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COMPARATIVE ANATOMICAL STUDIES ON THE STEMS OF FOUR ANNUAL HERBACEOUS SPECIES OF *Solanaceae*

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ABSTRACT

Four annual herbaceous species of *Solanaceae* were subjected to a comparative anatomical study on their stems at different growth successive stages. The main obtained results could be summarized as follows : 1- The extent of persistence of the epidermis during secondary growth of the stem differed according to the studied species. It may persist until the end of vegetative growth or may be substituted by a periderm initiated within timing of the periderm formation and extent of its development differed in the investigated species. 2- The clear manifestation dilatation growth in the extraxylary tissues of the stem differed according to species, depending probably on the rate of this process. 3- Pith diameter in the stem may remain unchanged after secondary growth, or it may largely decreased, according to species.

INTRODUCTION

Response of the primary tissues outside the xylem tissue to secondary growth, and sufficient information about the development of protective tissues in stems of the annual herbaceous dicotyledons is not sufficient in literature. The development of protective tissues in stems was stated by Eames and Mc Daniels (1947);Luhan (1955), Fahn (1977) and Carlsbecker and Helariutta (2005) to be restricted to the perennial plants where a true periderm arises at different levels.

Eames (1961); Fahn (1977); Carlsbecker and Helariutta (2005) and Bresinsky *et al.* (2008) pointed out that a variety of protective tissues was observed in plants with a limited amount of secondary growth as do the herbaceous dicotyledons growing in the Alpes : persisting thick-walled epidermis (*Ranunculus*); exodermis (*Primula*)); exogenously originating periderm (*Artemesia*) and other *Asteraceae*; dead and collapsed but persisting cortex (*Polygonum*); subdivided and superized endodermis (*Gentiana*); polydermal (*Potentilla*); periderm of deep seated origin (*Saxifragaceae*).

Metcalfe and Chalk (1983) recorded that in stems of the solanaceous plants, cork arises in the epidermis or sub-epidermis in species of several genera. It may also originate near the outer margin of the phloem in species of some genera. Secondary growth in the stem causes the xylem to be present in the form of a continuous cylinder, traversed by narrow rays in most of the genera and species examined, dissected by relatively broad rays in *Datura stramonium*. Vessels of the primary xylem tended to be in radial rows; those of the secondary variously distributed in different genera and species.

Some scientists studying the development of protective tissues in stems of the solanaceous plant species concluded that all investigated plant species belonging to the same genus have the phellogen in the same position, though no taxonomic importance may be as yet derived from it. The phellogen was described as epidermal, hypodermal or pericyclic (Rotera and Cristiani, 1957; Carlsbecker and Helariutta, 2005; Sieburth and Deyholos, 2006 and Bresinsky *et al*. 2008).

Esau (1965) and Metcalfe and Chalk (1979) stated that the amount of secondary growth varies in the different herbaceous plants as do the histology of the tissues and the distinction of periderm.

The development of protective tissues in stems of the *Solanaceous* plants concluded that all the *Argentine* spp. Studied, belonging to the one same genus have the phellogen in the same position, though no taxonomic importance may be as yet derived from it. The phellogen was described as epidermal, hypodermal or pericyclic.(Rotera and Christiani,1957).

Owing to the recorded diversity in the way by which the herbaceous dicotyledons build up protective tissues during their secondary growth beside the scarce literature on this histological character, it was highly appreciated to carry out the present work.

Four herbaceous annual species of the *Solanaceae* belonging to different genera were involved namely *Solanum melongena* L., *Solanum lycopersicum* L., *Datura stramonium* L.and *Capsicum frutescens* L. all of economic importance.

The main aim of the present investigation was to study in some detail the response of the primary body and the histological tissue changes accompanying secondary growth of the stems of four annual species of the *Solanaceae* i.e. eggplant, tomato, Thorn Apple and Hot Pepper plants. The anatomical structure of the stems was particularly described for the presence or absence of periderm and to time and site of its formation.

MATERIALS AND METHODS

The current work was carried out at the experimental station, Faculty of Agriculture, Cairo University, during the season 1974.

Seed lots of the four investigated solanaceous species listed before i.e. *Solanum melongena* L. (White Balady), *Solanum lycopersicum* L. (Pearl Harbor), *Datura stramonium* L. and *Capsicum frutescens* L. (California Wonder) were secured from the Agricultural Society, A.R.E. Seeds were sown on April 18. Forty pots 40 cm diameter filled with Nile Clay were used, 20 for each species. When plants were 22 days old, they were thinned out to 5 plants for each pot.

All plants were fertilized with a combination of NPK as 3:2:1 at a rate of 10 g/plot. This amount was equally applied at the two ages 25 and 50 days after sowing. While, some plants were devoted to secure the specimens needed for the histological study others were used for obtaining data for certain morphological characters.

For the histological study at every studied age, three samples from digging out plants of the main stem at middle of the 1st internode were killed and fixed in F.A.A. solution (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70 %).

From three weeks onwards, the stem samples were secured every fortnight until the age of 9 weeks and after every three weeks until end of the vegetative growth.

Fixed materials were dehydrated by normal butyl alcohol method and embedded in paraffin wax of 56-58 ^{o}C. m.p Sections 15 µ thick were cut by a rotary microtome (Gerlach, 1977).

Saffranin-light green combination was found to be the most suitable method for staining (Nassar and EL-Sahhar, 1998). Stained sections were mounted in Canada balsam and by light microscopy examined and photographed.

RESULTS AND DISCUSSION

***Solanum melongena* L.**

Age of 5 weeks :

At the age of 5 weeks the formation of a true periderm in the epidermis was obvious (Fig. 1). Thus, the periderm is superficial and its presence accompanied the development of a continuous vascular cylinder due to the active division of both intra and intrafascicular cambia. To accommodate the increase in circumference, the cortex underwent dilatation growth. The division of cells following their tangential elongation started from inside towards the periphery, the outer cortical layers were still devoid of any anticlinal walls.

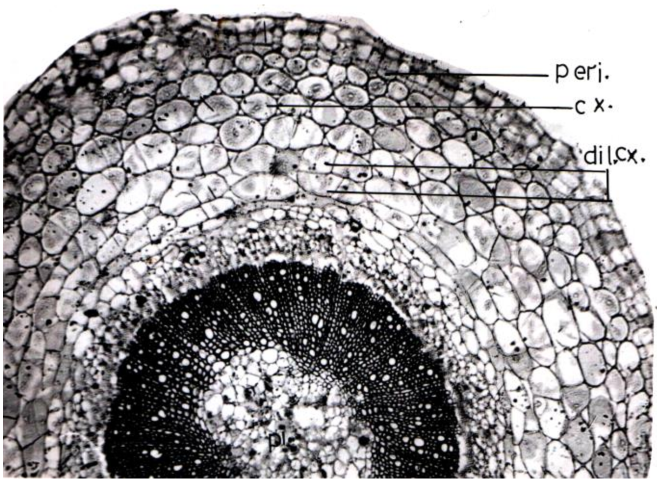

Fig. (1): Transection at the base of main stem of *Solanum melongena*, 5-weeks old plant (X77).

Peri.: Pericycle, Cx.: Cortex, dil.cx.: dilated cortex.

Age of 7 weeks :

At this age dilatation growth was typical in all the cortex and more layers of periderm were developed (Fig. 2). Vascular cylinder especially its xylem, increased considerably in width than in the previous age. Crystal sand was seen filling several cells of the phloem parenchyma.

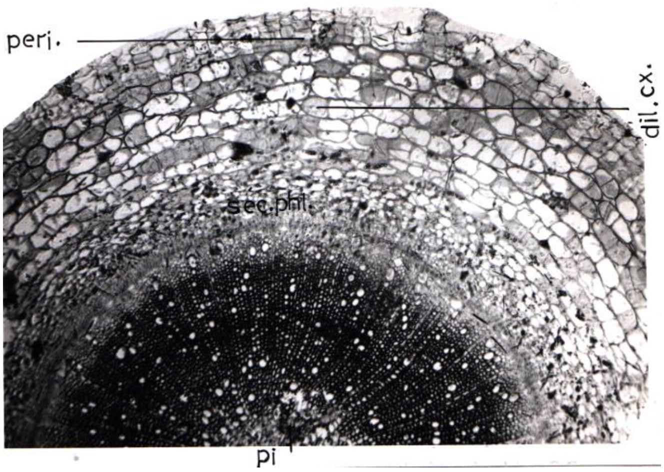

Fig. (2): Transection at the base of main stem of *Solanum melongena*, 7-weeks old plant (X77).

Peri.: Periderm, dil.cx.: dilated cortex, Pi.: Pith.

Age of 12 weeks :

It was clear that by nearly the end of vegetative growth, the vascular cylinder and the xylem region in particular had greatly increases in width (Fig. 3). However, the pith became deformed and decreased much in size probably by an inward pressure of the enlarging secondary body. Such phenomenon was recorded by Esau (1977).

The examination of sections until completion of the vegetative growth showed as in the root a very slow growth of the vascular cylinder. The periderm remained at its original place i.e. the epidermis and it increased vigorously in width, the cortex was much pressed, and it was too difficult to detect the outline of the individual cells or to count the number of its layers, due to the exaggerated dilatation growth.

The general structure of the xylem was as that described for the root. The main difference was in the bigger size of the vessels in the root, it compared with those in the stem, but in the latter this was compensated by bigger number of vessels.

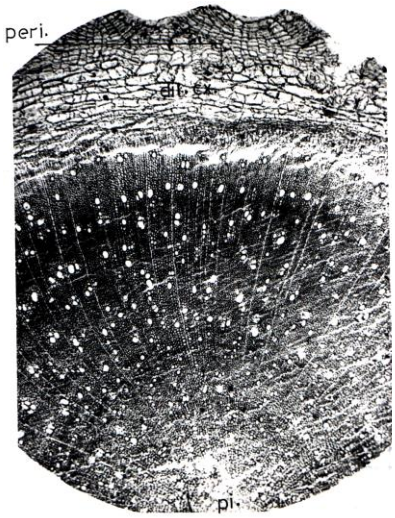

Fig. (3): Transection at the base of main stem of *Solanum melongena*, 12-weeks old plant (X77).

Peri.: Periderm.

***Solanum lycopersicum*, L. :**

Fig (4) illustrates the structure of the tomato stem at the age of 12 weeks after sowing i.e. after the cessation of vegetative growth. In spite of the presence of a wide continuous vascular cylinder formed by the activity of both inter and intrafascicular cambia. The epidermis was capable of persistence. It remained through dilatation growth the only protective tissue in the stem. Trichomes were still visible at few places. All cortical cells were seen dilated and divided with one or more radial walls.

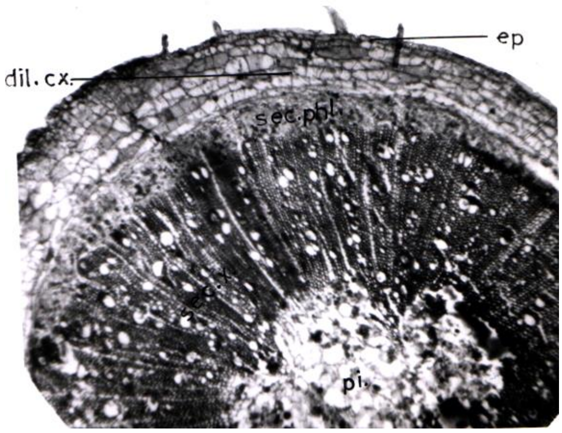

Fig. (4): Transection at the base of main stem of *Solanum lycopersicum*, 12-weeks old plant (X77).

ep.: epidermis, dil.cx.: Dilated cortex.

It was obvious that cells of the inner cortical layers were much more radially flattened than those of the outer ones and divided by more number of radial walls. It was difficult to distinguish the boundaries of many of these cells. This was probably due to their more exposure to the outward pressure which seemed to be weakened outwardly. Such phenomenon was also noticed in the eggplant.

The dissection of the xylem tissue into partitions by rather broad rays in the root was not shown in the stem.

The vessels were mainly solitary and smaller in diameter than those of the root.

The pith was narrowed during secondary growth, a phenomenon which was noticed before with *Solanum melongena* L. This was noticed when the stem at the age of 12 weeks was compared with that at 7 weeks (Fig. 5) where the pith was decidedly wider and consisted of large cells except at the vicinity of the xylem cylinder. An inward continuous pressure might be the reason for decreasing the pith. Many phloem parenchyma cells were seen filled with crystal sand.

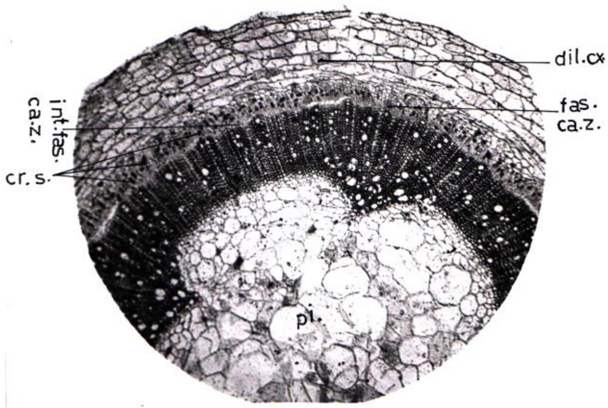

Fig. (5): Transection at the base of main stem of *Solanum lycopersicum*, 7-weeks old plant (X77).

dil.cx.: Dilated cortex, fas.ca.z.: Fascicular cambium zone, cr.s.: Crystal sand, int.fas.ca.z.: Interfascicular cambium zone.

***Datura stramonium* L.:**

It was not before the age of 7 weeks after sowing that tangential division of the epidermal cells started to build-up a periderm (Fig. 6) which, in the eggplant started earlier by about 2 weeks (Fig. 1). It appeared therefore, that in *Datura* the epidermis was less capable of long-durated dilatation growth. Also, in the eggplant at the age of 7 weeks, a complete vascular cylinder nearly of uniform width was developed due to an equal rate of cell division of both intra and intrafascicular cambia. The vascular cylinder enveloped a small pith area.

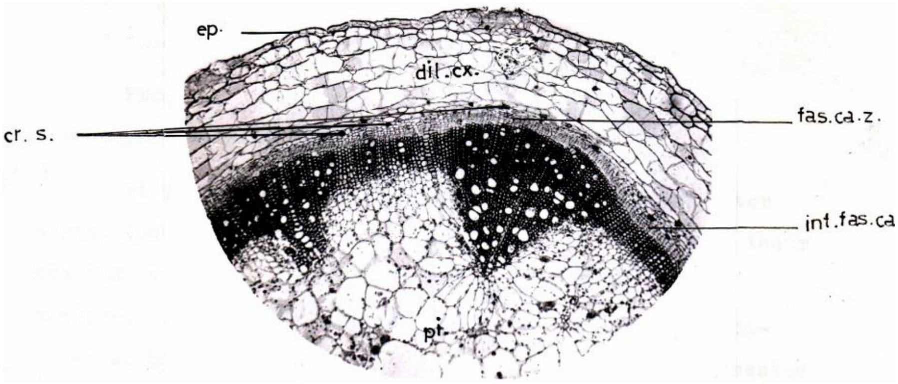

Fig. (6): Transection at the base of main stem of *Datura stramonium*, 7-weeks old plant (X100).

ep.: Epidermis, dil.cx.: Dilated cortex, int.fas.ca.z.: Interfascicular cambium zone, fas.ca.z.: Fascicular cambium zone, cr.s.: Crystal sand.

In Datura, the vascular cylinder was not regular in width due to the much more activity of the intrafascicular cambium which resulted in the appearance of wedge shaped structures at places of the original primary bundles.

The pith in contrast with that in the eggplant and in comparison with that in the tomato plant was too intensive. Another difference between the three plants at this age was that the cortex, while in the Jimson weed and the tomato plant showed a typical illustration of dilatation growth among all its layers, it was not so in the eggplant where this process was much less pronounced especially in the outer layers. This phenomenon was noticed in spite of the development of wider vascular tissues in the latter plant species. This might be attributed to a less capacity of the cell walls for tangential elongation with rapid cell division causing, therefore, quick appearance of newly formed cells which spread the cortex tissue (Fig. 7).

Presence of crystal sand was restricted to a few parenchyma cells of the phloem as in the eggplant.

At the end of vegetative growth i.e. 12 weeks after sowing (Fig. 7), secondary conducting tissues reached their maximum quantity either within or between the original bundles. The wedge-shaped structures formed by the fascicular cambium were still observed. They were interconnected by rather wide bands produced by the interfascicular cambium. These bands contained mainly lignified parenchyma and relatively few vessels in comparison with the wedge-shaped structures.

In the tomato stem, the vessels were of nearly same density through the whole vascular cylinder. The rays were mainly one cell wide; the broad rays recorded by Metcalf and Chalk (1950) to characterize the genus Datura were not observed.

Fig. (7): Transection at the base of main stem of *Datura stramonium*, 12-weeks old plant (X100).

Peri.: Periderm.

The cortex was largely dilated to the extent that some cells were divided anticlinally by 3 or more walls and it was difficult sometimes to identify their original outline.

The superficial periderm was poorly developed and consisted of a few layers in contrast to that of the eggplant stem.

As stated before, the pith in either the eggplant or the tomato plant decreased in diameter as growth proceeded such phenomenon was not recognized in the Jimson weed.

The vessels of the stem were smaller in size than in the root, a phenomenon showed also by the eggplant and the tomato plant.

***Capsicum frutescens* L. :**

Fig. (8) showed at the age of 7 weeks, the presence of a continuous vascular cylinder as a result of the activity of both inter- and intrafascicular cambia. It was difficult to define the site of the primary bundles. There was a considerable amount of secondary growth which seemingly was faced by the epidermis and the cortex, by increase in size of their cells.

A true periderm did not develop in the epidermis until a later age i.e. 12 weeks after sowing (Fig. 9). The epidermis, therefore, proved to be of rather long durated capacity for dilatation growth. The cortex underwent active dilatation growth due to the big increase in girth of the stem at this age.

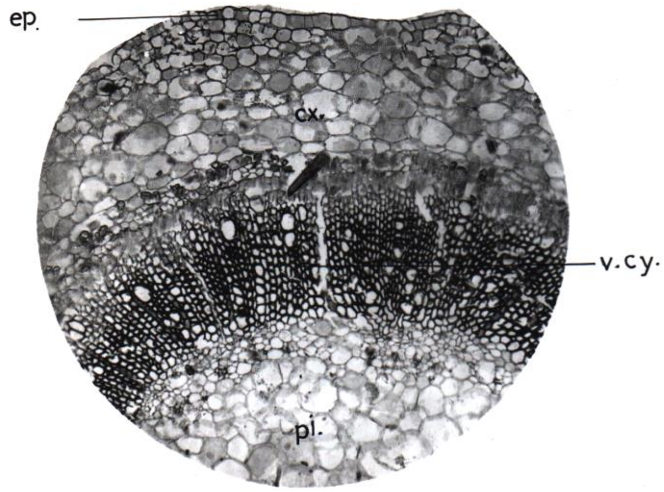

Fig. (8): Transection at the base of main stem of *Capsicum frutescens*, 7-weeks old plant (X100).

ep.: Epidermis, v.cy.: Vascular cylinder.

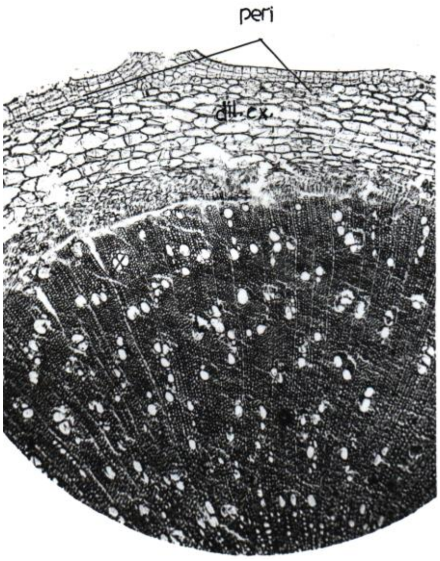

Fig. (9): Transection at the base of main stem of *Capsicum frutescens*, 12-weeks old plant (X100).

Peri.: Periderm, dil.cx.: dilated cortex.

At end of vegetative growth (Fig. 10) i.e. 15 weeks after sowing, the periderm increased somewhat in width and its cells were shown compressed or even ruptured due to the outward pressure exerted from the continued growth of the vascular cylinder during the last 3 weeks of vegetative growth. A new periderm was generated in the subepidermal layer.

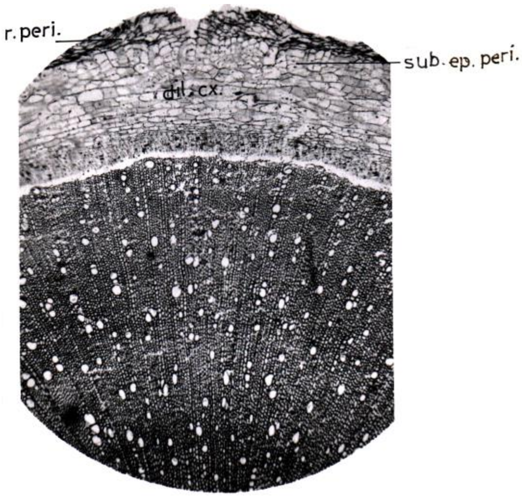

Fig. (10): Transection at the base of main stem of *Capsicum frutescens*, 15-weeks old plant (X77).

r. peri.: Ruptured periderm, sub.ep.peri.: Subepidermal periderm.

The vessels in the stem appeared smaller than those in the root. Their arrangement, the structure of the ground tissue, and the rays did not differ than described before in the root.

Comparing the xylem tissue at base of stem at end of vegetative growth, it was clear that the stem possessed bigger number of vessels as tabulated below :

	Mean No. Vessels	**Mean Vessel diameter (µ)**
Solanum melongena	1200	44.8
Solanum lycopersicum	940	48.0
Datura stramonium	800	51.8
Capsicum frutescens	1000	52.3

It was interesting to notice that the increase in amount of vegetative growth as estimated by dry weight appeared to go parallel with the increase in vessel diameter and not in number of vessels in the stems.

The investigated plant species could be arranged according to amount of vegetative growth, in two categories as shown below :

			g/plant
Eggplant	}	Small amount	4.779
Tomato			4.124

			g/plant
Thorn Apple	}	medium amount	7.378
Hot Pepper			6.880

The same arrangement could also be made with respect to the vessel diameter tabulated before. It seemed, therefore, that more nutritional requirements of bigger vegetative growth needed the development of wider vessels rather than their bigger number.

The investigated species varied in the presence, distinction and timing of formation of the periderm in their stems. In the species that developed a periderm this took place originally in the epidermis. This was in accordance with the statement of Metcalfe and Chalk (1950) for the *Solanaceous* species; they added that it may also occur in the subepidermis.

The description given for xylem characteristics in stems including density, size, shape, arrangement of vessels and width of rays may be of taxonomic value, since it may help in the identification of genera or species of the *Solanaceae*.

Finally, the other obtained results or histological observations might be of physiological evolutional or taxonomic value.

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دراسات تشريحية مقارنة على سيقان أربعة أنواع حولية عشبية من العائلة الباذنجانية

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قسم النبات الزراعى – كلية الزراعة – جامعة المنصورة

أربعة أنواع حولية عشبية من العائلة الباذنجانية كانت محل دراسة تشريحية مقارنة على سيقانها وذلك خلال مراحل نمو متتالية.

ويمكن تلخيص أهم النتائج المتحصل عليها كما يلى :

١- لوحظ أن قدرة البشرة على البقاء أثناء النمو الثانوي فى الساق تختلف تبعاً للنوع. إذا يمكن أن تستمر حتى نهاية النمو الخضري أو أن تستبدل بالبريدرم المتكون فيها. ولقد وجد أيضاً أن توقيت تكوين البريدرم وإمتداد تكونه يختلف في الأنواع محل الدراسة.

٢- وجد أن وضوح القابلية للتمدد فى الأنسجة الخارجية فى الساق يختلف تبعاً للنوع. ومن المحتمل أن يتوقف ذلك على معدل حدوث هذه العملية.

٣- وجد أن قطر النخاع فى الساق يمكن أن يظل بدون تغير بعد حدوث النمو الثانوي أو أن ينقص بوضوح وذلك تبعاً للنوع.