COMPARATIVE MORPHOLOGICAL, ANATOMICAL, CYTOLOGICAL AND PHYTOCHEMICAL STUDIES ON Capsicum frutescens Linn. and Capsicum annuum Linn. (SOLANACEAE1).

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ABSTRACT

The present study is set to investigate the comparative micro- and macro-morphological, anatomical, cytological and phytochemical properties of Capsicum frutescens Linn. and Capsicum annuum Linn., members of the family Solanaceae predominantly found in the Niger Delta Tropics, Nigeria. They are used as spices, vegetable and medicine. They are among the extremely pungent pepper. Their habits are annual sub woody plants which attain up to 65cm or more in height. The leaves are simple, glabrous, lanceolate to ovate with apex being acutely acuminate and the base being cuneate or abruptly acute and petiolate measuring 8.2 ± 1.67 cm in length and 4.1 ± 0.322 cm in width for Capsicum annuum Linn. and Capsicum frutescens Linn. Their glabrous stems are angular and the inflorescences terminal, flowers are axile in placentation borne at nodes. The petals are whitish and sepals greenish. The berry fruits are many seeded, globose shaped for C. annuum Linn. while linear for C. frutescens Linn. and borne at nodes. The epidermal studies reveal anomocytic stomata whereas the trichomes are simple uniseriate forms. The anatomy of mid-ribs and petioles showed bicollateral vascular systems. There are 2 vascular traces and node is unil a cunar in each species, their stems have 5 to 6 vascular bundles, their petioles are associated with 2 rib traces at primary growth phase. At secondary growth phase, their mid-ribs and petioles revealed vascular arcs and the stems have rings of open vascular systems. The cytological studies showed a diploid chromosome number of 2n = 24. Alkaloids, saponins, tannins, phlobatannins, flavonoids, combined anthraquinones, free anthraquinones and cardiac glycosides are present in both species.

KEYWORDS: Morphological, Anatomical, Cytological, Studies, Niger Delta.

INTRODUCTION.

The family Solanaceae is composed of 95 genera (1). It is widely distributed in temperate and tropical regions, but the centre of distribution is Central and South America. In West Africa however, there are 8 genera and 53 species of Solanaceae (2). Capsicum annuum Linn. and Capsicum frutescens Linn.are mostly annual sub-shrubs (3), though Capsicum frutescens Linn. is often found in the wild, according to (4), defined anomocytic (irregular-celled) as stoma surrounded by a limited number of cells that are indistinguishable in size, shape, or form from those of the remainder of epidermis; anisocytic (unequaledcelled) as stoma surrounded by three cells of which one is distinctly smaller than the other two; paracytic (parallel-celled) as stoma accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells while tetracytic as four subsidiary cells being present, two lateral and two terminal, and actinocytic as stoma surrounded by a circle of radially elongated subsidiary cells. Capsicum annuum Linn. has simple uniseriate trichomes (5). Trichomes are termed 'simple' when unbranched. Simple trichomes could be unicellular or multicellular (6). The type of hair can be of diagnostic value at species level, sometimes also at generic level, but rarely at family level (7). The word 'uniseriate' is really an anatomical term rather than morphological and does not describe the shape. 'Multiseriate' is not unique to trichomes, it could also mean multi layers as in epidermal and hypodermal axial parenchyma (6). Watson and Dallwitz (1) stated that members of Solanaceae have unilacunarnode. The primary vascular tissues of Solanaceae are bicollateral (1). Most members of Solanaceae are diploids for example the genus Solanum Linn. where 2n = 24 (8), (9). Capsicum spp. stimulate circulation and enhance blood flow, it is considered good for the circulatory system, a common condiment to the diet (10). As a cardiovascular stimulant, Capsicum spp. assistin lowering blood pressure and breaking down cholesterol build-up. The warming properties of *Capsicum* are useful for people suffering from poor blood circulation to the hands and feet and other related conditions (11). Capsicum has been used as a digestive aid to ease intestinal inflammation, stimulate protective mucus membrane of the stomach, and also relieve pain caused by ulcer (12). Capsicum is commonly used to buffer pain from other ailments, including arthritis, varicose veins, headaches, menstrual cramps and respiratory conditions such as asthma (13). The fruits contain capsaicin (methyl vanillylnonenamide) a lipophilic chemical that can produce a strong burning sensation in the mouth of the unaccustomed eater (14). Woody plants can accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides, phenolic compounds, lignin and lignans (15). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological actions on the human body (16). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more (17). These natural compounds formed the foundation of modern prescription drugs as we know today (18). Saponins are used as blood cleanser (19). The presence of tannins aid in wound healing (20). Cardiac glycosides have been shown to aid in treatment of congestive heart failure and cardiac arrhythmia (21).

The relevance of the study is to enhance information on the existing literature and taxonomic characteristics of *Capsicum* species, this is due to the fact that they are economic plants of high repute. Thus the objectives of the study is therefore aimed at considering: the comparative morphological, anatomical, cytological and phytochemical investigations of *Capsicum annuum* Linn. and *Capsicum frutescens* Linn.

MATERIALS AND METHODS.

The materials used for this study were collected from both cultivated or domesticated species and raised from seeds purchased from the fruit and vegetable markets in Rivers state. A study of the macromorphological features of the species were made using a 30cm ruler. The plants parts measured included: leaf length, leaf width, petiole length, sepal length, petal length, stamen length, style length, fruit length and width, flower stalk length and pedicel length, and average plant height. The presence or absence of trichomes was observed painstakingly under a light microscope, and microphotographs were taken where relevant.

Floral biology: The opening and closing time of the flowers of the species were studied.

The arrangement pattern of the petals and sepals (that is the aestivation type) was 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 (22). 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 (22). 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 (23). 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.

$$=\frac{S}{E+S}x\frac{100}{1}$$
 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 =
$$\frac{T}{E+T}x\frac{100}{1}$$
.

Comparative Anatomical Studies: Seeds of the plant materials were plated 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 (24) with some modifications. 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 um 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 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 series 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. The slides so prepared are as good as those of microtomes 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 *Capsicum annuum* 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 accumulate chromosomes at metaphase between 9 and 10 a.m. to be precise. The roots were treated with Carnoy'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- orceinstain (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 (25). Photomicrographs of the chromosomes were taken from good temporary slides, using a Sony digital camera (7.2 Mega pixels).

Phytochemical Studies (Qualitative analyses): The leaves of each species studied were sun dried for 72 hours (3 days) and weighed. Fifty grams (50g) of the leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter filtered and evaporated to dryness using a rotary evaporator set at 45°C to constant weight and later, an exhort extraction machine. Residue yields werenoted and a portion was used for the phytochemical screening. Phytochemical screening for saponin, frothing tests, was done following the method described by (26), (27) as shown below: The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. 0.5g of each plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. In order to remove 'false-positive' results, the blood haemolysis test was performed on those extracts that frothed in water. 0.5g of each extract was boiled briefly with 50ml phosphate buffer, pH 7.4, and then allowed to cool and filtered; 5ml of the filtrate was passed for 3 hours through an asbestos disc (1.5mm thick and about 7mm in diameter), which had been previously soaked with two or three drops of 1 percent cholesterol in ether and dried. After filtration the disc was washed with 0.5ml of distilled water, dried and boiled in 20ml of oxylol for 2 hours to decompose the complex formed between cholesterol and any saponins in the extract. The disc was then washed in ether, dried and placed on a 7 percent blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was taken as further evidence of presence of saponins.

Test for alkaloids: 0.5g of each extract was stirred with 5ml of 1 percent aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (28), (29). A confirmatory test designed to remove non-alkaloidal compounds capable of eliciting 'false-positive' results was carried out as follows with all extracts which gave preliminary positive tests for alkaloids. A modified form of the tin-layer chromatography (TLC) method as described by (30) was used. 1g of the extract was treated with 40 percent calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. The extracts were combined and concentrated in vacuo to 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvent systems (of widely varying polarity) were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatograms (indicated by an orange or darker coloured spot against a pale yellow background) was confirmatory evidence that the plant extract contained an alkaloid.

Test for tannins: 5g of each portion of plant extract was stirred with 10ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (29).

Test for anthraquinones: Borntrager's test was used for the detection of anthraquinones. 5g of each plant extract was shaken with 10ml benzene, filtered and 5ml of 10 per cent ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones.

For combined anthraquinones, 5g of each plant extract was boiled with 10ml aqueous tetraoxosulphate vi acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10 per cent ammonia solution added. A pink, red, or violet coloration in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract (29).

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1 per cent aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (29).

Test for cardiac glycosides: Lieberman's test was used. 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice. Tetraoxosulphatevi acid was carefully added. A colour change from

violet to blue to green indicated the presence of a steroidal nucleus (i.e. aglycone portion of the cardiac glycoside) (31).

RESULTS:

MORPHOLOGICAL CHARACTERISTICS: The geographic location of the parent plants studied were 04⁰52¹3377¹¹N and 006⁰54¹860¹¹E at 18m altitude for *Capsicum annuum* Linn. and 04⁰54¹558¹¹N and $006^{0}35^{1}1181^{11}E$ at 6m altitude for Capsicum frutescens Linn.. The opening and closing times of the flowers was studied. It was revealed that the flowers commenced opening at 6:50 a.m. and opened completely at 8:30 a.m. while the closing time started at 5:00 p.m. and closed completely at 8:50 p.m. for C. annuum Linn, while C. frutescens Linn, flowers started opening at 6:50 a.m., opened completely at 9:00 a.m. and commenced closing at 4:30p.m. and closed completely at 9:00p.m. This feature is of taxonomic relevance. The germination test conducted was 60%. The distributional pattern of the species has been recorded by (3). Capsicum annuum Linn. (Plate 1) and Capsicum frutescens Linn. (Plate 2). Pepper as commonly known is an annual stout branched sub woody plant attaining up to a height of 65cm or more. The sub-sessile leaves are simply ovate, apex acutely acuminate and cuneate or abruptly acute at base measuring up to 19cm in length and 4cm wide. The petioles are 0.2cm in length. The glabrous stemsare also angular in shape. Inflorescences are terminal and flowers are borne singly at nodes and measuring up to 0.4cm in diameter. The petals are 5 and 6 in number for Capsicum annuum Linn., while petals are 5 for Capsicum frutescens Linn.pale whitish and up to 0.5cm long and 0.3cm wide. The greenish sepals are 5 and 6 in number but not separated, up to 0.2cm long and 0.1cm wide. The stamens are also 5 and 6 in number up to 0.3cm long. It is discovered that both species are both pentamerous while Capsicum annuum Linn. in addition hashexamerous flowers. The fruits comprised many seeded berry, borne singly at nodes, globose in shape for Capsicum annuum Linn. while linear in shape for Capsicum frutescens Linn, and when unripe green and red, orange, yellow, brown, or purplish when ripe up to 1 to 2.5cm in diameter for the former and 3 to 4cm in length, and 1 to 1.5cm in width. The seeds are 0.2 to 0.3cm 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: Capsicum annuum Linn. Foliar epidermal study revealed the presence of anomocytic stomata and uniseriate trichomes at both the adaxial and abaxial foliar surfaces (Plates 3 and 4). It is shown that the adaxial foliar layer has 25.95% stomatal index and 6.02% for the abaxial surface. Trichome index is also studied revealing 1.61% for the adaxial and 7.69% for the abaxial surfaces, while Capsicum frutescens Linn. also revealed anomocytic stomata and uniseriatetrichomes at both adaxial and abaxial surfaces. Plates 5 and 6. Shows stem epidermal study of Capsicum annuum Linn. showed presence of paracytic stomata, uniseriate trichomes and irregularly-shaped cells (Plate 7). Capsicum frutescens Linn. stem epidermal study revealed presence of contiguous stomata with paracytic structure and uniseriate trichomes (Plate 8).

Anatomical Investigation:

Anatomy of *Capsicum annuum* Linn.mid-rib shows uniseriate trichomes in 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 2 vascular traces having bicollateral arrangement with no rib bundle wings in both growth phases (Plate 9). The mid-rib of *Capsicum frutescens* Linn is similar to those of *C. annuum* Linn.. See plate 10. The petiole of *Capsicum annuum* Linn. is made of a layer of cells in the epidermis, 2 to 4 layers of collenchyma in the hypodermis, the general cortex is predominated by parenchymatous cells. The primary growth phase reveals 2 vascular traces from 1 gap having bicollateral arrangement with 2 rib bundle wings (Plate 11). The petiole anatomy for *C. frutescens* Linn.is as described for *C. annuum* Linn. (Plate12). The internodal anatomy of *Capsicum annuum* Linn. shows a four-sided or rectangular structure with swollen protuberances at each end. The epidermal layer has a layer of sclerenchymatous cells made of secondary walls. The hypodermis is made of 5 layers of

collenchymatous cells, and the general cortex comprises 3 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 3 to 4 cell-layers. The nodal pattern is unilacunar. The pith region is made of large parenchymatous cells. The internodal anatomy is shown in Plate 13. *C. frutescens* Linn.intermodal anatomy revealed 5-sided roughly shaped structure having same structural arrangement from the epidermis to the pith (Plate14). The nodal pattern for *C. annuum*Linn.is revealed in Plate 15 while the nodal pattern for *C. frutescens* Linn. is also unilacunar (Plate 16). Root anatomy of *Capsicum annuum* Linn. showed the piliferouslayer is single-cell thick. The vascular bundles are radially symmetrical with exarch xylary cells. Centralized parenchymatous cells occupy the pith region of the root (Plate 17). While the root anatomy of *C. frutescens* Linn. has radial symmetry with exarhxylary pattern and similar to those of *C. annuum* Linn. (Plate 18). Ovary anatomy of *Capsicum annuum* Linn. and *C. frutescens* Linn. revealed the placentation as axile type. Their ovaries are bilocular and 2-celled.

Cytological Investigation.

Cytological Studies of *Capsicum annuum* Linn.and *Capsicum frutescens* Linn. showed the mitotic chromosome number as 2n=24 at early metaphase for the former and late prophase for the latter (Plates 19 and 20).

Phytochemical studies: Qualitative analysis carried out revealed the presence of the following phytochemical constituents: alkaloids, saponins, tannins, phlobatannins, flavonoids, combined anthraquinones, free anthraquinones and cardiac glycosides respectively.

DISCUSSION.

Observations on vegetative and floral features of Capsicum annuum Linn. and Capsicum frutescens Linn. revealed the habits of the species as either annual sub woody plants or short-lived perennial sub-shrubs which are of taxonomic significance, Capsicum species are mostly annual sub-shrubs as also recorded by (3). In other words, fruits of Capsicum annuum Linn, and Capsicum frutescens Linn, are very pungent and as such are used as spices and preservatives, as supported by (2). Capsicum annuum Linn. and Capsicum frutescens Linn. possess simple uniseriate trichomes. Their stem epidermal studies revealed paracytic stomata including contiguous stomata present in stem epidermis of C. frutescens Linn.. Some of these morphological variations supported the findings of (1), (8) and (5). Capsicum annuum Linn.is observed having pentamerous and hexamerous flowers while C. frutescens Linn. is observed pentamerous. The structure of the stamens and carpels, and mostly their pilose nature are of taxonomic relevance in delimitations at the generic and species level. Their stem investigations revealed that Capsicum annuum Linn. is angular or tetra-angular structure, also possessing swollen protuberances at the tetra angular ends while C. frutescens Linn.is penta angular with undulating rough structure. The primary nodes of the Capsicum species are unilacunar and the roots vascular system revealed radial symmetry. The species investigated are bisexual, hypogynous and placentation are axile which is also in accordance to the observation of (3). The fruit globose for C. annuum Linn.as supported by the findings of (3) and C. frutescens Linn. has linear fruits. Anatomically, studies on the primary growth phase revealed the midribs and petioles of the Capsicum species are observed with 2 vascular traces and also have bicollateral vascular system. It was observed that the departures of the rib-bundle wings are towards the position of the open vascular system. The secondary growth phase revealed vascular arc structure in the mid-ribs and petioles, while the stems and roots showed a complete ring structure of an open vascular system in all the species investigation. Cytologically, the basic chromosome number for members of Solanaceaeis x = 12. Omidiji (8), Okoli and Osuji (9) and Okoli (25) also supported the chromosome basic number as x = 12. and diploids of 2n = 24.

The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological actions on the human body as also recorded by (16). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more as also related by (17). These natural compounds formed the foundation of modern prescription drugs as we know today as observed by (18).

CONCLUSION.

Capsicum species are essential ingredients in most African dishes. Their pungency and all-round seasonal fruiting period make them interestingly worth noting. Having worked extensively on their morphological, anatomical, cytological, and phytochemical properties, other areas of interest needs are DNA barcoding, Palynology, proximate analysis and quantitative aspect of phytochemistry. Interested researchers could carry on work in these areas.

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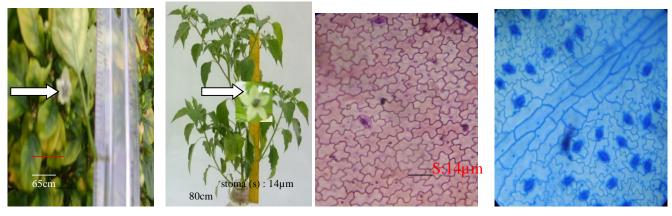


Plate 1: CapsicumannuumLinn.Plate 2: Capsicum frutescensLinn.Plate 3: C. annuumAdaxialfoliar surface Plate 4: C. annuumAbaxialArrow shows hexamerousArrow reveals pentamerous with anomocytic stomata surface with anomocytic stomata flowerflower

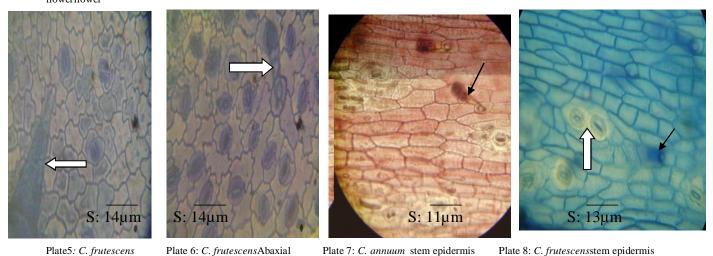


Plate 5: C. frutescens
Adaxial foliar surface
Arrow reveals tapering
Uniseriatetrichome. Trichome

Plate 6: C. frutescens Abaxial
foliar surface
Flack Arrow showsuniseriatetrichome.
Black Arrow showsuniseriatetrichome.
White Arrow reveals clavateuniseriate

howsuniseriatetrichome.

White Arrow reveals contiguous stomata.

0.2cm 0.2cm 0.2cm 0.2cm

Plate 9: *C. annuum* Plate 10: *C.frtescens* mid-rib
Mid-rib
Arrow shows vascular arc
Double-way arrow reveals
2 vascular traces.

Plate11: *C. annuum* petiole
Arrow shows vascular arc.

Plate 12: *C. frutescens* petiole Arrow reveals vascular arc .



Plate13: C. annuum Internodal stem anatomy Double-way arrow reveals Protuberances at each end. bundle wings

Plate 14 : C. frutescens intermodal stem anatomy

Black arrow shows developing rib-

Plate 15: C. annuumndodal pattern Plate 16: C. frutescensnodal pattern Arrow shows 2 vascular traces from White arrow reveals 2 vascular traces from 1 gap one (1) gap.

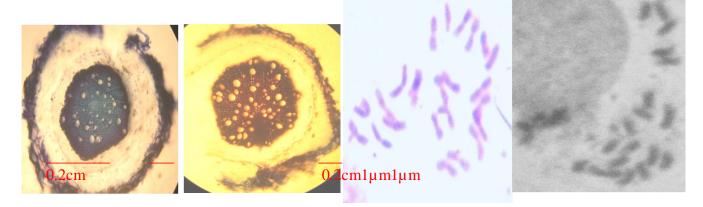


Plate17: C. annuum root Anatomy frutescensmitoticChromosomes at early metaphase

Plate 18: C. frutescens root anatomy

Plate19: C. annuum mitotic

chromosomes at late prophase

2n = 24

Plate 20: C.

2n = 24