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The Impact of Changing Climate on the Cambial Activity during Radial Growth in Some *Citrus* Species

Moin Ahmad Khan and M. Badruzzaman Siddiqui

Abstract

This study on radial growth in the stem of *Citrus* was carried out with an aim to notice the behavior of vascular cambium with respect to climatic and age effects. The fusiform initials vary in length from 137 to 363 μm in *C. limon*, 100 to 463 μm in *C. paradisi*, 137 to 413 μm in *C. reticulata* var. *kinnow*, and 137 to 375 μm in *C. sinensis*. The length rises with age, followed by decline and then again increase in *C. limon*. In *C. paradisi*, there is increase up to maximum and after decline is soon followed by constancy. In *C. reticulata* var. *kinnow*, increase in length from top to base in *C. sinensis*, increase up to maximum followed by a decline. Swelling of cambial cells occurs in the third week of March in *C. limon*, last week of March in *C. paradisi*, third week of April in *C. reticulata* var. *kinnow*, and second week of April in *C. sinensis*. The cambium turns dormant in early October in *C. limon*, late December in *C. paradisi*, early December in *C. reticulata* var. *kinnow*, and early November in *C. sinensis*. Thus, the cambium remains active for about 6 months in *C. limon* and *C. sinensis*, 9 months in *C. paradisi*, and 7 months in *C. reticulata* var. *kinnow*.

Keywords: cambium, radial growth, fusiform initials, ray initials

1. Introduction

In most dicotyledons and gymnosperms, a layer of procambial cells between the primary phloem and primary xylem matures into fascicular cambium, while the cells of pith or medullary rays which lie in between the edges of the fascicular cambium divide accordingly to form a new layer of cambium across the medullary rays, known as interfascicular cambium, resulting in the formation of a complete ring of cambium. In this way, a new lateral meristem, the vascular cambium, which is responsible for the “growth in thickness by the formation of secondary vascular tissues (radial growth),” is formed and adds secondary phloem toward the outer side and secondary xylem toward the inner side.

2. Cambium

In three-dimensional view, the cambium is a continuous cylindrical sheath about the xylem. In most of the plants, the vascular cambium is reported to exhibit

successive active and dormant phases during a calendar year. This behavior of cambium is believed to be regulated by several internal and external factors that include heredity constitution, physiological phenomenon, and environmental conditions of the habitat [1]. Therefore, there is further need to investigate the influence of different physical and climatic factors on cambial makeup and its activity and then to suggest measures for the maintenance of desirable growth pattern to ensure a vigorous production of derivative tissues and their content, although in the past, several workers have conducted such type of studies in different species growing in tropical and subtropical regions [2–12].

Butterfield [13] defines **cambium** as a “multiseriate zone of periclinally dividing cells lying between the differentiating secondary xylem and phloem, with distinct initials capable of both periclinal and anticlinal divisions lying somewhere within each radial file of cells.” The same terminology has been adopted for describing cambium in the present study.

In spite of the fact that Indian subcontinent is one of the richest tropical tree flora on earth, the studies on the radial growth of these trees, that is, the activity of cambium, its structure, and behavior are still meager. Much, therefore, remains to be known about the growth phenomenon of Indian tropical trees, particularly the vascular cambium and its derivative tissues, xylem, and phloem, their cellular organization with age and varying climatic conditions.

The tropical trees in general exhibit a continuous growth unlike temperate ones where the growth phenomenon is sharply rhythmic. A majority of tropical trees grow in multiple flushes or in an intermittent manner due to the prolonged favorable climatic conditions that prevail in the tropical belt. Keeping in view the afore-said fact, the present anatomical studies are an attempt to elaborate the structure and behavior of vascular cambium and its derivative tissues in some **Rutaceae** members in relation to various seasonal conditions of the study site and age of the trees. My study includes the following aspects:

1. Structure and activity of vascular cambium.
2. The effect of climate on the activity and structure of vascular cambium.
3. The effect of age on the activity and structure of vascular cambium.

In fact, no information is available with regard to the cambial activity and formation of its derivative tissues in *Citrus* species of Rutaceae family. It is noteworthy that *Citrus* is of immense medicinal importance as well as economic value.

2.1 *Citrus*

A genus of evergreen, usually armed, aromatic shrubs or small trees distributed in the Indo-Malayan region, South-east Asia, and China but cultivated throughout the tropical and temperate regions for fruits. Currently, *Citrus* is commercially grown primarily between the latitudes 40°N to 40°S.

Four species of genus *Citrus*, available in and around district Aligarh, Uttar Pradesh, India, have been selected for a comparative anatomical study on the aspects as described earlier.

1. *Citrus limon* (Linn.) Burm.f.

Classification [14]

Class: Dicotyledons

Series: Lignosae
Order: Rutales
Family: Rutaceae
Genus: *Citrus*
Species: *limon*
Hindi: Baranibu, Jambira, Paharikaghzi, Paharinimbu, Kinnanibu

2. *Citrus paradisi* Macf.

Classification [14]
Class: Dicotyledons
Series: Lignosae
Order: Rutales
Family: Rutaceae
Genus: *Citrus*
Species: *paradisi*
Hindi: Chakotra

3. *Citrus reticulata* var. *kinnow*

Classification [14]
Class: Dicotyledons
Series: Lignosae
Order: Rutales
Family: Rutaceae
Genus: *Citrus*
Species: *reticulata* var. *kinnow*
Hindi: Kinnow

4. *Citrus sinensis* (Linn.) Osbeck

Classification [14]
Class: Dicotyledons
Series: Lignosae
Order: Rutales
Family: Rutaceae
Genus: *Citrus*
Species: *sinensis*
Hindi: Mosammi, Malta

3. The vascular cambium: structure

The term cambium was coined by Grew [15] who presented the classification of plant tissues. In the year 1863, Sanio [16] recognized vascular cambium, its origin and function as a lateral meristem. The vascular cambium in all the species investigated forms a continuous cylinder between the xylem and phloem. Vascular cambium as a rule is made up of exclusively mononucleate elongated spindle-shaped elements with long tapering end walls, the fusiform initials, and almost isodiametric or rectangular ray initials [17]. In the presently investigated species, the arrangement of end walls of the adjacent cambial initials overlaps to a considerable extent depicting a clear non-storied (non-stratified) structure in all species investigated (**Figure 1(i)**).

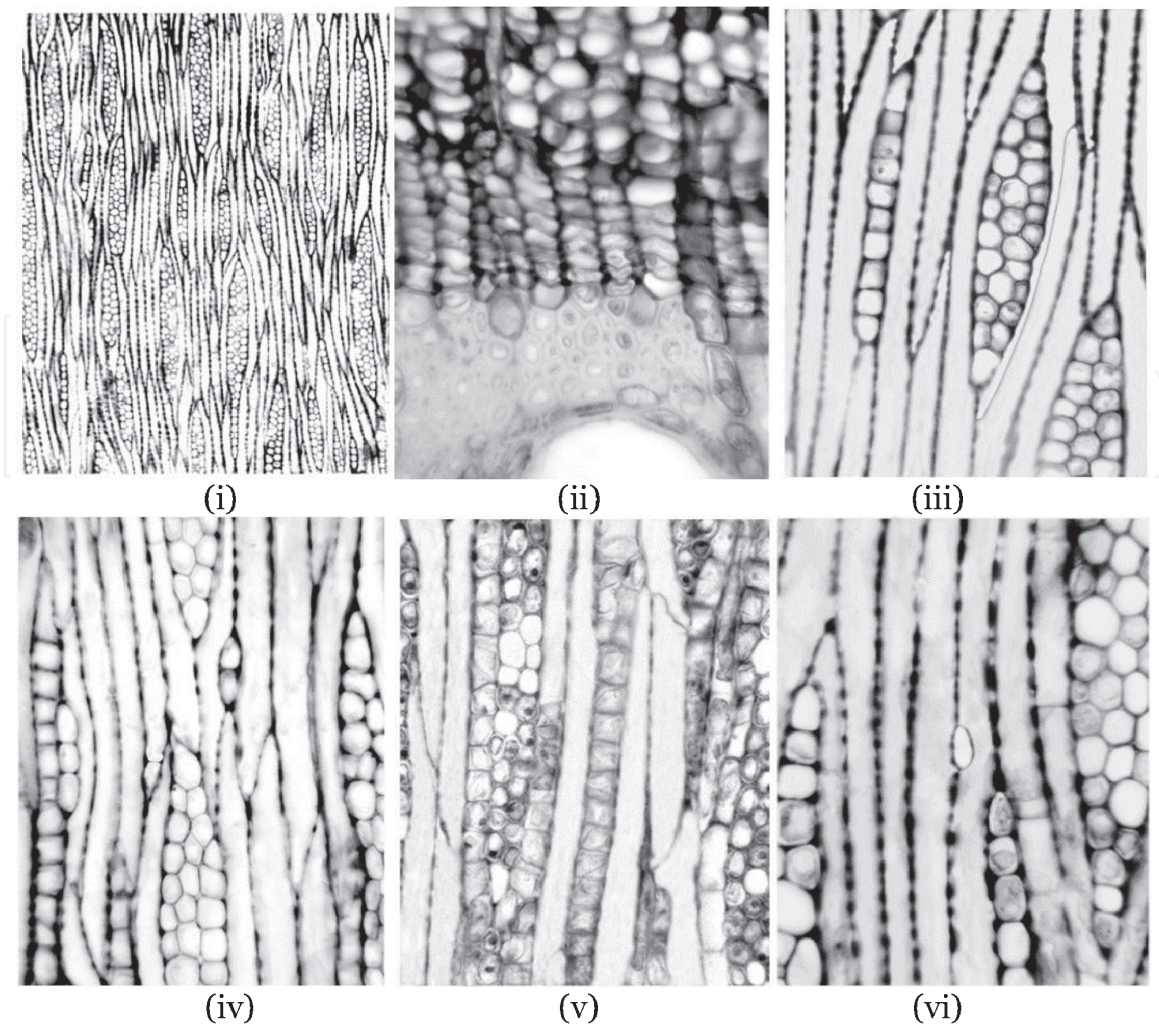


Figure 1.

(i) *C. sinensis* T.L.S. through dormant cambium showing arrangement of various components of cambium, (ii) *C. limon* T.S. showing dormant cambial zone, (iii) *C. paradisi* T.L.S. through active cambium showing pseudotransverse wall in dividing fusiform initial, (iv) *C. sinensis* T.L.S. through active cambium showing two-celled newly formed ray, terminally cut ray cell and lateral fusion of rays, (v) *C. sinensis* T.L.S. through active cambium showing terminal and lateral fusion of rays and transverse septation of fusiform initial, (vi) *C. paradisi* T.L.S. through active cambium showing laterally cut ray cell.

After measuring cambial initials of a wide variety of tropical as well as temperate trees Bailey [18] concluded that the fusiform initials vary in length from 460 to 4400 μm showing non-stratified cambium. The observations regarding this aspect indicate that in the presently investigated species, the length of fusiform initials ranges from 137 to 363 μm in *Citrus limon*, 100 to 463 μm in *C. paradisi*, 137 to 413 μm in *C. reticulata* var. *kinnow*, and 137 to 375 μm in *C. sinensis*, which is contrary to the findings of Bailey [18]. But the present findings are in agreement with the results of some earlier workers like Ghouse and Iqbal [19] in some arid zone species of *Acacia* and *Prosopis*, Kojs [20] in selected woody species, Khan [10] in *Jacaranda mimosifolia*, *Pterospermum acerifolium*, and *Terminalia arjuna* who have found fusiform initial length to fall shorter than Bailey's [18] reported limit for non-storied cambium.

Among the species investigated in this study, *C. reticulata* var. *kinnow* has been found to possess comparatively short fusiform initials while *C. limon* having the longest with the other two species falling in between these. If the size of fusiform initials is taken as a criterion for phylogenetic advancement, then obviously *C. reticulata* var. *kinnow* appears to be the most evolved form among the presently investigated species.

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 have been observed to be usually thicker than tangential walls in the present study in all the species investigated. Especially during dormancy, the primary pit fields appear deeply depressed in tangential longitudinal view giving a beaded look to the radial walls. Similar situation has been noticed by Iqbal [21], Khan et al. [22], and Khan and Siddiqui [12].

The cambial initials have been reported to undergo anticlinal and periclinal divisions periodically [23]. Anticlinal division which is also known as multiplicative division increases the cambial population, whereas the periclinal or additive division increases the number of cambial derivatives emanating new phloem and xylem elements [24]. The anticlinal division in the cambial initials has been noted to be pseudotransverse wall formation takes place running askew intersecting the two radial walls at two different levels [12, 22, 23, 25, 26].

The pseudotransverse wall formation observed in this study varies in the length from short to long in all the species investigated. Sometimes, the dividing wall almost extending from one end of the cell to the other, as it has been reported earlier by Khan and Siddiqui [12] in *Alstonia* species.

The ray initials may arise primarily as a single cell, which is cut at the ends of fusiform initials as terminal segments [27] or as lateral segments [20, 27] or they may arise by transverse segmentation of fusiform initials [20, 27]. In the presently investigated species, the first and last types of ray development are found to be more frequent than the lateral segments. Once the ray initials get established, they continue to undergo multiplication resulting in expansion of rays in height and width [27]. The rays also increase in height and width by fusion of two or more vertically and radially aligned rays. These fusions result due to the transverse segmentation of the intervening fusiform initials or by multiplication of already existing ray initials of the adjacent panel of rays [20, 27]. Apart from the above fact, some long and broad rays get split into smaller units by intrusion of adjacent fusiform initials in all the species investigated as has already been reported by [20, 27–29].

The ray initials form an integral part of the cambial cylinder in all the species investigated. The relative proportion of ray initials to that of fusiform initials has been found to vary from species to species. A maximum of 23% has been observed in *C. paradisi* and *C. reticulata* var. *kinnow* and minimum of 18% in *C. limon*, whereas in *C. sinensis*, the ray initials constitute about 22% of tangential area of cambial cylinder in adult trees [30–34].

4. The vascular cambium: developmental changes in the structure

In transverse sections of the young shoots, the cambial zone consists of three to five layers of cells in all the species, whereas the number of cell layers in the cambial zone of adult trees varies from three to nine (**Figure 1(ii)**). It is evident that the fusiform cambial initial experience considerable length variation as the tree grows in thickness in all the species investigated in the present research. Average length of fusiform initials has been noticed to vary from 158.78 to 259.71 μm in *C. limon*, 228.53 to 257.59 μm in *C. paradisi*, 118.00 to 246.70 μm in *C. reticulata* var. *kinnow*, and 156.00 to 232.43 μm in *C. sinensis* in different age group samples (**Table 1**). On close observations, it is seen that the average length rises with age from 158.78 to 232.46 μm that is followed by a decline to 220.31 μm and then again increase in the old stem to 259.71 μm in case of *C. limon* which coincides with the findings of Cumbie [35] and Ajmal et al. [36]. In *C. paradisi*, there is a gradual increase up to

Circumference of axis in mm	Length (μm) <i>C. limon</i>				Circumference of axis in mm	Length (μm) <i>C. paradisi</i>			
	Range	Mean \pm SE	SD	CV%		Range	Mean \pm SE	SD	CV%
20	200–325	158.78 \pm 1.81	36.25	22.83	20	137–325	228.53 \pm 2.43	48.63	21.27
40	225–363	184.21 \pm 1.94	38.85	21.09	40	137–338	234.68 \pm 2.68	53.61	22.84
65	250–400	210.28 \pm 2.02	40.46	19.24	65	112–350	249.65 \pm 2.85	57.04	22.84
95	275–400	232.46 \pm 1.80	36.19	15.56	95	175–375	257.59 \pm 2.17	43.50	16.89
150	262–400	220.31 \pm 1.79	35.91	16.29	164	175–363	238.56 \pm 2.51	50.35	21.10
195	250–363	259.71 \pm 1.80	36.01	13.86	204	125–363	239.00 \pm 2.79	55.83	23.35
LSD at 5%		= 44.60			LSD at 5%		= 73.33		
LSD at 1%		= 59.73			LSD at 1%		= 98.19		
Circumference of axis in mm	Length (μm) <i>C. reticulata</i> var. <i>kinnow</i>				Circumference of axis in mm	Length (μm) <i>C. sinensis</i>			
	Range	Mean \pm SE	SD	CV%		Range	Mean \pm SE	SD	CV%
20	50–200	118.00 \pm 2.00	40.14	34.01	20	100–213	156.00 \pm 1.79	35.94	23.03
45	75–263	124.18 \pm 2.04	40.94	32.96	45	87–250	178.15 \pm 2.03	40.60	22.78
67	100–250	129.50 \pm 1.95	39.19	30.26	67	137–288	204.81 \pm 1.92	38.45	18.77
96	112–263	132.62 \pm 1.96	39.26	29.60	96	150–313	232.43 \pm 1.96	39.36	16.93
147	187–338	235.54 \pm 2.01	40.25	17.08	143	150–288	219.57 \pm 1.88	37.64	17.14
199	125–400	246.70 \pm 3.59	71.92	29.15	198	137–263	205.12 \pm 1.84	36.94	18.00
LSD at 5%		= 68.48			LSD at 5%		= 73.33		
LSD at 1%		= 91.70			LSD at 1%		= 98.19		

Table 1.

Changes in the length size of fusiform initials (as observed in tangential longitudinal section) of cambial zone along tree axis of varying girth.

maximum from 228.53 to 257.59 μm and after slight decline to 238.56 μm is soon followed by constancy in the adult region which coincides with the report of Evert [37, 38] and Ghouse and Yunus [39]. In *C. reticulata* var. *kinnow*, a significant impact of age is seen on the vascular cambium as the fusiform initials increase in length from top to base, that is, from 118.00 to 246.70 μm with an increase in girth of the axis, which coincides with the report of Khan [40], Khan [10], and Mahmood [11]. Whereas in *C. sinensis*, there is an initial increase up to maximum from 156.00 to 232.43 μm with the advancing age of stem axis which is followed by a decline toward the basal region which goes in agreement with the results of Ghouse and Iqbal [41], Khan et al. [42], Ajmal [43], and Mahmood [11].

In general, fusiform initials are found longer and broader in stouter axes than in the slender ones. The rate of increase happens to be high in young shoots and low in older ones. It appears, therefore, that the ability of newly formed initials to elongate in size depends on the age of the meristem. The younger is the cambium, greater is the ability of the initials to elongate, and conversely, the older is the meristem, the lesser is the ability of the initials to undergo expansion. A similar comparative analysis of the data obtained on the width of the fusiform initials has revealed that they do not show any significant change with the increase in the circumference of the stem axes.

A similar analysis of the ray initials of the investigated species (**Table 2**) shows a slight initial increase from 12.44/9.86 to 15.98/13.80 μm which is followed by constancy in anticlinal and periclinal diameters of *C. limon* as has already been reported by Khan [10] in *Terminalia arjuna* and slight increase from 8.97/7.82 to 11.96/10.13 μm with the increasing diameter of axis in *C. reticulata* var. *kinnow* which coincides with the findings of Khan [10] in *Jacaranda mimosifolia*, whereas a slight initial increase followed by a decrease in the basal region is seen in anticlinal diameter of *C. sinensis* as has been reported by Khan [10] in *Pterospermum acerifolium*. The ray initials do not show any appreciable change in their dimension in relation to age of the axis in *C. paradisi* as has been reported by earlier researchers like Ajmal [43]. However, they undergo multiplication to become multiseriate in older axis [11, 41]. As a consequence of various developmental changes in cambial zone, relative proportion of fusiform and ray initials also varies with age of stem axis. The ray initials occupy a relatively greater area in the cambial cylinder in the old axis as compared to younger ones. The ray initials occupy 14–17% in *C. limon*, 7–25% in *C. paradisi*, 14–23% in *C. reticulata* var. *kinnow*, and 12–18% in *C. sinensis* of the total tangential area of the cambial cylinder.

With the growing girth of the axis, the cambial cylinder also expands by adding more cells. The fusiform initials undergo pseudotransverse divisions and give rise to sister initials (**Figure 1(iii)**). Similarly, the ray initials also divide and give rise to new ray initials (**Figure 1(iv)**). All this happens in order to cope with the expansion of the axis. The new ray initials are also produced by the fusiform initials and this happens either by the transverse septation of the fusiform cells (**Figure 1(v)**) or by the formation of new initials as terminal (**Figure 1(vi)**) or lateral segments (**Figure 1(vi)**). Occasionally, the rays are seen fusing with one another to form tall and wide bodies (**Figure 1(v)**). This is brought about by the conversion of the intervening fusiform initials into a group of ray initials, which forms the bridge between the two already existing groups of ray initials. The newly produced rays having a limited height in the beginning grow into tall structures by the divisions of the existing initials. At times, the fusiform initials are found to intrude into a panel of ray initials, resulting in the division of a broad or tall ray into a number of smaller entities (**Figure 2(i)**).

Vascular cambium, therefore, constantly undergoes changes in its composition, as an accommodative measure to meet the increasing circumference of the vascular

Circumference of axis in mm	Anticlinal diameter (μm)				Periclinal diameter (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
<i>In Citrus limon</i>								
20	6-17	12.44 \pm 0.11	2.31	18.56	6-14	9.86 \pm 0.10	2.17	22.00
40	10-17	14.41 \pm 0.12	2.50	17.34	10-17	13.12 \pm 0.12	2.45	18.67
65	10-21	15.30 \pm 0.17	3.48	22.74	10-17	13.60 \pm 0.11	2.35	17.27
95	10-21	15.98 \pm 0.18	3.74	23.40	10-17	13.80 \pm 0.11	2.29	16.59
150	10-17	13.46 \pm 0.11	2.35	17.45	10-17	11.49 \pm 0.08	1.78	15.49
195	10-21	14.75 \pm 0.16	3.30	22.37	10-17	12.17 \pm 0.09	1.81	14.87
LSD at 5%		= 4.46				= 3.10		
LSD at 1%		= 5.97				= 4.15		
<i>In Citrus paradisi</i>								
20	6-14	8.63 \pm 0.12	2.47	28.62	6-14	7.95 \pm 0.11	2.21	27.79
40	6-17	10.47 \pm 0.13	2.62	25.02	6-14	8.50 \pm 0.11	2.38	28.00
65	6-14	10.60 \pm 0.12	2.42	22.83	6-14	8.84 \pm 0.12	2.54	28.73
95	6-14	10.20 \pm 0.14	2.80	27.45	6-14	8.36 \pm 0.91	1.82	21.77
164	6-14	9.92 \pm 0.11	2.24	22.58	6-14	9.18 \pm 0.09	1.95	21.24
204	6-14	9.72 \pm 0.11	2.25	23.14	6-14	8.97 \pm 0.13	2.61	29.09
LSD at 5%		= 3.55				= 3.04		
LSD at 1%		= 4.75				= 4.07		
<i>In Citrus reticulata var. kinnow</i>								
20	6-14	8.97 \pm 0.13	2.70	30.10	6-14	7.82 \pm 0.10	2.17	27.74
40	6-14	9.52 \pm 0.10	2.04	21.42	6-14	7.68 \pm 0.09	1.90	24.73
67	6-17	9.99 \pm 0.10	2.08	20.82	6-14	8.43 \pm 0.11	2.38	28.23
96	6-14	10.54 \pm 0.11	2.38	22.58	6-14	8.77 \pm 0.13	2.64	30.10
147	6-14	10.33 \pm 0.12	2.45	23.71	6-14	8.29 \pm 0.09	1.82	21.95
199	6-17	11.96 \pm 0.18	3.68	30.76	6-14	10.13 \pm 0.13	2.76	27.24
LSD at 5%		= 3.73				= 3.32		
LSD at 1%		= 4.99				= 4.45		
<i>In Citrus sinensis</i>								
20	10-17	12.92 \pm 0.12	2.54	19.65	6-14	8.84 \pm 0.13	2.72	30.76
45	10-21	14.96 \pm 0.17	3.47	23.19	6-14	9.65 \pm 0.11	2.29	23.73
67	10-21	14.82 \pm 0.16	3.38	22.80	6-14	10.88 \pm 0.12	2.54	23.34
96	10-17	14.28 \pm 0.12	2.54	17.78	6-14	11.22 \pm 0.10	2.17	19.34
143	10-17	13.26 \pm 0.11	2.38	17.94	6-14	10.20 \pm 0.14	2.80	27.45
198	6-17	11.83 \pm 0.18	3.74	31.61	6-14	9.79 \pm 0.09	1.88	19.20
LSD at 5%		= 4.23				= 3.42		
LSD at 1%		= 5.67				= 4.58		

Table 2. Changes in the cell size of ray initials (as observed in tangential longitudinal section) of cambial zone along tree axis of varying girth.

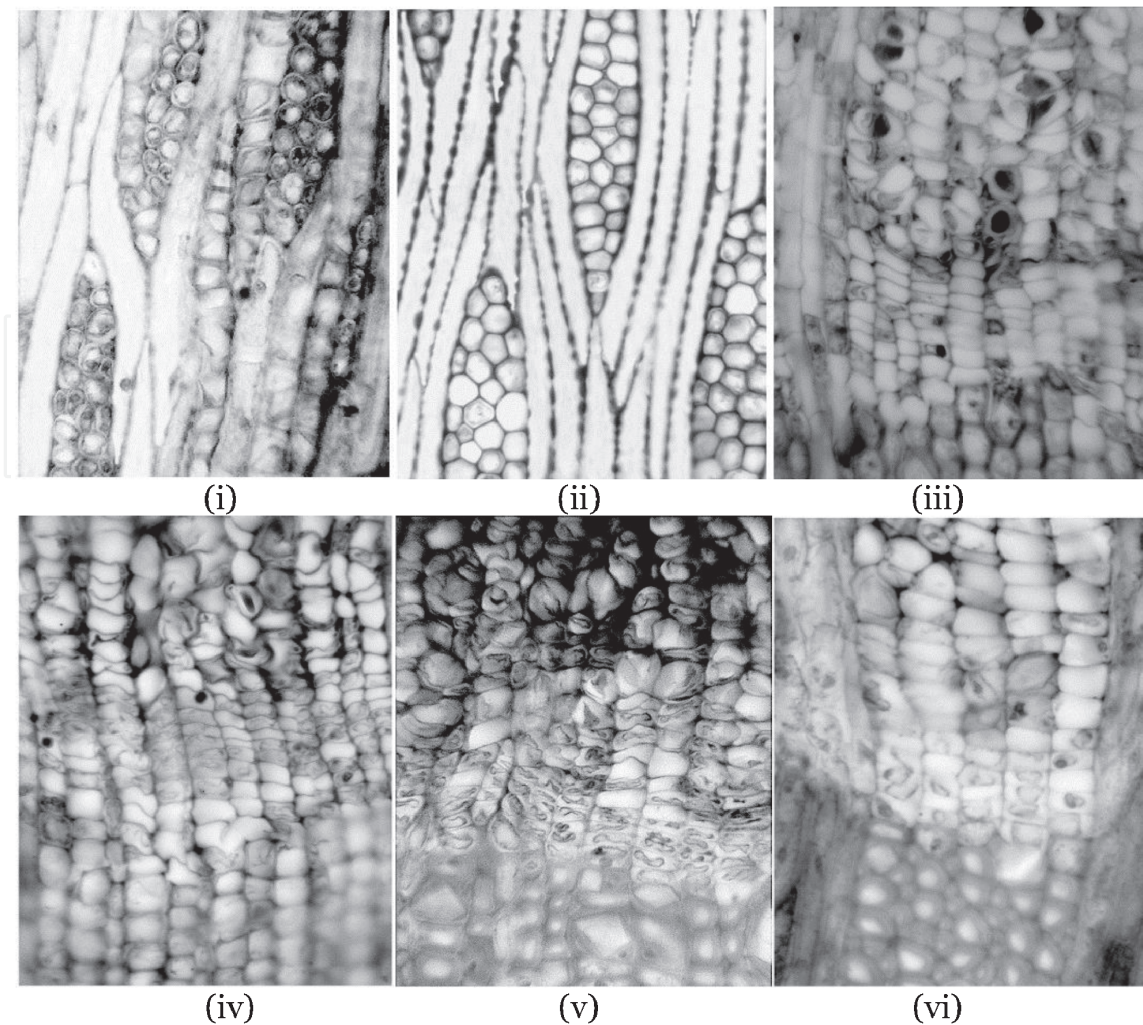


Figure 2.

(i) *C. reticulata* var. *kinnow* T.L.S. through active cambium showing transverse septation of fusiform initial and splitting of ray, (ii) *C. paradisi* T.L.S. through dormant cambium showing beaded radial walls, (iii) *C. limon* T.S. showing swelling of cambial zone, (iv) *C. paradisi* T.S. showing swelling of cambial zone, (v) *C. reticulata* var. *kinnow* T.S. showing swelling of cambial zone, (vi) *C. sinensis* T.S. showing swelling of cambial zone.

cylinder. This usually resulted in a considerable change in the corresponding volume of the different initials. Thus, in the young shoots, the fusiform initials have been found to occupy 86% of the total area of the cambial cylinder in *C. limon*, 93% in *C. paradisi*, 86% in *C. reticulata* var. *kinnow*, and 88% in *C. sinensis*, while the corresponding area of fusiform initials in the cambial cylinder gets reduced toward the mature stem.

5. The vascular cambium: seasonal changes in the structure

As far as the impact of seasonal changes on the dimensions of fusiform initials is concerned, it has been noted that length, width, and tapering ends averages of fusiform initials as well as magnitude of ray initials vary to some extent depending on the time of development of new cambial initials and the period of their growth. Analysis of the data obtained during three consecutive years has revealed that both the structure and the contents of the cambial initials vary from season to season. Short fusiform initials with narrow width and comparatively tapering ends coincided with the activity of the cambium in all the species investigated [44]. Also, the size of ray initials show smaller diameter during the height of cambial activity as has

earlier been reported by Catesson [3], Kitin et al. [8], Espinosa et al. [45], Gricar et al. [46], and Begum et al. [47].

In *C. limon*, the averages of length and width of fusiform initials varies from 233.72 to 278.00 and 17.47 to 21.28 μm , respectively, while the average size of their tapering ends ranges from 76.75 to 90.75 μm . Comparatively shorter fusiform initials occur in May to August and then rest of the months (**Table 3**). The size of ray initials also shows minor variation in different seasons. The mean value of the anticlinal and periclinal diameters ranges from 12.64/11.22 to 16.18/14.82 μm during a calendar year (**Table 4**). In *C. paradisi*, the averages of length and width of fusiform initials range from 217.74 to 284.00 and 16.38 to 21.48 μm , respectively and the end walls range from 72.75 to 91.50 μm . Comparatively shorter fusiform initials are found from June to November (**Table 5**). Similarly, anticlinal and periclinal diameters of ray initials range from 8.63/8.43 to 15.50/14.96 μm in different seasons (**Table 6**). In *C. reticulata* var. *kinnow*, the averages of length and width of fusiform initials range from 211.64 to 256.03 and 16.25 to 18.97 μm , respectively. The size of their tapering ends varies from 72.25 to 82.25 μm . Comparatively, shorter fusiform initials are found from June to October (**Table 7**). The mean value of anticlinal and periclinal diameters of ray initials varies from 10.47/9.24 to 15.23/13.32 μm (**Table 8**). In *C. sinensis*, the averages of length and width of fusiform initials range from 205.36 to 270.50 and 15.98 to 19.51 μm , respectively. The size of their tapering ends varies from 71.25 to 84.25 μm . Comparatively, shorter fusiform initials occur from May to September (**Table 9**). The mean value of anticlinal and periclinal diameters of ray initials varies from 11.76/10.67 to 15.16/12.44 μm (**Table 10**).

The frequency of ray types, when studied in fortnightly collections has revealed that the size as well as the formation of their development happens to be highly influenced by the seasonal conditions. The short and medium sized rays are more frequent during the activity of the cambium in all the species investigated [44]. The distribution of uniseriate to multiseriate rays is found to be influenced by the weather conditions. Frequency of uniseriate and short cambial rays has been found higher in active period than in the inactive phase of the cambium in the species investigated presently. In *C. limon*, the multiseriate rays are dominant in number and constitute 45–65% of the cambial zone in different months lowest being in June and highest in January. Following this, the uniseriate rays generally vary from 23 to 34% and biseriate rays generally vary from 12 to 21% of the total rays of the cambial zone. In *C. paradisi*, multiseriate and uniseriate rays occur more frequently than the biseriate ones. The uniseriate rays are more frequent, that is, 22–30%, whereas the biseriate ones are from 16 to 28%. The highest number of multiseriate rays noticed is 62% (February) and lowest is 42% (June). In *C. reticulata* var. *kinnow*, uniseriate rays vary from 4 to 14%, whereas the biseriate ones are from 13 to 23%. Multiseriate rays are very common in this species and occur frequently within the range of 63–83% minimum being found in May and maximum in February. In *C. sinensis*, uniseriate rays vary from 22 to 30%, biseriate from 16 to 28% and multiseriate from 42 to 62% maximum in the month of March and minimum in June. Earlier workers have also reported such changes both in size and magnitude of different types of cambial initials in tropical trees [48–53].

The amount of ray and fusiform initials shows some minor fluctuations in different months of a calendar year. In *C. limon*, the percentage area occupied by ray initials varies from 14 to 22%, the maximum being in November, while the minimum occurring in May. In *C. paradisi*, it is found to vary from 18 to 27% with lowest being in May and highest in January. In *C. reticulata* var. *kinnow*, 19 to 28% with minimum in June and maximum in December and in *C. sinensis* from 18 to 31% with highest value occurring in January and lowest in July. Thus, *C. sinensis* shows

Months	Length (µm)				Width (µm)				Tapering ends (µm)			
	Range	Mean ± SE	SD	CV%	Range	Mean ± SE	SD	CV%	Range	Mean ± SE	SD	CV%
January	200–313	265.29 ± 1.81	36.22	13.65	17–21	18.63 ± 0.08	1.70	9.12	75–88	81.00 ± 0.31	6.25	7.71
February	200–338	264.00 ± 1.78	35.65	13.50	17–24	19.65 ± 0.11	2.28	11.60	75–100	84.75 ± 0.42	8.41	9.92
March	200–350	269.67 ± 2.01	40.35	14.96	17–24	20.06 ± 0.12	2.47	12.31	75–100	86.25 ± 0.45	9.11	10.56
April	212–325	264.55 ± 1.65	33.07	12.50	17–24	18.97 ± 0.09	1.81	9.54	75–100	82.25 ± 0.33	6.66	8.09
May	175–300	238.32 ± 1.63	32.67	13.70	13–21	17.74 ± 0.10	2.07	11.66	62–88	77.75 ± 0.38	7.62	9.80
June	137–313	233.72 ± 2.02	40.58	17.36	13–21	17.47 ± 0.12	2.45	14.02	62–88	76.75 ± 0.45	9.02	11.75
July	150–313	241.40 ± 1.93	38.66	16.01	13–21	17.88 ± 0.11	2.23	12.47	62–88	78.25 ± 0.41	8.22	10.50
August	150–325	247.00 ± 1.85	37.09	15.01	13–24	18.36 ± 0.10	2.15	11.71	62–100	80.00 ± 0.39	7.91	9.88
September	187–338	263.44 ± 1.79	35.92	13.63	13–24	18.70 ± 0.10	2.07	11.06	62–100	81.25 ± 0.38	7.61	9.36
October	200–338	269.34 ± 1.89	37.87	14.06	17–24	19.44 ± 0.11	2.26	11.62	75–100	84.00 ± 0.41	8.31	9.89
November	250–263	274.84 ± 1.79	35.97	13.08	17–24	21.28 ± 0.11	2.23	10.47	75–100	90.75 ± 0.41	8.22	9.05
December	225–350	278.00 ± 1.66	33.39	12.01	17–24	20.40 ± 0.11	2.25	11.02	75–100	87.50 ± 0.41	8.30	9.48
LSD at 5%		= 50.48				= 3.03				= 11.14		
LSD at 1%		= 67.60				= 4.05				= 14.92		

Table 3. Changes in the cell size of fusiform initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in Citrus limon.

Months	Anticlinal diameter (μm)				Periclinal diameter (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	10–21	15.23 \pm 0.17	3.42	22.45	10–21	14.62 \pm 0.16	3.21	21.95
February	10–21	15.16 \pm 0.16	3.35	22.09	10–17	12.78 \pm 0.12	2.41	18.85
March	10–21	15.43 \pm 0.17	3.55	23.00	10–21	14.82 \pm 0.16	3.38	22.80
April	10–21	13.46 \pm 0.13	2.63	19.53	10–14	11.22 \pm 0.07	1.56	13.90
May	10–17	12.64 \pm 0.10	2.15	17.00	10–14	11.35 \pm 0.08	1.61	14.18
June	10–17	12.85 \pm 0.12	2.48	19.29	6–14	11.69 \pm 0.09	1.82	15.56
July	10–17	12.92 \pm 0.12	2.54	19.65	10–17	12.10 \pm 0.10	2.17	17.93
August	10–17	14.48 \pm 0.12	2.43	16.78	10–17	12.30 \pm 0.10	2.03	16.50
September	10–17	13.46 \pm 0.11	2.35	17.45	10–17	12.17 \pm 0.09	1.81	14.87
October	10–21	15.57 \pm 0.16	3.34	21.45	10–17	13.60 \pm 0.11	2.35	17.27
November	10–21	16.18 \pm 0.19	3.82	23.60	10–17	14.55 \pm 0.11	2.36	16.21
December	10–21	15.84 \pm 0.18	3.64	22.97	10–21	13.94 \pm 0.16	3.21	23.02
LSD at 5%		= 4.34				= 3.38		
LSD at 1%		= 5.81				= 4.53		

Table 4.

Changes in the cell size of ray initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus limon*.

maximum fluctuation as compared to others [44]. The ray initials multiply considerably to become multiseriate in older axis in all the investigated species as has been reported by earlier workers [10, 11, 52].

6. Periodicity of the vascular cambium

The activity of vascular cambium is not uniform but shows great variation depending on the genetic constitution of plants and difference in the internal and external environment [1]. There are plants whose cambium is active throughout the entire life of the plant, that is, the cells of cambium divide continuously and the resulting cells undergo gradual differentiation to form xylem and phloem. Such type of activity usually occurs in plants growing in tropical regions [54]. However, not all tropical trees exhibit continuous cambial activity [55–58].

In the present study, it has been observed that the vascular cambium of all three species shows a periodic activity rather than a continuous growth as reported in other tropical species of Indian subcontinent [10, 11, 52, 57–61]. During the dormant stage, the cambial zone is represented by a narrow zone of tangentially flattened cells constituting of three to seven layers in *C. limon*, seven to nine layers in *C. paradisi*, four to six layers in *C. reticulata* var. *kinnow*, and five to seven layers in *C. sinensis* (**Figure 1(ii)**). The radial walls of cambial cells during dormant stage are found comparatively thicker than what they are during the active phase. In tangential view, the radial walls are found prominently beaded during the resting period (**Figure 2(ii)**) due to the alternatively thickened areas and the deeply depressed primary pit fields, through which they communicate by plasmodesmata connections with the contiguous elements. The fusiform cambial cells during their active phase possess relatively thin and almost smooth radial walls due to the absence of thickened areas, alternating with the primary pit fields (**Figure 1(v)**).

Months	Length (μm)				Width (μm)				Tapering ends (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	187–438	284.00 \pm 3.18	63.72	22.43	13–28	20.12 \pm 0.16	3.25	16.15	62–113	86.50 \pm 0.59	11.96	13.82
February	187–463	281.35 \pm 3.12	62.53	22.22	13–31	21.48 \pm 0.18	3.63	16.89	62–125	91.50 \pm 0.66	13.34	14.57
March	125–450	272.87 \pm 3.15	63.18	23.15	10–31	19.65 \pm 0.18	3.74	19.03	50–125	84.75 \pm 0.68	13.77	16.24
April	237–343	270.73 \pm 1.76	35.35	13.05	17–24	20.53 \pm 0.12	2.45	11.93	75–100	88.00 \pm 0.45	9.01	10.23
May	137–400	266.37 \pm 2.90	58.17	21.83	13–24	19.38 \pm 0.15	3.13	16.15	62–100	83.75 \pm 0.57	11.53	13.76
June	100–425	217.74 \pm 2.98	59.65	27.39	10–28	16.38 \pm 0.15	3.02	18.43	50–113	72.75 \pm 0.55	11.10	15.25
July	125–313	220.32 \pm 2.26	45.28	20.55	10–21	16.86 \pm 0.13	2.72	16.13	50–88	74.50 \pm 0.50	10.00	13.42
August	150–375	227.06 \pm 2.31	46.32	20.39	13–24	16.86 \pm 0.12	2.45	14.53	62–100	74.50 \pm 0.45	9.01	12.09
September	125–363	239.00 \pm 2.79	55.83	23.35	10–24	17.61 \pm 0.16	3.31	18.79	50–100	77.25 \pm 0.60	12.18	15.76
October	125–363	243.58 \pm 2.70	54.08	22.20	10–24	17.74 \pm 0.16	3.28	18.48	50–100	77.75 \pm 0.60	12.08	15.53
November	137–338	244.09 \pm 2.56	51.35	21.03	13–24	18.36 \pm 0.14	2.96	16.12	62–100	80.00 \pm 0.54	10.91	13.63
December	150–450	267.41 \pm 3.19	63.95	23.91	13–28	19.24 \pm 0.18	3.64	18.91	62–113	83.25 \pm 0.66	13.38	16.07
LSD at 5%		= 81.05				= 4.61				= 16.95		
LSD at 1%		= 108.53				= 6.17				= 22.70		

Table 5. Changes in the cell size of fusiform initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus paradisi*.

Months	Anticlinal diameter (μm)				Periclinal diameter (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	10–21	15.36 \pm 0.16	3.35	21.80	10–21	14.96 \pm 0.16	3.33	22.25
February	10–17	14.14 \pm 0.12	2.48	17.53	6–14	10.13 \pm 0.13	2.76	27.24
March	10–21	14.55 \pm 0.12	2.55	17.52	10–17	12.85 \pm 0.12	2.48	19.29
April	10–17	14.21 \pm 0.12	2.42	17.03	10–17	12.51 \pm 0.10	2.19	17.50
May	6–17	10.54 \pm 0.14	2.90	27.51	6–14	9.45 \pm 0.10	2.18	23.06
June	6–14	9.72 \pm 0.11	2.25	23.14	6–14	8.97 \pm 0.13	2.61	29.09
July	6–14	8.63 \pm 0.12	2.47	28.62	6–14	8.43 \pm 0.09	1.83	21.70
August	6–14	8.77 \pm 0.13	2.64	30.10	6–14	8.56 \pm 0.11	2.38	27.80
September	10–17	13.46 \pm 0.12	2.45	18.20	6–14	10.33 \pm 0.11	2.25	21.78
October	10–21	15.43 \pm 0.16	3.35	21.71	10–17	14.55 \pm 0.12	2.45	16.83
November	10–17	14.34 \pm 0.12	2.48	17.29	10–21	13.87 \pm 0.15	3.18	22.92
December	10–21	15.50 \pm 0.17	3.48	22.45	10–21	14.82 \pm 0.16	3.31	22.33
LSD at 5%		= 3.77				= 3.77		
LSD at 1%		= 5.05				= 5.06		

Table 6.

Changes in the cell size of ray initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus paradisi*.

The beaded nature of radial walls, if at all present during the active period, is not as prominent as in the dormant period. The cambial zone as a whole during the active phase takes light stain due to the absence of colored contents and loss of chromaticity of protoplasm (**Figure 1(v)**).

The vascular cambium in all the species appears to undergo activation once in a year, after undergoing a definite period of rest. The first sign of activity appears in April in *C. limon* and *C. paradisi* and in May in *C. reticulata* var. *kinnow* and *C. sinensis*. The cells in the cambial zone undergo radial expansion in the third week of March in *C. limon* (**Figure 2(iii)**) and in the last week of March in *C. paradisi* (**Figure 2(iv)**), in the third week of April in *C. reticulata* var. *kinnow* (**Figure 2(v)**), and in the second week of April in *C. sinensis* (**Figure 2(vi)**). As a result of this enlargement, the cambial zone swells up from 32 to 40 μm in *C. limon*, 51 to 79 μm in *C. paradisi*, 17 to 64 μm in *C. reticulata* var. *kinnow*, and 34 to 40 μm in *C. sinensis*. Several criteria have been employed in the past to judge the initiation and the duration of cambial activity in tropical as well as in the different temperate species. Firstly, Priestly et al. [62] demonstrated the case with which the bark separate itself from wood of a tree trunk during the active period, a phenomenon what they named as “slippage of the bark.” Subsequent workers later employed several other criteria to recognize the reactivation of cambium after its winter dormancy. The important finding in this connection is Frankenstein et al. [63]. In the present study, however, a number of criteria have been used in combination while studying periodicity of cambium. The initiation of cambial reactivation has been taken from the time of radial expansion of cambial initials, but the activity of cambium has been counted from the actual cell division and not from the date of histochemical changes or physical expansion of initials. The ceassation of activity has been taken by stopping of cell division which normally proceeds to the histochemical change in the initials.

Months	Length (μm)				Width (μm)				Tapering ends (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	162–325	252.51 \pm 2.02	40.43	16.01	13–21	18.42 \pm 0.10	2.05	11.12	62–88	80.25 \pm 0.37	7.54	9.39
February	150–325	251.13 \pm 1.68	33.61	13.38	17–24	18.56 \pm 0.09	1.82	9.80	75–100	80.75 \pm 0.33	6.72	8.32
March	150–300	248.00 \pm 1.97	39.57	15.95	13–21	17.88 \pm 0.11	2.33	13.03	62–88	78.25 \pm 0.43	8.60	10.99
April	137–400	246.61 \pm 3.60	72.00	29.19	13–28	18.36 \pm 0.19	3.97	21.62	62–113	80.00 \pm 0.72	14.59	18.23
May	200–325	242.00 \pm 1.66	33.29	13.75	17–24	18.97 \pm 0.09	1.81	9.54	75–100	82.25 \pm 0.33	6.66	8.09
June	150–288	230.42 \pm 1.88	37.60	16.31	13–21	17.40 \pm 0.11	2.32	13.33	62–88	76.50 \pm 0.42	8.54	11.16
July	150–413	226.38 \pm 2.08	41.67	18.40	13–28	17.06 \pm 0.12	2.59	15.18	62–113	75.25 \pm 0.47	9.52	12.65
August	137–338	211.64 \pm 1.88	37.73	17.82	13–24	16.25 \pm 0.11	2.28	14.03	62–100	72.25 \pm 0.42	8.41	11.64
September	137–275	216.52 \pm 1.95	39.11	18.06	13–21	16.59 \pm 0.12	2.42	14.58	62–88	73.50 \pm 0.44	8.89	12.09
October	175–300	237.00 \pm 1.69	33.85	14.28	13–21	17.68 \pm 0.11	2.25	12.72	62–88	77.50 \pm 0.41	8.30	10.70
November	175–300	245.69 \pm 1.62	32.56	13.25	13–21	18.22 \pm 0.94	1.89	10.37	62–88	79.50 \pm 0.34	6.97	8.76
December	150–338	256.03 \pm 2.01	40.33	15.75	13–24	18.83 \pm 0.10	2.06	10.93	62–100	81.75 \pm 0.37	7.59	9.28
LSD at 5%		= 58.27				= 3.37				= 12.40		
LSD at 1%		= 78.03				= 4.51				= 16.61		

Table 7. Changes in the cell size of fusiform initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus reticulata* var. kinnow.

Months	Anticlinal diameter (μm)				Periclinal diameter (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	10–21	15.23 \pm 0.17	3.48	22.84	10–17	13.32 \pm 0.12	2.53	18.99
February	10–21	14.68 \pm 0.12	2.58	17.57	10–17	13.26 \pm 0.11	2.38	17.94
March	10–17	12.44 \pm 0.10	2.11	16.96	6–14	10.94 \pm 0.12	2.48	22.66
April	10–21	13.53 \pm 0.12	2.59	19.14	6–17	11.56 \pm 0.15	3.12	26.98
May	10–17	13.73 \pm 0.12	2.45	17.84	10–14	11.28 \pm 0.07	1.58	14.00
June	6–14	10.47 \pm 0.11	2.34	22.34	6–14	9.38 \pm 0.11	2.21	23.56
July	6–17	11.69 \pm 0.14	2.98	25.49	6–14	9.45 \pm 0.09	1.96	20.74
August	6–17	10.74 \pm 0.14	2.83	26.35	6–14	9.24 \pm 0.10	2.04	22.07
September	6–17	11.96 \pm 0.18	3.68	30.76	6–14	10.13 \pm 0.13	2.76	27.24
October	10–17	13.80 \pm 0.12	2.49	18.04	10–14	11.15 \pm 0.07	1.52	13.63
November	10–21	14.75 \pm 0.16	3.23	21.89	10–17	11.76 \pm 0.09	1.95	16.58
December	10–17	13.94 \pm 0.11	2.38	17.07	10–17	12.51 \pm 0.11	2.30	18.38
LSD at 5%		= 3.91				= 3.26		
LSD at 1%		= 5.24				= 4.36		

Table 8.

Changes in the cell size of ray initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus reticulata* var. *kinnow*.

In all the species investigated, the reactivation of vascular cambium has been indicated by radial expansion of cambial initials which has been described as “swelling” of cambial cell by earlier workers [52, 60, 64]. This phenomenon has been observed in the present study to occur a few days before the cells start dividing to produce new derivatives in all the species presently investigated. The present study shows swelling of the cambial zone in March in *C. limon* and *C. paradisi* and in April in *C. reticulata* var. *kinnow* and *C. sinensis*. However, the extent of radial expansion was found varying in different species.

After swelling, the cell divisions start within a week or two in the cambial zone which in turn is followed by a number of histochemical changes in the initials. A decrease in the density of cell protoplast coupled with the loss of chromaticity and the leaning of cell wall as a result of reduction in wall thickening and in the size of beads of radial walls. More or less similar changes in the nature and structure of cambial initials have been described by Oribe et al. [65–67]. The initiation of cell division during hot weather conditions shows that this phenomenon depends upon high temperature and low humidity as has been reported earlier by Mellerowicz et al. [68] in *Abies balsamea*, Barnett and Miller [69] in *Picea sitchensis*, Oribe and Kubo [70] in Conifers, and Espinosa et al. [45] in 83 tropical trees.

In *C. limon*, the cells start dividing in the first week of April which causes an increase in the layers of cells up to nine layers. In *C. paradisi*, cambial cell division begins from the second week of April, increasing the number of cambial layers up to 12. Similarly, in *C. reticulata* var. *kinnow* and in *C. sinensis*, the cells start dividing in the first week of May, causing an increase in the layers of cambium up to 10 in both the species. The newly produced derivatives differentiate first into xylary elements in all the species investigated as a result of which new xylem is being added in *C. limon* and *C. paradisi* in the month of April, in *C. reticulata* var. *kinnow* and *C. sinensis* in May. The phloem production, out of the newly produced cambial derivatives, is observed in two flushes first in May, June, and then again in September in

Months	Length (μm)				Width (μm)				Tapering ends (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	175–325	264.75 \pm 1.95	39.06	14.75	13–24	18.08 \pm 0.11	2.30	12.72	62–100	79.00 \pm 0.42	8.46	10.70
February	137–300	261.00 \pm 2.02	40.53	15.52	13–21	17.40 \pm 0.12	2.42	13.90	62–88	76.50 \pm 0.44	8.89	11.62
March	200–338	263.63 \pm 1.72	34.45	13.06	17–24	19.04 \pm 0.09	1.92	10.08	75–100	82.50 \pm 0.35	7.07	8.56
April	150–363	260.39 \pm 2.18	43.79	16.81	13–24	18.83 \pm 0.12	2.47	13.11	62–100	81.75 \pm 0.45	9.09	11.11
May	175–350	235.33 \pm 2.04	40.84	17.35	13–24	17.54 \pm 0.12	2.48	14.13	62–100	77.00 \pm 0.45	9.14	11.87
June	137–275	215.45 \pm 1.93	38.74	17.98	13–21	16.52 \pm 0.12	2.45	14.83	62–88	73.25 \pm 0.45	9.02	12.31
July	137–300	205.36 \pm 1.84	36.96	17.99	13–21	15.98 \pm 0.10	2.17	13.57	62–88	71.25 \pm 0.40	8.01	11.24
August	137–300	227.04 \pm 2.10	42.07	18.52	13–21	17.27 \pm 0.12	2.43	14.07	62–88	76.00 \pm 0.44	8.96	11.78
September	162–325	238.58 \pm 1.68	33.75	14.14	13–21	17.81 \pm 0.10	2.10	11.79	62–88	78.00 \pm 0.38	7.73	9.91
October	187–325	256.00 \pm 1.88	37.73	14.73	13–24	18.70 \pm 0.09	1.95	10.42	62–100	81.25 \pm 0.35	7.18	8.83
November	150–325	262.66 \pm 2.07	41.40	15.76	13–24	18.02 \pm 0.11	2.38	13.20	62–100	78.75 \pm 0.43	8.76	11.12
December	200–375	270.50 \pm 2.05	41.04	15.17	17–24	19.51 \pm 0.11	2.33	11.94	75–100	84.25 \pm 0.43	8.60	10.20
LSD at 5%		= 56.77				= 3.24				= 11.91		
LSD at 1%		= 76.03				= 4.34				= 15.95		

Table 9. Changes in the cell size of fusiform initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus sinensis*.

Months	Anticlinal diameter (μm)				Periclinal diameter (μm)			
	Range	Mean \pm SE	SD	CV%	Range	Mean \pm SE	SD	CV%
January	10–17	14.14 \pm 0.12	2.48	17.53	10–17	12.30 \pm 0.11	2.24	18.21
February	10–17	12.85 \pm 0.12	2.48	19.29	10–14	11.15 \pm 0.07	1.52	13.63
March	10–17	13.32 \pm 0.11	2.34	17.56	10–14	11.56 \pm 0.08	1.66	14.35
April	10–17	13.19 \pm 0.10	2.11	15.99	10–17	12.24 \pm 0.11	2.25	18.38
May	10–17	12.78 \pm 0.09	1.87	14.63	6–14	10.88 \pm 0.12	2.54	23.34
June	6–17	11.76 \pm 0.16	3.20	27.21	6–17	10.67 \pm 0.15	3.04	28.49
July	10–14	12.58 \pm 0.07	1.56	12.40	6–14	10.74 \pm 0.09	1.84	17.13
August	10–17	12.64 \pm 0.10	2.15	17.00	6–14	10.94 \pm 0.11	2.28	20.84
September	10–17	13.26 \pm 0.11	2.38	17.94	6–14	11.22 \pm 0.10	2.17	19.34
October	6–21	13.53 \pm 0.19	3.88	28.67	6–14	11.90 \pm 0.11	2.28	19.15
November	10–17	14.34 \pm 0.12	2.48	17.29	10–14	11.49 \pm 0.08	1.65	14.36
December	10–21	15.16 \pm 0.16	3.28	21.63	10–17	12.44 \pm 0.12	2.41	19.37
LSD at 5%		= 3.77				= 3.16		
LSD at 1%		= 5.05				= 4.24		

Table 10.

Changes in the cell size of ray initials (as observed in tangential longitudinal section) of cambial zone during various months of a calendar year in *Citrus sinensis*.

C. limon. In *C. paradisi*, new phloem is added in the last week of October followed by another addition of new phloem in November and December. In *C. reticulata* var. *kinnow*, new phloem is added in October and November. In *C. sinensis*, first flush of new phloem is added in June and July. The second flush of phloem differentiates in October.

The cessation of cambial activity occurs in early October in *C. limon*, early November in *C. sinensis*, early December in *C. reticulata* var. *kinnow*, and late December in *C. paradisi*. Thus, it appears that in the investigated species, extreme fall in temperature brings down the dormancy as reported earlier by Paliwal and Paliwal [71] in *Rhododendron arboreum*, Khan [10] in *Jacaranda mimosifolia*, *Pterospermum acerifolium*, *Terminalia arjuna*, and Mahmood [11] in *Alstonia scholaris*, *Emblica officinalis*, *Putranjiva roxburghii*.

Thus, in the presently investigated species, the cambium remains active for about 6 months in *C. limon* and *C. sinensis*, 9 months in *C. paradisi*, and 7 months in *C. reticulata* var. *kinnow*. More or less similar prolonged tends of duration of 5–9 months of radial growth has been reported earlier by Fahn [72], Zhang et al. [57], Rao and Rajput [60], Khan [10], and Mahmood [11].

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