Journal of Spices and Aromatic Crops 7 (2) : 111-123 (1998)

Anatomy of *Piper colubrinum* Link.

P N RAVINDRAN & A B REMASHREE

Indian Institute of Spices Research
Marikunnu P. O., Calicut - 673 012, Kerala, India.

Abstract

Piper colubrimum exhibits dimorphic branching (orthotropic and plagiotropic shoots) as in many other *Piper* species. The stem exhibits anomalous secondary growth. The number and size of peripheral and medullary bundles show variations in the two types of shoots. Secondary growth occurs only in the peripheral bundles. In a mature stem (7 mm thickness), the average length and width of xylem vessel is 258 µm and 73.8 µm respectively, in peripheral bundles, whereas in medullary bundles it is 195 µm and 81.5 µm, respectively. Vessels are pitted with simple perforation plates. Tracheids have scalariform and annular thickening. Libriform unseptate fibres are also present. Comparative stem anatomy of *P. nigrum* and *P. colubrimum* shows similarities. However, the aerial and underground roots show variations especially in the number of vascular elements. Secondary thickening in root is comparable to that in dicots. The aerial roots undergo transformation in internal structure when they enter soil and grow as underground roots.

Key words : anatomy, *Piper colubrimum, P. nigrum.*

Introduction

Piper colubrimum Link. (Piperaceae), a woody shrub, is a native to northern part of South America. This species is of great importance becase of its resistance to *Phytophthora capsici* Leonian and *Radopholus similis* Thorne (burrowing nematode), the causal organisms of foot rot and slow decline, respectively. Both being soil-borne pathogens, it was felt by many that grafting black pepper (*P. nigrum* L.) on *P. colubrimum* would solve these problems. Trials carried out in Brazil, Sarawak and India indicated high initial success but poor survival later which was interpreted to be due to the anomalous secondary growth present in *Piper* spp. (De Waard & Zeven 1969). The anomalous anatomy of *Piper* spp. has attracted the attention of many workers. The vascular organization in *P. longum* L. and *P. betle* L. (Murthy 1959), the nature of development of medullary and peripheral bundles of the genus *Piper* from their ontogenetic and histogenetic stand point (Pal 1961) and comparison of the vegetative anatomy of

Piperales (Datta & Dasgupta 1977) were studied in these perennial climbers. *P. colubrinum,* because of its resistance to soil-borne pathogens, is of great practical significance in crop breeding. Efforts are being made at Indian Institute of Spices Research, Calicut to derive a grafting technique to offset the incompatibility caused by its anatomical features. In this context a detailed knowledge of the anatomical features of *P. colubrinum* and a comparison with *P. nigrum* becomes essential.

Materials and methods

Orthotropic stems of 4 mm and 7 mm thickness and plagiotropic stems of 4 mm thickness and aerial and underground roots of *P. colubrinum* and *P. nigrum* were collected from the Germplasm Conservatory of Indian Institute of Spices Research at Peruvannamuzhi (Kerala). The samples were fixed in Formalin-Acetic acid-Alcohol mixture. The materials were processed for microtomy as per standard procedures and cut at 5 µm thickness in an electric sledge microtome and stained with toluidine blue O for histological studies (Johansen 1940; Krishnamoorthy 1988).

Results and discussion

P. colubrinum, as in many other species of *Piper,* exhibits a dimorphic branching pattern. The orthotropic shoots show monopodial growth and the plagiotropic (flowering) branches sympodial growth. During the course of development, vegetative apical buds of the latter modify into spikes and vegetative growth is continued by axillary buds. The spikes therefore appear leaf opposed.

Stem

Vascular structure of *P. colubrinum* stem is basically similar to that of other *Piper* species. There are 5-10 medullary bundles and 39 to 49 peripheral bundles in a 4-5 mm thick stem. Secondary thickening is restricted to the peripheral vascular bundles (Fig. 1 A). The epidermis of both plagiotropic and orthotropic stems are single layered having a thick cuticle (Fig. 1 B). The cortex possesses three types of cells - chlorenchyma, collenchyma and parenchyma (Fig. 1 B). Mucilage canals are present in outer cortex while there is no central mucilage canal as in *P. nigrum* (Fig. 1 C). The cortex possesses 15 to 17 layers of cells. Endodermis with casparian strips and pericycle are distinct (Fig. 1 D). Vascular bundles are conjoint, collateral and open. In each peripheral vascular bundle, a continuous wavy band of 3-5 layered sclerenchymatous conjunctive tissue is observed and the lower part of xylem region gets merged with these layers (Fig. 1 C). The medullary bundles are large and 2 or 3 layers of sclerenchymatous sheaths are present on the upper side of the bundles during maturation (Fig. 2 A). The vascular bundles in the peripheral ring consists of small and large bundles arranged alternately.

Phloem consists of sieve tube elements, companion cells, phloem parenchyma and phloem fibres (Fig. 2 B). Cambium is two to three layered and produces xylem towards inside and phloem outside (Figs. 1 C & 2 B). Xylem is highly lignified but the newly produced metaxylem elements are unlignifed (Fig. 1 C). Xylem consists of xylem vessels, tracheids and fibres, having pitted thickening and simple perforation plates. Tracheids are of scalariform and annular types. Libriform fibres are also present.

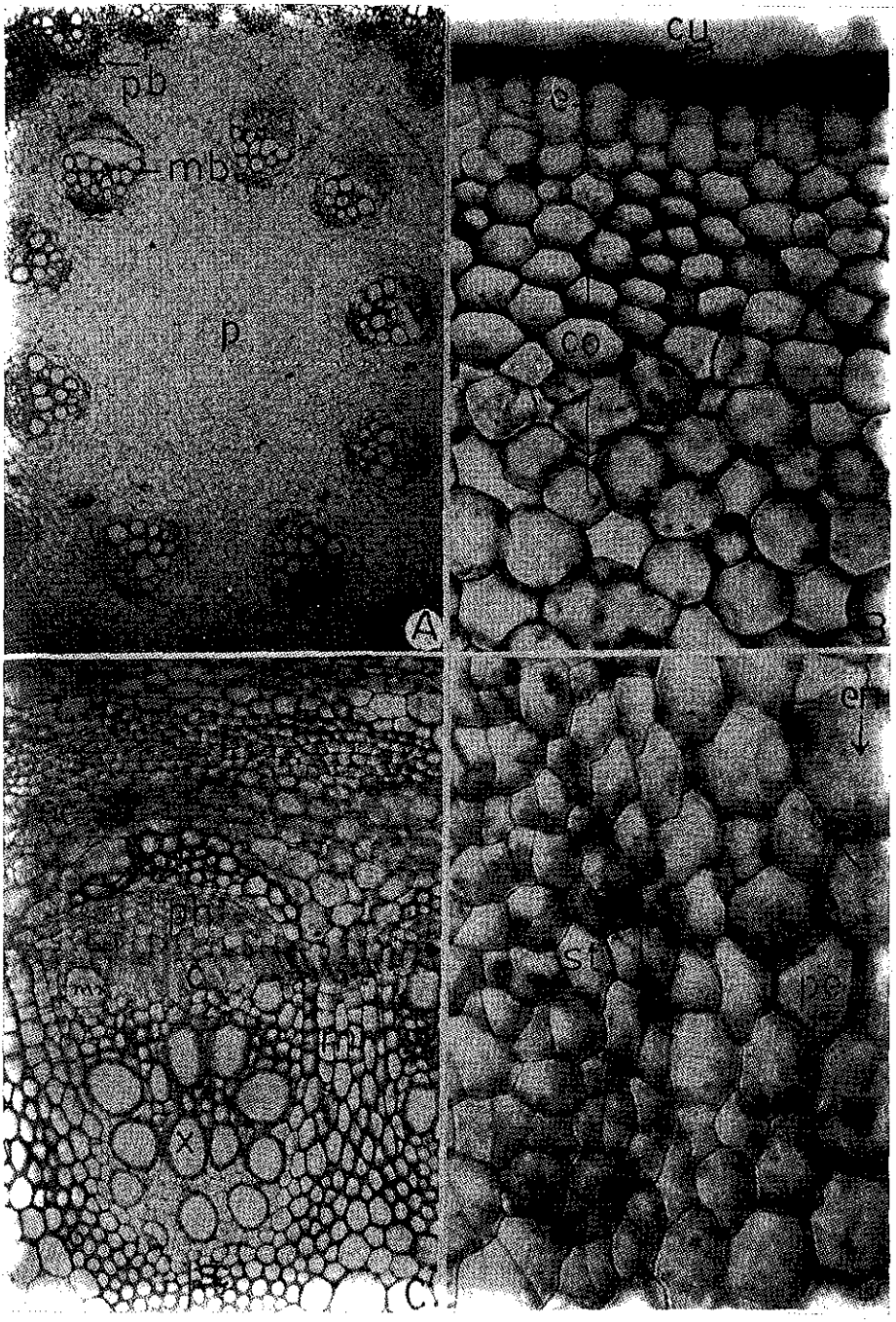

Fig. 1. T S of stem of *Piper colubrinum*
A. Peripheral and medullary bundles (x 200) B. Cuticle epidermis and cortex (x 400) C. Single peripheral vascular bundle (x 200) D. Endodermis with casparian strips, pericycle and phloem (x 400) (c-cambium; co-cortex; cs-casparian strips; cu-cuticle; e-epidermis; en-endodermis; f-fibre; mb-medullary bundle; mr-medullary ray; mx-metaxylem; p-pith; pb-peripheral bundle; ph-phloem; pe-pericycle; st-sieve tube; x-xylem.)

Variations are observed in vascular bundles found in plagiotropic and orthotropic shoots. The average number of peripheral bundles in mature plagiotropic stem is 39 and medullary bundles 9 in a 4 mm thick stem, whereas young othrotropic stems possess an average of 42 peripheral bundles and 11 medullary bundles in stems of same thickness. The mature orthotropic stem of 7 mm thickness shows an average of 49 peripheral bundles and 15 medullary bundles (Table 1).

In the plagiotropic stem, in each bundle, xylem tracheid and vessels are in a proportion of 3:10 in peripheral bundle and 3:8 in medullary bundle. In the case of orthotopic stem, it is 4:8 in peripheral bundles and 3:5 in medullary bundles. The orthotropic stem with secondary growth exhibits a xylem : phloem ratio of 7:9 in peripheral bundle and 5:7 in medullary bundle (Table 2).

The diameter of medullary bundles increase proportionately with growth. The traceary elements of orthotropic stems are larger than the plagiotropic stem; however increase in diameter of these elements during growth is accompanied by decrease in length (Table 3 & 4).

The vessels show greater wall thickness than tracheids. Both in plagiotropic and orthotropic stems, medullary bundles have greater wall thickness than peripheral bundles and orthotropic stems have greater wall thickness than plagiotropic stems (Table 5). Secondary thickening is observed only in peripheral bundles (Fig. 2 C). A clear cambial ring is formed during secondary growth. Fascicular cambium present between primary xylem and primary phloem cuts off xylem elements towards inside and phloem elements towards outside. But interfascicular cambium, which is present between vascular bundles, produce medullary rays and axial parenchyma towards inside and parenchymatous tissue toward outside (Fig. 2 D). Medullary rays and pith regions contain numerous starch grains (Fig. 2 D).

Aerial root

Externally, the aerial root is bound by the epidermis with a thick cuticle. Below the epidermal cell, 2 to 3 layered closely packed thick walled hypodermal cells with a few sclerenchymatous patches are present (Fig. 3 A) The cortex consists of 20-25 layers of chlorenchyma and parenchyma cells containing starch grains (Fig. 3 B). In the cortex, 10-12 mucilage canals surrounded by epithelial cells are found and there is no central mucilage canal (Fig. 3 C). The inner most most layer of the cortex constitutes the endodermis and consists

Table 1. Number of vascular bundles in stem of *Piper colubrinum*

Category	Peripheral bundles	Medullary bundles
Orthotropic stem (4 mm thickness)	42	11
Orthotropic stem (7 mm thickeness)	49	1
Plagiotropic stem (4 mm thickness)	39	9

Values are means of 10 samples

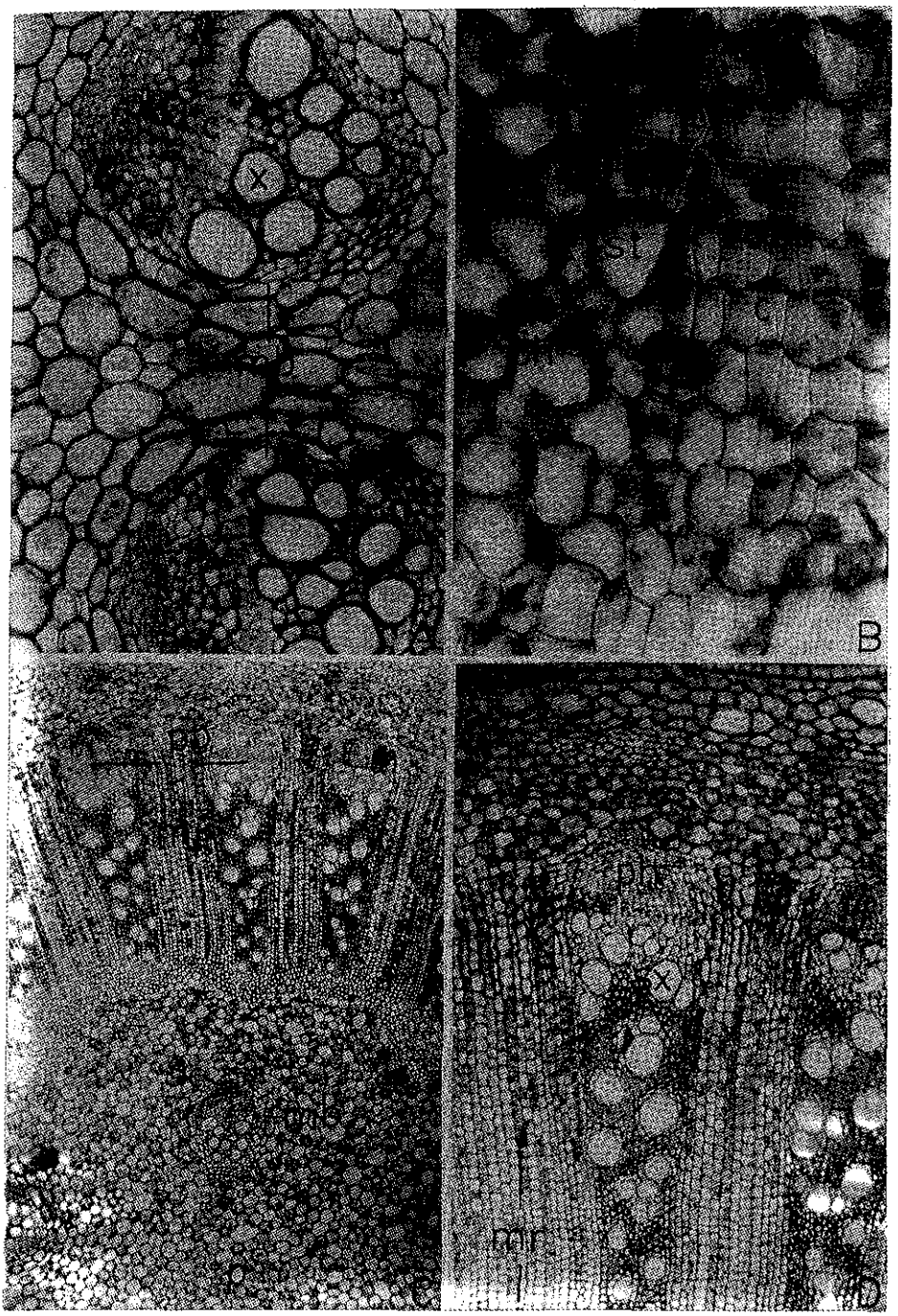

Fig. 2. T S of stem of *Piper colubrinum*
A. Medullary bundles (x 400) B. Cambial layers and phloem (x 400) C. Peripheral and medullary bundles (x 100) D. Secondary growth in peripheral bundles (x 200) (c-cambium; mb-medullary bundle; mr-medullary ray; p-pith; pb-peripheral bundle; ph-phloem; st-sieve tube; sc-sclerenchyma; x-xylem)

of cells having distinct casparian strips (Fig. 3 C & D).

In the aerial root tip, vascular bundles are radially arranged with 40-46 groups of xylem and phloem (Fig. 3 B). During development, secondary growth takes place and a cambial strand is first formed below the phloem and then above the protoxylem region. Later these cambial strands join together forming a continuous cambial ring (Fig. 3 C). In the fascicular region, it is 2 to 4 layered and in the interfascicular region it is 1 to 2 layered. The secondary cambium of the aerial root functions like that in the stem and cuts off xylem towards inside and pholem towards outside giving the configuration of conjoint, collateral and open type of vascular bundle.

Phloem consists of sieve tubes, companion cells, phloem parenchyma and a few phloem fibres. As in the case of stems, after secondary growth, phloem is arranged exterior to xylem (Fig. 3 D). The interfascicular cambium produces parenchyma tissues towards outside and conjunctive sclerenchymatous tissues towards inside. Xylem consists of fewer vessels and more of tracheids. Pith is constituted by thick walled parenchyma with mucilage ducts and cells contain starch grains (Fig. 3 D).

Transition of aerial root

When the root is above the soil, the cortex and the pith as equal in size but when it reaches the soil a gradual decrease in the size of the pith and an increase in the cortical region is observed. As the roots grow in soil, the number of vasular bundles gets reduced. A transition from aerial root to normal underground root sets in and shows the typical structure of a normal root. The changes taking place during the transition of the aerial root to normal root is represented in Fig. 5.

Underground root

The underground root has a thick cuticle with root hairs. Just below the epidermis, 2-3 layered hypodermis exists without sclerenchymatous patches. The cortex consists of 25-30 layers of lacunar collenchymatous cells. Most of the cells contain starch grains (Fig. 4 A). Endodermis and pericycle are present, but casparian strips are not clearly visible. There are six groups of radially arranged vascular elements that occupy the central part of the root (Fig. 4 B & D). Xylem is exarch consisting of 2-3 vessels and the rest tracheids. Phloem cells are arranged in patches with sieve tubes, companion cells and phloem

Table 2. Proportion of tracheids and vessels in each vascular bundle of *Piper colubrinum*

Category	Peripheral bundles	Medullary bundles
Orthotropic stem (4 mm thickness)	4:8	3:5
Orthotropic stem (7 mm thickeness)	7:9	5:7
Plagiotropic stem (4 mm thickness)	3:10	3:8
Aerial root	6:3	Nil
Underground root	8:2	Nil

Values are means of 10 samples

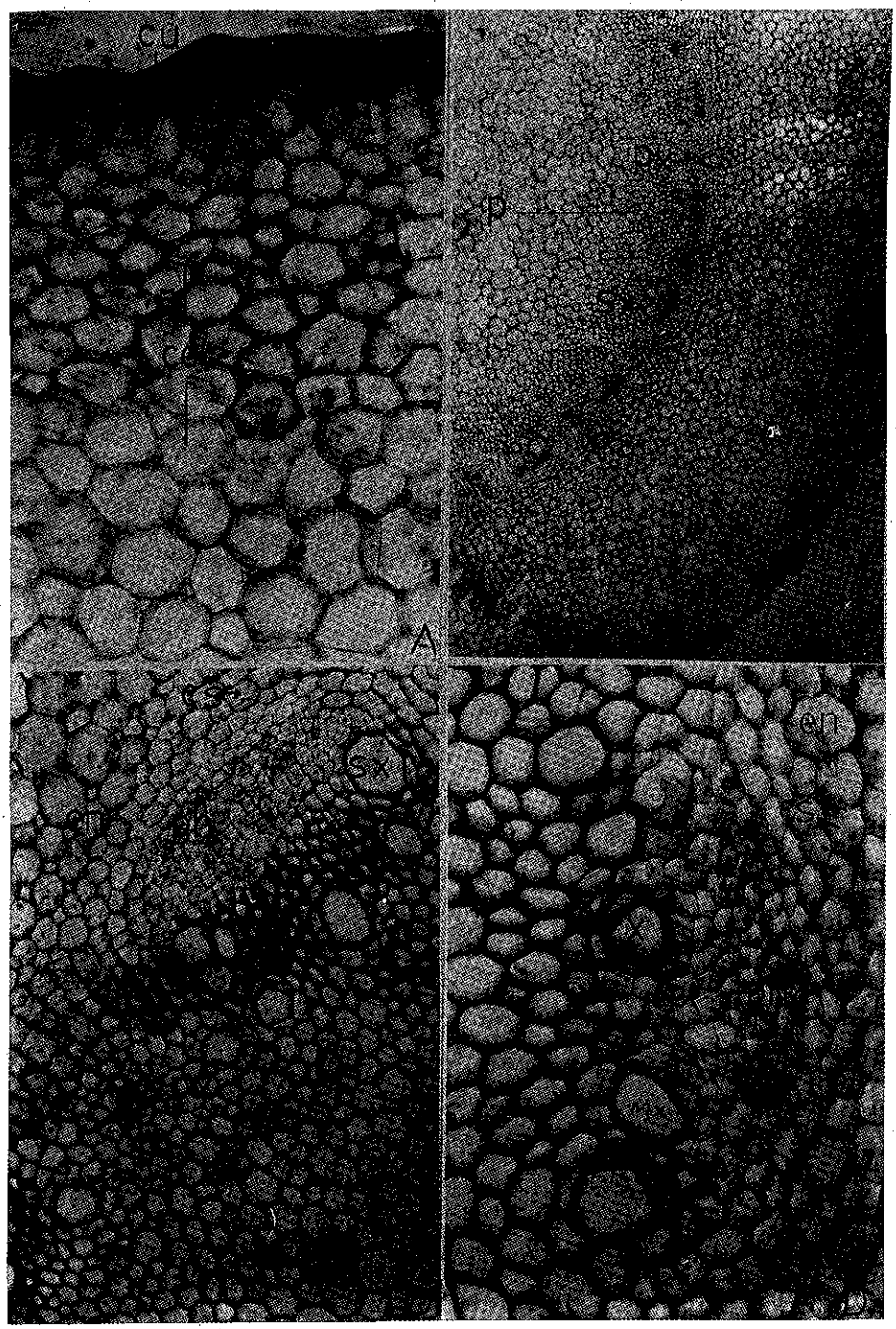

Fig. 3. T S of aerial root of *Piper colubrinum*
A. Thick cuticle, epidermis and cortex (x 400) B. Vascular bundles, pith and cortex (x 100) C. Secondary growth (x 200) D. Enlarged view of single vascular bundle (x 400) (c-cambium; co-cortex; cs-casparian strip; cu-cuticle; en-endodermis; mx-metaxylem; p-pith; ph-phloem; px-protoxylem; sx-secondary xylem; x-xylem.)

Table 3. Diameter (in μm) of vessels, tracheids and fibres in *Piper colubrinum*

Category	Peripheral bundle			Medullary bundle		
	Min	Max	Av	Min	Max	Av
Orthotropic stem (4 mm thickness)						
Vessel	15.0	62.0	36.3	28.0	79.0	51.6
Tracheid	13.0	28.0	17.8	15.5	25.5	20.5
Fibre	10.0	22.0	15.3	10.0	23.4	18.7
Orthotropic stem (7 mm thickness)						
Vessel	68.0	83.0	73.0	68.0	90.8	81.2
Tracheid	24.0	58.0	34.3	26.0	57.0	38.4
Fibre	15.0	28.0	25.2	15.5	28.3	26.3
Plagiotropic stem (4 mm thickness)						
Vessel	20.0	52.5	30.9	21.5	70.5	48.3
Tracheid	8.1	20.0	13.5	8.5	22.3	17.7
Fibre	6.8	16.3	12.38	9.3	18.8	14.3

Min=Minimum; Max=Maximum; Av=Average

Values are means of 10 samples

parenchyma (Fig. 4 B & D). The central part possesses parenchymatous pith (Fig 4 D). Matured root shows secondary thickening and produce secondary phloem and xylem (Fig. 4 C).

The aerial and underground roots differ in the following aspects : 1. Root hairs develop in the underground root but are absent in the aerial root 2. Absence of sclerenchymatous and chlorenchymatous cells in the hypodermis and cortical layers of undeground root 3. Size of cortex increases and pith size decreases when aerial root reaches the soil 4. Number of primary xylem and phloem group is 40-46 in aerial root tip and decreases to 6 in underground root tip 5. Well developed secondary thickening like in the stem in aerial root.

The proportion of tracheids and vessels in each bundle of the aerial root is 6:3 and 8:2 in the normal root (Table 2) and the average lengths of tracheid and vessel are 168 μm and 410 μm in aerial root and 105 μm and 325 μm in normal root, respectively (Table 4).

Datta & Dasgupta (1977) reported species differences in the genus *Piper* with regard to medullary bundle, nature of tracheary element, primary xylem and secondary xylem, though the basic structure of both stems are more or less similar to *P. nigrum* (Table 6). The number and size of peripheral as well as medullary bundles showed variations between orthotropic and plagiotropic shoots. Secondary thickening is only observed in peripheral bundles. Datta & Dasgupta (1977) reported that specialization of primary xylem in Piperales showed two major lines, one showing the absence of scalariform perforations, the former being more primitive. In *P. colubrimum,* xylem shows scalariform perforation. The distribution of sclerenchyma also shows species differences. Sclerenchyma cap occurs exter-

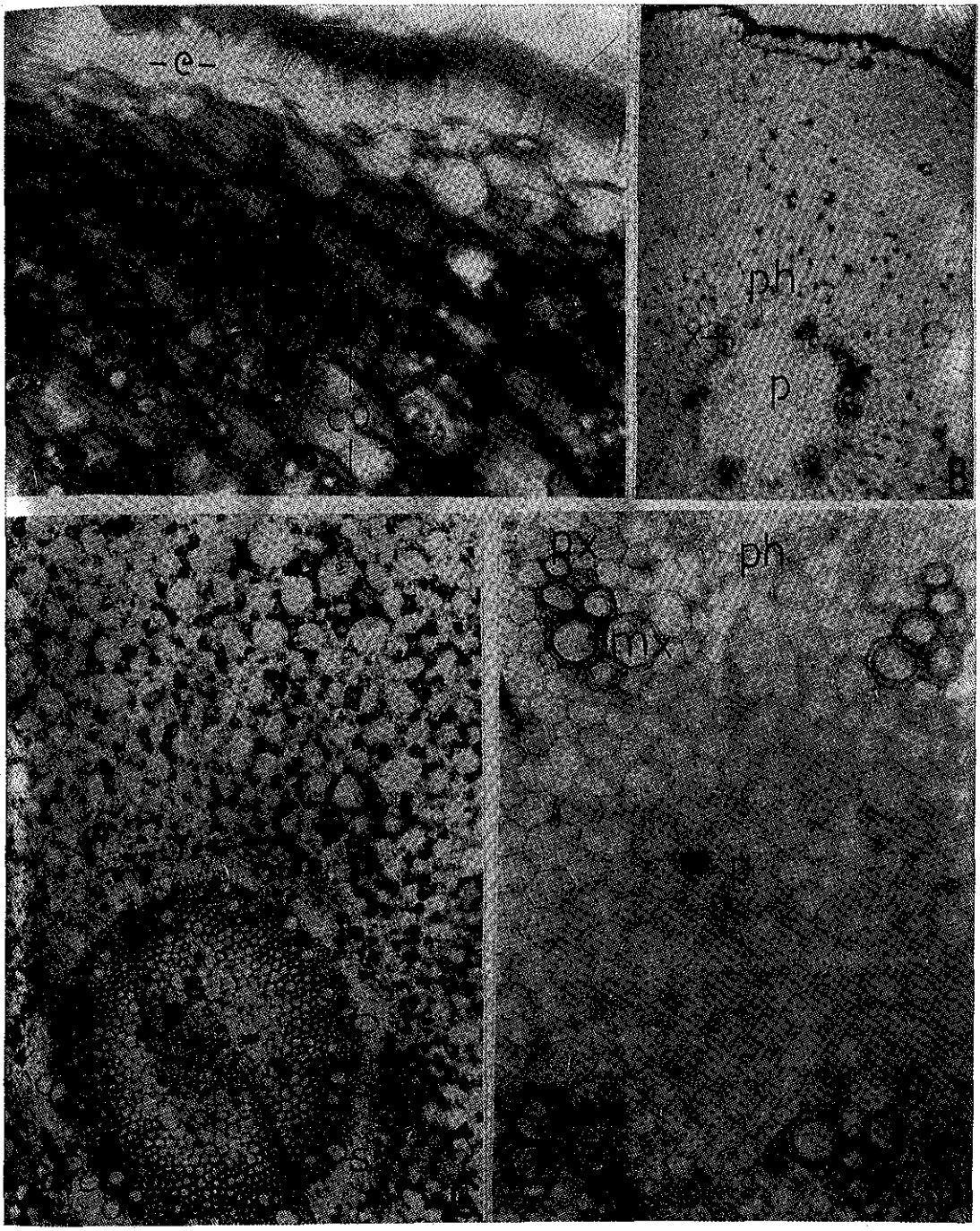

Fig. 4. TS of underground root of *Piper colubrinum*
A. T S of underground root (x 400) B. Secondary growth in root (x 200) C. Primary root showing six groups of xylem and phloem in radial arangement (x 40) D. Primary root with exarch xylem (x 400) (co-cortex; cu-cuticle; e-epidermis; mx-metaxylem; p-pith; ph-phloem; px-protoxylem; sx-secondary xylem; sph-secondary phloem; x-xylem.)

Table 4. Length (in μm) of fibres, tracheids and vessels in *Piper colubrinum*

Category	Peripheral bundle			Medullary bundle		
	Min	Max	Av	Min	Max	Av
Orthotropic stem (4 mm thickness)						
Vessel	105	306	187	95	265	160
Tracheid	500	650	595	475	600	533
Fibre	450	900	656	358	780	595
Orthotropic stem (7 mm thickness)						
Vessel	204	395	258	125	315	195
Tracheid	525	780	650	489	675	563
Fibre	600	980	725	506	925	685
Plagiotropic stem (4 mm thickness)						
Vessel	75	203	106	60	98	72
Tracheid	365	506	438	205	415	338
Fibre	380	720	535	300	620	410
Aerial root						
Vessel	90	198	168	--	--	--
Tracheid	388	538	410	--	--	--
Underground root						
Vessel	68	168	105	--	--	--
Tracheid	280	410	325	--	--	--

Min=Minimum; Max=Maximum; Av=Average

Values are means of 10 samples

nal to phloem and a continuous wavy band below the peripheral ring. In this respect *P. colubrimum* shows similarity to *P. nigrum*. In the aerial root, a polyarch condition with 40-46 groups of vascualr elements may be found, but when it transforms to normal root it is hexarch condition due to fusion of strands. This transformation is also

Table 5. Wall thickness (in μm) of vessels, tracheids and fibres in *Piper colubrinum*

Category	Peripheral bundle			Medullary bundle		
	Vessel	Tracheid	Fibre	Vessel	Tracheid	Fibre
Orthotropic stem (4 mm thickness)	3.03	2.08	2.56	3.70	2.30	2.70
Orthotropic stem (7 mm thickness)	4.62	3.62	4.01	4.90	3.90	4.32
Plagiotropic stem (4 mm thickness)	3.62	1.70	1.96	2.80	1.80	2.01

Values are means of 10 samples

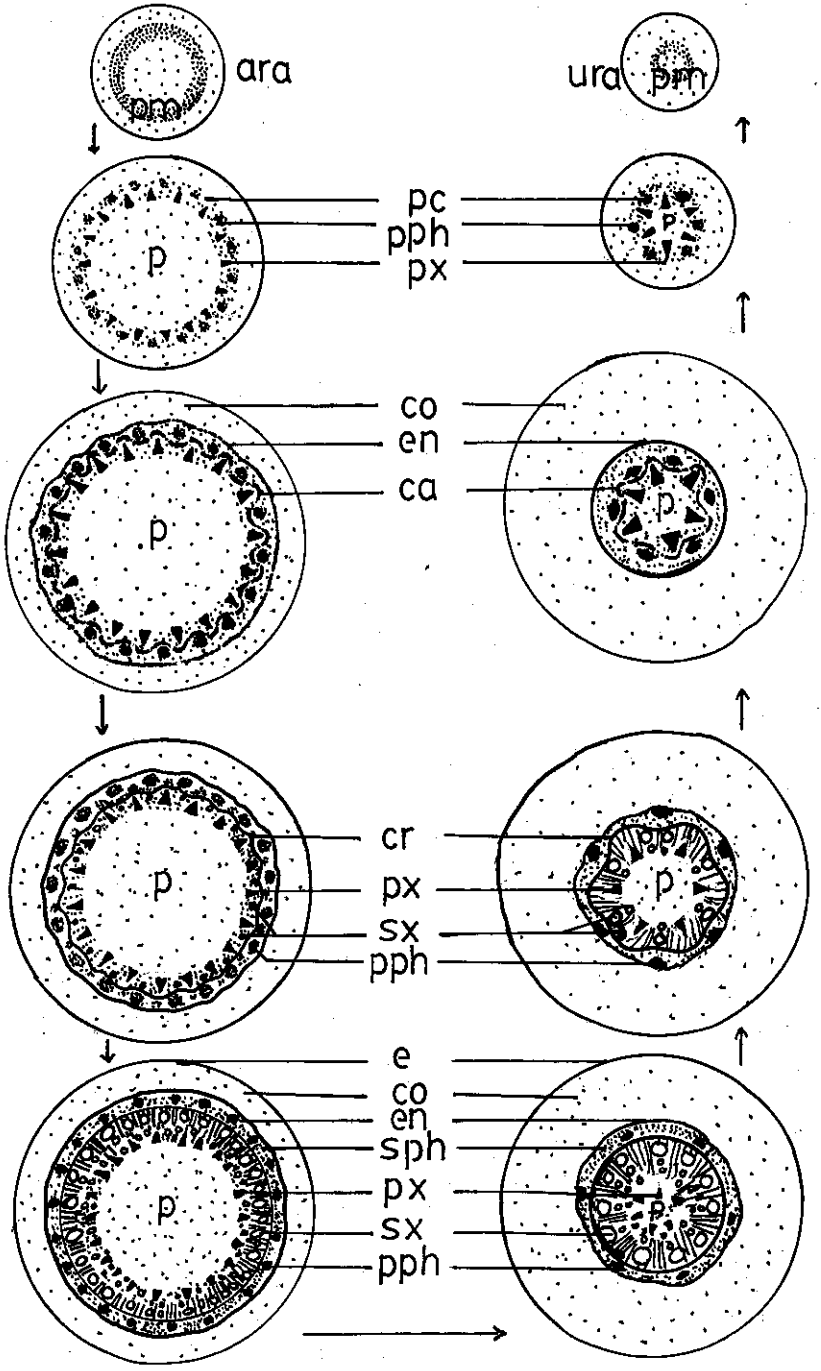

Fig. 5. Diagrammatic representation of transition of aerial root to normal root (ara-aerial root; ca-cambial arc; co-cortex; cr-cambial ring; e-epidermis, en-endodermis; p-pith; pc-procambium; pph-primary phloem; pm-promeristem; px-primary exarchxylem; sph-secondary phloem; sx-secondary endarch xylem ura-underground root apex)

Table 6. Comparative stem anatomy of *Piper nigrum* and *P. colubrinum*

Tissue	*P. nigrum*	*P. colubrinum*
Epidermis	Uniseriate with thick cuticle	Uniseriate with thick cuticle
Cortex	Collenchymatous, chlorenchymatous, sclerenchymatous and parenchymatous cells are present	Sclerenchymatous, chlorenchymatous and parenchymatous cells are present
Endodermis	Present	Present
Casparian strips	Present	Present
Pericycle	Present	Present
No. of peripheral vascular bundles	34-38	42-46
No. of medullary bundles	9 bundles arranged like a broken ring in the pith	11-14 bundles scattered in the pith
Sclerenchymatous ring below peripheral bundle	Present	Present
Type of bundle	Conjoint, collateral and open	Conjoint, collateral and open
Type of vessels	Pitted thickening with simple perforation plates	Pitted thickening with simple perforation plates
Phloem	Phloem parenchyma, sieve tubes and companion cells	Phloem parenchyma, sieve tubes and companion cells
Central mucilage canal	Present	Absent
Cortical mucilage canal	Present	Present

accompanied by other changes. The cortical and pith regions also show variations. Variations in aerial root anatomy was found in *P. nigrum* and *P. betle*. In these species, the aerial roots function as clinging roots to anchor the weak stem to support trees. The structure of these roots show distinct features different from normal roots.

From our observations based on anatomical features it is clear that the orthoropic stem showed dominance over plagiotropic stem. The diameter and width of the xylem element is higher in the medullary bundle than in the peripheral bundle. But the length of the tracheary element of medullary bundles is less than in peripheral bundle, that is, increase in diameter is accompanied by decrease in length. All the dimensional values increase proportionately to growth. The aerial root showed secondary thickening and more number of vascular elements than the normal root.

Acknowledgement

We are thankful to Dr. K. V. Peter, Director, Indian Institute of Spices Research, Calicut for encouragement during the course of the study.

References

Datta P C & Dasgupta A 1977 Comparison of vegetative aratomy of Piperales. I. Juvenile xylem of twigs. Acta. Biol. Acad. Sci. Hung. 28 : 81-96.

De Waard P W F & Zeven D C 1969 Pepper In : Ferwaeeda F P & Wit F (Eds.) Outlines of Perennial Crop Breeding (pp. 409-426). H Veenman and Zorern, NV Wageningen.

Johansen D 1940 Plant Microtechnique. Mc Graw Hill Book Co. Inc., New York, USA.

Krishnamoorthy K V 1988 Plant Histochemistry. S. Viswanathan Pvt. Ltd., Madras.

Murthy Y S 1959. Studies in the Order Piperales IV. A contribution to the study of vegetative anatomy of three species of *Piper*. Proc. Nat. Inst. Sci., India. 25 B : 31-88.

Pal P 1961 Development studies. VII. The origin and courses of the vascular systems in the shoot apices of six species of the genus *Piper* (Piperaceae). Bull. Bot. Soc. Bengal 15 : 17-29.