal study revealed the presence of anisocytic stomata and stellate trichomes at both the adaxial and abaxial foliar surfaces. Plates 3a and 3b shows that the adaxial foliar layer has 22.50% stomatal index and 24.19% for the abaxial surface. Trichome index is also studied, revealing 31.29% for the adaxial and 27.47% for the abaxial surfaces, stomatal characteristics revealed adaxial stomatal length of 5.6±0.239µm with 4.16% coefficient of variation (C.V.) and width of 3.6±0.176µm with 5.02% CV and abaxial stomatal length as 5.4±0.172µm with 3.19% (C.V.) and stomatal width of 3.7±0.224 with 6.09% (C.V.) while *S. nigrum* Linn. also revealed anisocytic stomata and the stomatal characteristics showed adaxial stomatal length of 4.3±0.161µm with 3.74% CV and width of 3.2±0.141µm with 4.39% CV and that of abaxial stomatal length of 4.5±0.176µm with 3.96% C.V. and width of 2.4±0.757µm with 31.54% CV respectively.

Anatomical Investigation: Anatomy of *S. aethiopicum* Linn. mid-rib shows stellate trichomes in the epidermis made of a layer of cells. The collenchymatous cells occupy the region of the hypodermis. Parenchymatous cells occupy the ground meristem. The primary growth phase reveals 3 vascular traces with no rib bundle wings in both growth phases (Plate 5). The secondary growth phase has vascular arc (Plate 6). The mid-rib of *S. nigrum* Linn. is similar to that of *S. aethiopicum* Linn. (See plate 6). The petiole of *S. aethiopicum* Linn. is made of a layer of cells in the epidermis, the cell and tissue arrangements in the petiole is similar to that of the mid-rib except that there are 2 to 4 rib-bundle wings present in the petiole (Plate 8). The nodal pattern is unilacunar (Plate 10). The anatomy between two successive nodes, the internode, has a pericyle of many layers of cells below the endodermis (the innermost part of the cortex) and a large pith occupied by parenchymatous cells (plate 12). Root anatomy of *S. aethiopicum* Linn. reveals epiblema made of one layer. The vascular bundles have radial symmetry (Plate 14). Anatomy of *S. nigrum* Linn. mid-rib: There are numerous simple multicellular trichomes on the epidermal layer made of a roll of cells. The hypodermis is made of few layers of thick wall cells termed collenchyma, the rest of the general cortex is composed of parenchymatous cells which are larger and made of thin walls (plate 7). The petiole of *S. nigrum* Linn. has the same pattern of cell arrangements as in *S. aethiopicum* Linn. except that there are less tanniferous cells in *S. nigrum* Linn. than the former. Two rib-bundle wings also observed in the petiole of *S. nigrum* Linn. alongside 3 vascular traces (Plate 9). The nodal pattern is also unilacunar (plate 11). The internodal anatomy of *S. nigrum* Linn. shows an epidermis of a layer of cells. The hypodermis is made of about 3 to 4 layers of collenchymatous cells, and the general cortex comprises 3 to 4 layers of parenchyma of thin walls. The endodermis is made of a layer of barrel-shaped cells clearly-marked. The pericycle just below the endodermis is composed of 2 to 3 cell-layers. The pith region is wide and made of large parenchymatous cells (plate 13). Root anatomy of both species has exarch xylary structure. The piliferous layer is single-cell thick. The vascular bundles are radially symmetrical. Few centralized parenchymatous cells occupy the pith region of the root (Plate 14 and 15). The ovary anatomy of both species revealed the placentaion as axe type. Their ovaries are trilocular and 3-celled (See Plates 16 and 17). The species investigated are bisexual, hypogynous with axe placentaion which is also in accordance to the observation of Hutchinson and Dalziel (1958).

Cytological Investigation: Cytological Studies of *S. aethiopicum* Linn. shows the mitotic chromosome number as 2n=24 (plates 18), while that of *S. aethiopicum* Linn. as 2n=4x=48, a tetraploid (plate 19). Cytologically, the basic chromosome number for members of Solanaceae is n=x=12 Omidiji (1985), Okoli and Osuji (2008) also supported the chromosome basic number as x = 12 and diploids of 2n = 24. *S. nigrum* Linn. is a teraploid as also observed by Okoli (1988).

CONCLUSION

*S. nigrum* Linn. possesses simple uniseriaterichomes and anisocytic stomata while *S. aethiopicum* Linn. showed stellate trichomes and anisocytic stomata. The structure of the stamens and carpels, and mostly their pilose nature are of taxonomic relevance in delimitations at the generic and species level. The fruit of *S. aethiopicum* Linn. is reddish and that of *S. nigrum* Linn. is blue-black in colour and the sizes of the fruits are averagely the same, 1.2 to 1.3cm in diameter. The stem of both species have 3 vascular traces at primary growth phase while the secondary growth phase showed vascular arcs for the mid-ribs and petioles. The nodal pattern is unilacunar for both species and the roots’ vascular system revealed radial symmetry. The species investigated are bisexual, hypogynous with axe placentaion. Cytologically, the basic chromosome number for members of Solanaceae is n=x=12. *Solanum nigrum* Linn.is a teraploid.

*S. aethiopicum* Linn and *S. nigrum* Linn. are used as vegetable in most African dishes and in tradomedicine. Having worked extensively on their morphological, anatomical and cytological properties, other areas of interest need are DNA barcoding, Palynology, proximate analysis and quantitative aspect of phytochemistry. Interested researchers could carry on with the work in these areas.

REFERENCES


Wahua et al / Greener Journal of Agricultural Sciences


Plate 1: Solanumaethiopicum Linn. Arrow shows ripe red fruit (1cm) in size.

Plate 2: Solanumnigrum Linn. Arrow revealed ripe blue-black fruit 1cm in size.

Plate 5 & 6: Mid-rib section of S. aethiopicum Linn. Plate 7: Mid-rib section of S. nigrum Linn. Plate 8: Petiole section of S. aethiopicum Linn. (The sections from plates 5 to 17 are 2cm in sizes)
Plate 9: Petiole section of *S. nigrum*Linn. Plate 10: Unilacunar nodal anatomy of *S. aethiopicum*Linn.
Plate 11: Unilacunar nodal anatomy of *S. nigrum*Linn.

Plate 12: Stem section of *S. aethiopicum*Linn.
Plate 13: Stem section of *S. nigrum*Linn.
Plate 14: Root section of *S. aethiopicum* Linn.
Plate 15: Root section of *S. nigrum*Linn
Plate 16: Ovary section of *S. aethiopicum* Linn.
Plate 17: Ovary section of *S. nigrum*Linn.
Plate 18: Mitotic chromosomes of *S. aethiopicum* Linn.
Plate 19: Mitotic chromosomes of *S. nigrum*Linn.