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# Pollution Effect on the Cambial Structure of *Citrus reticulata* var. *tangelos*

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**Abstract:** This study includes investigation of the effect of pollution (radiations and coal-smoke) on the structure of vascular cambium of *Citrus reticulata* var. *tangelos* of family Rutaceae. The results obtained from the observations clearly shows that the plants under investigation shows a retarded radial growth under the influence of radiations as the length of fusiform initials reduces from 240 μm under normal condition to 204 μm under irradiated condition while the width of fusiform initial shows a similar decrease from 12 to 9 μm. A similar trend is observed for ray initials where the anticlinal and periclinal diameter decreases from 12/10 to 10/9 μm, respectively. In case of coal smoke pollution results obtained were almost similar to those under normal condition. The length of fusiform initials decreased considerably from 240 to 216 μm while the width of fusiform initials is almost constant and is 11 μm. Similarly the periclinal diameter shows constancy and is 10 μm while the anticlinal diameter decreases slightly to 11 μm. The ratio of ray to fusiform initials also shows variation from 23% under normal condition to 28% under radiations and 24% under coal smoke pollution.

**Key words:** Vascular cambium, fusiform initials, ray initials, *C. reticulata* var. *tangelos*, coal-smoke pollution, radiation pollution

## INTRODUCTION

With increasing industrialization, forest are being affected adversely because of contamination of air, water and soil by growth inhibiting or generally toxic substances that include gases, acids and particles etc. Most air pollutants decrease photosynthetic activity of woody plants; affect internal physiological changes leading to growth inhibition and cause visible injury and death of the plants. Air pollutants rarely exist singly; the combined pollutants may have synergistic, additive or antagonistic effects.

Considerable literature is available on the cambial activity of Indian tropical trees (Dave and Rao, 1982a, b; Ghouse and Iqbal, 1975, 1977; Ghouse *et al*., 1980; Han and Woong, 1993; Iqbal, 1990; Khan, 1977; Khan *et al*., 1988; Khan, 1980; Khan, 2001; Khattak and Majeed, 1993; Lim and Woong, 1997; Mahmood, 2001; Paliwal and Yadav, 1999; Paliwal *et al*., 2002; Rajput, 1997; Rao, 1988; Rao and Rajput, 1999; Rao *et al*., 1996) but little is known about the activity of vascular cambium in trees growing under pollution (Berlyn and Battey, 1985; Creber and Chaloner, 1990; Khan, 1982) though a few attempts have been made to explain the formation of xylem in conditions affected by air pollution (Bauch, 1986; De Kort, 1986; Evertsen *et al*., 1986; Ghouse *et al*., 1984a, b; Kottzenburg and Knigge, 1987; Mahmooduzzafar and Iqbal, 1993; Mahmooduzzafar *et al*., 1986; Wahlmann *et al*., 1986).

These evidences are very much insufficient and much remains to be known for which critical research is needed. The authors have attempted to elucidate in the present report the effects of environmental pollution on the structural anatomy of dormant vascular cambium of *Citrus reticulata* var. *tangelos* belonging to family Rutaceae. Through investigation of the vascular cambium of pollution effected as well as plants growing naturally in plantations far from the reach of pollutants showed structural differences between the two types.

## MATERIALS AND METHODS

The study was conducted in the Plant Anatomy Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, India. Dormant cambial samples along with some bark and sapwood of 1-2 inch$^2$ size were collected from the main trunk of *Citrus reticulata* var. *tangelos* belonging to family Rutaceae growing under the environmental stress of coal smoke pollution near Kasimpur thermal power plant, Harduaganj, District, Aligarh, Uttar Pradesh, India and from plants growing under environmental stress of radiation pollution near Atomic Power Plant, Narora, District Bulandshar, Uttar Pradesh, India while the healthy plants growing in natural conditions from Government Jawahar Park, Dist. Aligarh, India. Twenty samples were taken from 5 plants of comparable age and vigour under one environmental

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condition on the same day of the month of December. Samples were fixed on spot in FAA and finally put after 4-5 days in 70% ethanol for preservation.

Sections in transverse and tangential longitudinal planes were obtained at a thickness of 6-8 μm on a Reichert's sliding microtome. Staining was done following the method of Foster (1934).

Measurements of cambial initials were carried out from tangential longitudinal sections with the help of an ocular micrometer scale under the specific magnification of a compound microscope. An average of 500 measurements were taken on random basis. The mean and range of cells dimensions were determined after pooling the readings obtained from different samples. The ratio of ray to fusiform initials was calculated following Ghouse and Iqbal (1975).

## RESULTS

Plants grow in height (primary growth) and diameter (secondary growth) through the activity of meristems, which comprise a very small fraction of the total mass. The diameter of the plant axis increases chiefly by the activity of the vascular cambium, a cylindrical lateral meristem that is located between the xylem and phloem in the stem. The cambium is 5-7 cell layers thick in TS and usually produces xylem on its inner side and phloem on the outer side; the new annual increments of xylem and phloem are inserted between old layers of these tissues and the cambium (Fig. 1A-C).

The cambium as seen in TLS is composed of two morphologically different types of cell, ray initials and fusiform initials which are interchangeable (Fig. 1D-H). The vertically elongated spindle shaped elements with long tapering ends are known as fusiform initials while the radially aligned almost isodiametric angular elements are known as ray initials. Fusiform cells, through transverse or lateral division, can give rise to ray cells, whereas the latter may be elongated to transform into the former (Fig. 1D-I).

The pattern of arrangement of fusiform initials is non-storied or non-stratified as the tapering ends overlap those of the adjacent cells in vertical alignment (Fig. 1 D-F). The walls of the fusiform initials bear primary pit fields and have distinct plasmodesmata connections with the contiguous elements, especially with the ray initials. The radial walls of fusiform initials are slightly thicker than the tangential ones and appear distinctly beaded during dormancy (Fig. 1G).

The length of fusiform cells ranges from 200-288 μm under normal growth conditions and 164-252 and 176-264 μm under environmental stress conditions caused by radiation pollution and coal-smoke pollution, respectively (Table 1). The tapering end walls of these cells measure 67, 56 and 60 μm, respectively. The width of these cells is found to vary from 10-14 μm in normal plants as well as under coal smoke pollution plants while under radiation pollution it varies from 7-11 μm (Table 1).

The ray initials which occur in distinct groups generally form cambial rays which vary in width and height to a considerable extent in the different environmental conditions (Fig. 1D-F). Two or more cambial rays often fuse together to form complex aggregates running to a greater height and covering the length of one or more fusiform initials. A fusiform initial can also intrude into a unit of ray initials by apical intrusive growth thus slitting it into two parts (Fig. 1D- F). Their height varies from 1-28 cells in plants under investigation (Table 1). The size of ray initials also varies in the different environmental conditions. The anticlinal and periclinal diameter of these initials ranges from 7/7-20/14 μm in normal plants and under coal-smoke pollution while 7/7-17/11 μm in plants exposed to radiations (Table 1).

The cambial rays are classified into three groups based on their height-short (1-10 cells), medium (11-20 cells) and tall (21-above cells). The rays of different height occur in different proportion in the plants investigated. Short rays occur more frequently in plants growing in irradiated conditions, while their percentage is less than that of normal plants under coal-smoke pollution (Fig. 2). In width the rays varies from uniseriate to multiseriate under different conditions of environmental pollution (Fig. 1D-F). The multiseriate rays are predominant in plants under radiation pollution and constitute about 67% of the total number of rays in a unit area (Fig. 3). While in

Table 1: Dimensions of cambial initials under different environmental conditions

| Condition | | Fusiform initials Length (μm) | Width (μm) | Tapering ends (μm) | Ray initials Anticlinal diameter (μm) | Periclinal diameter (μm) | Cambial rays height in No. of cells |
|---|---|---|---|---|---|---|---|
| Normal | Mean | 240 | 12 | 67 | 12 | 10 | 1-25 |
| | Range | 200-288 | 10-14 | 50-88 | 7-20 | 7-14 | |
| Radiation pollution | Mean | 204 | 9 | 56 | 10 | 9 | 1-24 |
| | Range | 164-252 | 7-11 | 38-75 | 9-17 | 7-11 | |
| Coal-Smoke pollution | Mean | 216 | 11 | 60 | 11 | 10 | 1-28 |
| | Range | 176-264 | 10-14 | 38-75 | 7-20 | 7-14 | |

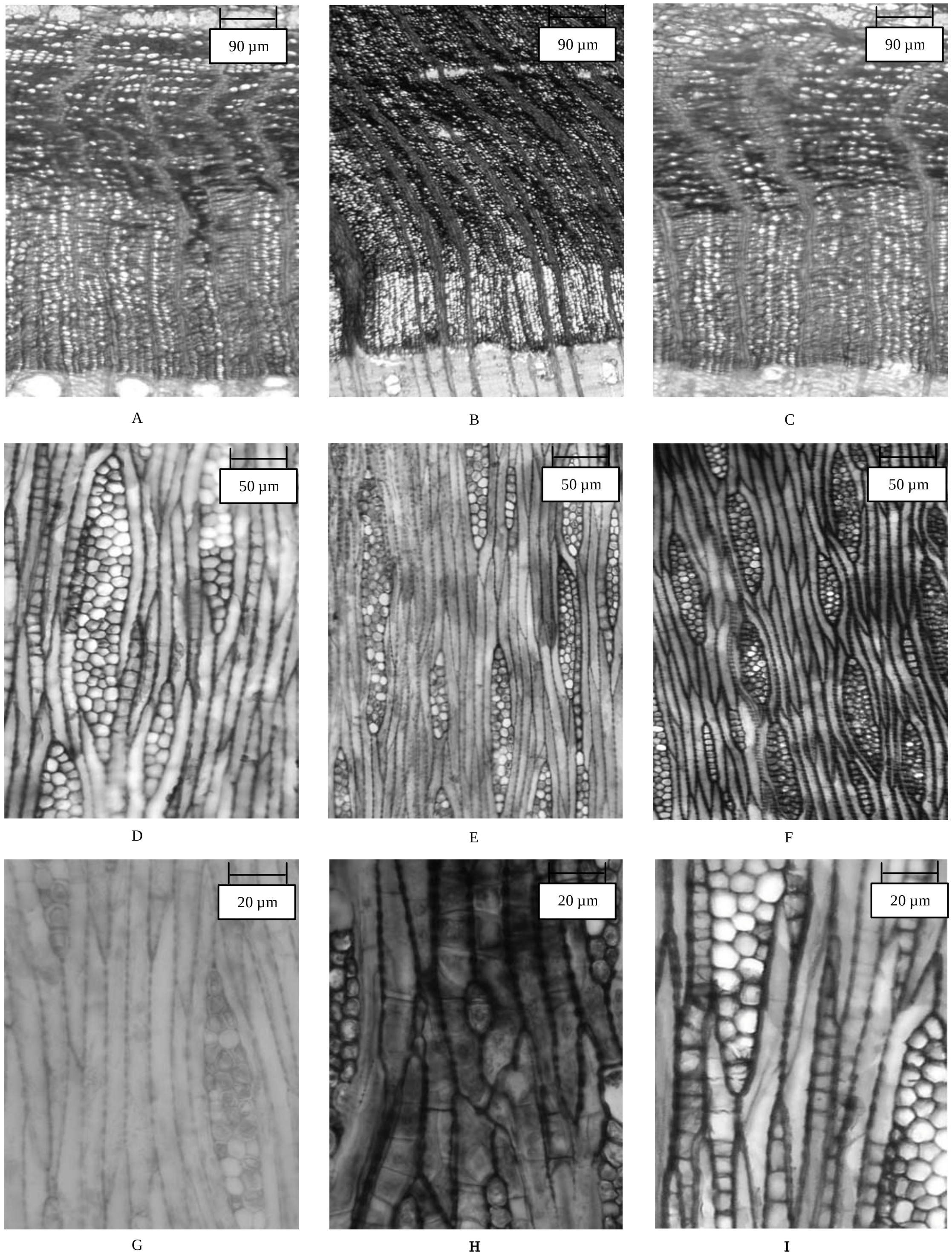


Fig. 1: T.S. normal plant (A), plant under coal-smoke pollution (B), plant under radiation pollution (C) at 10X. TLS through cambium normal plant (D), plant under coal-smoke pollution (E), plant under radiation pollution (F) at 40X. TLS through dormant cambium of normal plant showing distinct beaded structure of primary pit fields (G), formation of ray (H), pseudo-transverse division of fusiform initial at 80X (I)

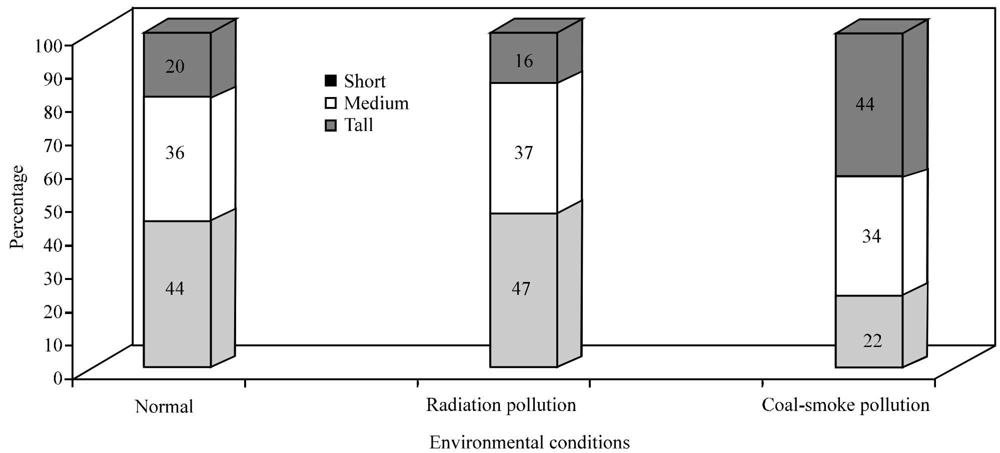


Fig. 2: Percentage of short, medium and tall rays under different environmental conditions

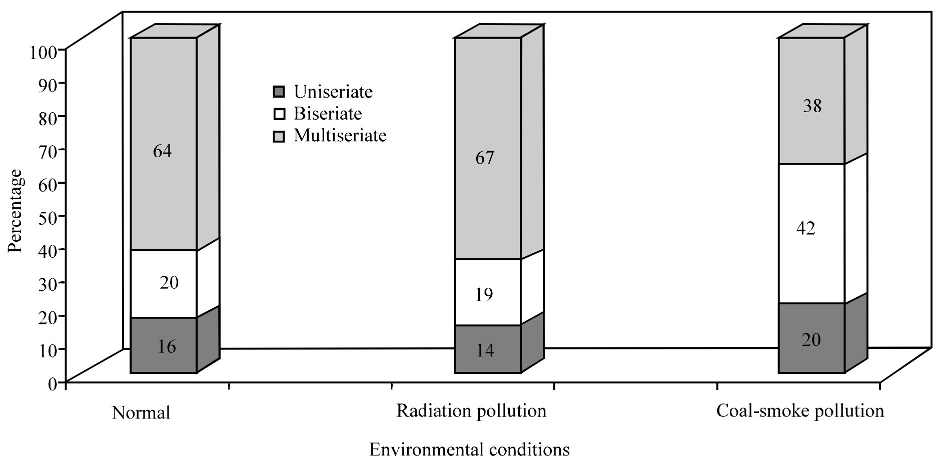


Fig. 3: Percentage of Uni, Bi and multi-seriate rays under different environmental conditions

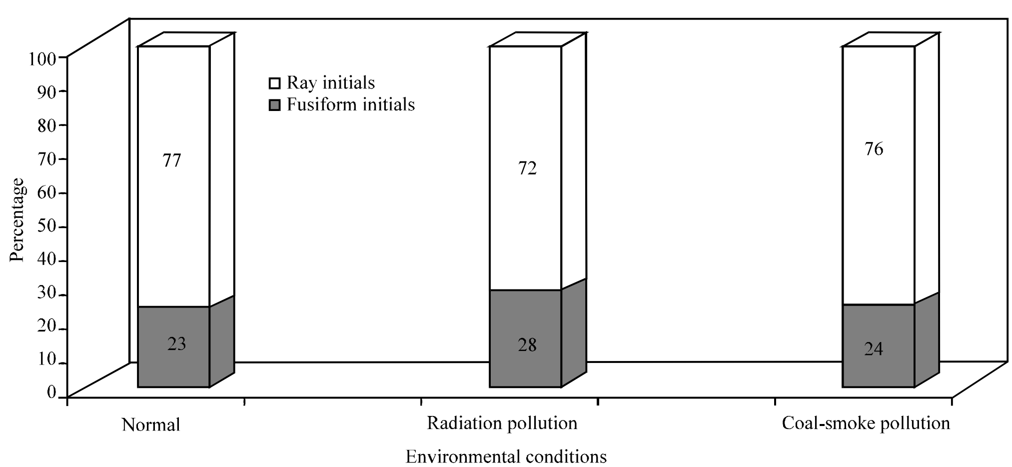


Fig. 4: Ratio of ray initials of fusiform initials under different environmental conditions

plants exposed to coal smoke multiseriate rays constitute 38% in a similar count and is less than that of normal plants which has 64% multiseriate rays. Biseriate rays constitute 20% of the total count in normal plants, 19% under irradiated conditions and 42% under coal smoke pollution. The uniseriate rays are 16, 14 and 20%, respectively (Fig. 3). Depending on the cambial make-up, the ratio of ray to fusiform initials differs in different conditions of the environmental pollution. In normal plants, the ray initials constitute about 23% in the tangential view of the dormant cambial zone, 28% in plants growing in radiation zone and 24% in plants near coal smoke pollution (Fig. 4).

## DISCUSSION

Trees are considered as active bioindicators of air pollution (Braun and Lewark, 1986; Eckstein *et al.*, 1981) and they react sensitively to all interferences. The interference may severely reduce carbohydrate contents of the roots and disturb cell differentiation of the secondary xylem. Air pollutants such as $SO_2$, HF and $NO_x$ influence plant physiology on their own or in association with certain climatic factors (e.g., drought). $SO_2$ affects leaves of trees and, therefore, hinders the assimilation process (Linzon, 1978; Maas, 1987; Rennenberg and Herschbach, 1996). The reduced photosynthetic activity retards cambial activity and consequently xylem and phloem production (Wahlmann *et al.*, 1986). Transpiration rate decreases and assimilation processes are starved of minerals (Halbwachs, 1970). The activity of cambium depends on availability of water, starch, soluble sugars, minerals and growth hormones etc. (Berlyn and Battey, 1985; Iqbal, 1995; Riding and Little, 1984). The amount of starch in the xylem varies during the year (Puls and Rademacher, 1988; Rajput and Rao, 1999). The concentration of soluble sugars (sucrose, glucose, fructose) also varies during the active season (Puls and Rademacher, 1988). Along the radius of the trunk, the carbohydrate content decreases with increasing distance from the cambium (Puls and Rademacher, 1986).

The change in sapwood portion may give an idea of the rate of radial growth in plants and can thus be a good indicator of the influence of air pollution on cambial activity and wood formation (Wahlmann *et al.*, 1986). Sapwood width decreases with increasing damage to trees. A reduction of the sap wood width and sap wood percentage in cross section is frequently combined with narrow annual rings in peripheral position (Kucera and Bosshard, 1989), thus illustrating the interaction between photosynthesis, cambial activity and sap wood production. No changes arise due to pollution in the heartwood of plants investigated.

The term cambium owes its origin from Grew (1682). Sanio (1863) happens to be the first botanist to recognize cambium as a lateral meristem. In the present study, the term cambium incorporates the whole lot of undifferentiated phloem and xylem mother cells in addition to the true cambial initials which in each radial file of cambial cells lie somewhere between the formers and is responsible for the production of cambial derivatives on both outer and inner sides. This view is mainly advocated by Fahn (1997), Ghouse and Iqbal (1975), Iqbal (1990), Paliwal and Yadav (1999), Paliwal *et al.* (2002) and Rao *et al.* (1996) has been supported by ultra-structure studies of Murmanis (1970) pertaining to tangential wall characteristics.

The vascular cambium in general is made up of elongated fusiform initials and roughly isodiametric ray initials. On the basis of the arrangement of the different initials, two basic patterns of cambial structures have been recognized by Bailey (1923). In one of them the fusiform initials occur in horizontal tiers with the ends of cells appearing approximately at the same level in a given tier and in the other, the end walls of the adjacent initials overlap to a considerable extent. The former is known as stratified or storied cambium, while the latter as non-stratified or non-storied cambium. In the presently investigated plants, arrangement of cambial initials depicts a clear non-stratified structure as in the majority of vascular plants.

Bailey (1923) found that the length of fusiform initials in non-stratified form vary from 460-4400 μm. The present observations regarding this aspect do not agree with the limits of Bailey's above observations. Bartwal *et al.* (1983), Ghouse and Iqbal (1975), Ghouse *et al.* (1980), Khan (1980), Khan (2001) and Mahmood (2001) have found the length of fusiform initials to fall shorter than Bailey's reported limits for the non-stratified cambium. Among the plants investigated in the present study, *Citrus reticulata* var. *tangelos* growing under the influence of radiation pollution has been found to possess comparatively short fusiform initials followed by plants under influence of coal-smoke pollution while the control has the longest. As an impact of the decreased transpiration, the water content in the stem is reduced and the fusiform cambial initials readjust their dimensions to decrease in size. The short size of fusiform initials is coupled with structural shortening of other features of bark and wood namely the size and structure of sieve-tube members, vessel elements, xylem and phloem rays and the amount and distribution of parenchyma so as to support the capillary action in the lumen (Esau, 2002; Fahn, 1997). Reduction in length of fusiform initials due to radiation pollution is significant while the coal-smoke pollution do not have such a heavy impact. The present findings go in agreement with earlier workers (Creber and Chaloner, 1990; Ghouse *et al.*, 1985, 1989; Iqbal *et al.*, 1987a,b; Khan, 1982; Mahmooduzzafar *et al.*, 1986; Yunus and Iqbal, 1996). Radial walls of fusiform initials have been reported to be usually thicker than tangential walls, specially during dormancy with the primary pit fields appear deeply depressed in tangential longitudinal view (Riding and Little, 1984). Similar observations have been made in the present study too, in cambium of plants under all conditions investigated, if not at the same level but in different degrees.

The cambial initials have been reported to undergo anticlinal and periclinal divisions periodically (Esau, 2002; Fahn, 1997; Han and Woong, 1991). The anticlinical divisions add to the cambial population while the periclinal ones increase the number of cambial derivatives emanating new phloem and xylem elements. Bailey (1923) recognized two fundamental types of anticinal divisions in the different vascular plants investigated by him. In one type, the anticlinal division occurs in a radial longitudinal plane and in the other pseudotransverse wall formation takes place running askew intersecting the two radial walls at two different levels (Han and Woong, 1991; Iqbal, 1990; Venugopal and Krishnamurthy, 1994). The pseudotransverse anticlinal division usually leads to the formation of two unequal daughter cells. In the present study the anticlinal divisions in the cambial initials have been noted to be pseudotransverse, as it has been the case in the majority of forms having non-stratified cambium (Bailey, 1923). The Pseudotransverse wall observed in the present study varies in length from short to long in all the three conditions, sometimes the dividing wall almost extending from one end of the cell to the other as has been reported by Esau (2002), Fahn (1997), Iqbal (1979), Khan (1977), Khan (1980), Khan (2001) and Zagorska-Marek (1984).

The ray initials may originate as single cells which are cut at the ends as terminal segments (Khan, 1977) or as lateral segments (Ghouse and Iqbal, 1977; Khan, 1977). They may also arise by the transverse segmentation of the fusiform initials (Khan, 1977). In the investigated Rutaceae members, first two modes of origin of ray development are found to be less frequent than the later type. The growing cambial rays quite often fuse with each other resulting in long rays complex. The fusion of different rays into a single complex unit has often been brought about by the development of new groups of ray initials out of the intervening fusiform cells or by the multiplication of the already existing ray initials of the adjacent panels of rays (Ghouse and Iqbal, 1977). Contrary to the above, some of the long and broad rays get splitted up into smaller units by the intruding action of adjacent fusiform initials in the citrus worked out (Bartwal *et al.*, 1983; Esau, 2002; Ghouse and Iqbal, 1977; Iqbal, 1990; Khan, 1977; Khan *et al.*, 1983; Rao, 1988; Zagorska-Marek, 1984). In the present study, the plants growing in radiation zone have more number of short ray than the taller ones which is an indication of less frequency of fusion of rays, while the reverse is true for plants growing in coal smoke pollution region.

The analysis of dimensions of ray initials in the present study indicates that they undergo a decrease in the polluted environmental conditions as compared to healthy trees. While the relative proportion of the ray initials to that of fusiform initials has been observed to increase from 23% in normal condition to 28% under irradiated condition and 24% under coal-smoke pollution. This clearly shows an increase in the number of ray initials with decrease in their anticlinal and periclinal diameters under the polluted environment (Creber and Chaloner, 1990; Romberger *et al.*, 1993; Yunus and Iqbal, 1996). Earlier workers show the fusiform initials to constitute more than 90% of the cambial cylinder (Bailey, 1923; Butterfield, 1972; Margaris and Papadogianni, 1977). But this report goes in agreement with Bhat *et al.* (2005), Ghouse and Iqbal (1975, 1977), Khan *et al.* (1982, 1983), Khan and Siddiqui (2007a-d) and Khan *et al.* (2005).

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