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Structure of Sieve Area in Some Ferns

M N B NAIR and J J SHAH, FNA
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388120

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Sieve plates are absent but sieve areas are found either scattered or variously aggregated on all walls of the sieve cells in seven ferns studied. In *Cyathea spinulosa* Wall. ex Hook. and *Cyathea nilgirensis* Holtum sieve pores on end walls are sometimes larger than those on the lateral walls; rarely sieve areas with a single large pore are seen on the lateral wall. There is a mathematical correlation between diameter and number of pores per sieve area in *Cyathea*.

Key Words: Sieve Cells, Sieve area, Ferns

Introduction

The morphology of sieve area in the sieve cells of pteridophytes is little explored and the present knowledge is mostly from electron microscopic study undertaken recently by Burr and Evert (1973), Dute and Evert (1977), Evert (1976), Kruatrachue and Evert (1974, 1978), Perry and Evert (1975) and Warmbrodt and Evert (1974).

Generally, the vascular cryptogams are reported to have no sieve plate (Esau 1969) because sieve areas do not show varied degree of differentiation. Presence of sieve plate has been reported in *Equisetum* (Agashe 1968, Lamoureux 1961) and *Cyathea gigantea* (Shah & Fotedar 1974a). Perry and Evert (1975) observed occasional solitary pores on the lateral wall of sieve element of *Psilotum nudum* which are consistently larger than the sieve pores on the end wall.

Sieve pore development in angiosperms is generally associated with callose except in *Lemna minor* (Walsh & Melaragno 1976). In *Lycopodium lucidulum* (Warmbrodt & Evert 1974), *Isoetes muricata* (Kruatrachue & Evert 1974), *Selaginella kraussiana* (Burr & Evert 1973), Ophioglossaceae (Evert 1976, Lamoureux 1961) and some other ferns callose is absent. Dute and Evert (1977) found association of callose with sieve pores in *Equisetum hyemale*.

It has been demonstrated both by light microscope (Evert & Derr 1964) and electron microscope studies (Cronshow & Anderson 1969) that the amount of callose found in association with mature sieve plate pore decreases considerably when materials are frozen and fixed in cold. It is also proved that even mechanical stimulation like ultrasound causes heavy deposition

of callose (Eschrich 1975). Eschrich (1975) is of the view that callose controls the flow rate and movement of assimilate through the sieve pores. For a better understanding of the sieve area morphology a study of its form, variation, development and modification is necessary.

Materials and Methods

The structure of sieve area in the sieve cells of seven ferns has been studied (table 1). The material was fixed in FAA and preserved in 70% ethanol. Samples were dehydrated in TBA series, infiltrated with para TBA and paraffin and embedded in tissue prep (Sass 1958). Longitudinal sections (of 6–8 μm thickness) and transverse sections (of 10–12 μm thickness) were stained with tannic acid ferric chloride—Resorcin blue (Cheadle et al. 1953), Safranin O (Sass 1958) and Toluidine blue O (O'Brien et al. 1964). For statistical analysis of the sieve area, drawings were made on graph paper and the data obtained from 600 readings were pooled. Graphs were prepared showing: (1) number of sieve pores vs. percentage of their occurrence; (2) diameter of the pore vs. percentage of its occurrence; (3) number of pores vs. area of the sieve area; (4) number of pores vs. diameter of the pores. For the third and fourth graphs the following curves were tried.

$$y = a+bx$$
$$y = a+bx+cx^2$$
$$\log y = a+bx$$
$$y^2 = a+bx$$
$$1/y = a+bx.$$

Photomicrographs were taken on a Carl Zeiss photomicroscope with planapochromatic objectives. Kodak panatomic-X and ORWO NP—15 type 35 mm films were used with yellow filter.

Table 1 *Diameter of the sieve pores*

Name of the Taxon	Maximum diameter of the pore (μm)	
	(Rachis)	(Rhizome)
Botrychium lanuginosum Wall.	1.2	1.6
Cyathea nilgirensis Holtum	2.4	6.8
Cyathea spinulosa Wall. ex Hook	2.8	6.4
Dennstaedtia appendiculata (Wall.) J. Smith	0.8	3.2
Pteris wallichiana JG Agardh	2.0	2.4
Woodwardia radicans (L.) Kühn	2.4	—
Pteridium aquilinum (L.) Smith	2.8	3.2

Results

Mature sieve cells of all taxa studied are elongated with oblique to very oblique end walls. Sieve areas are present on the end walls (figures 1–4, 28–30) and on lateral walls (figures 5–27, 31–41). Sieve areas are lightly stained areas on the sieve cell wall and are easily recognised when the sieve cell wall is relatively thick (figures 5–35). As the sieve cell ages, its wall becomes thin (figures 39–43) and sieve areas lose their distinct delimitation (figures 42–43).

The sieve areas develop from primary pit-fields on the wall of developing sieve cell. They become prominent as the sieve cell wall thickens. During development the primary pit-fields enlarge and may compartmentalize by deposition of additional wall material.

The distribution of sieve areas may be solitary, aggregate or reticulate. When solitary, the sieve areas are well spaced from each other (figures 9, 16, 18, 23, 24) and may

either be arranged in a single row or be scattered (figures 23, 24). The wall portion separating adjacent sieve areas may be more or less uniformly thick (figures 9, 23, 24). When aggregated, two or more sieve areas are grouped together (figures 6, 12, 17, 19–21, 25–28) in a scalariform (figure 28) or scalariform-reticulate (figures 26, 27) pattern. The sieve areas may extend horizontally through the entire width of the wall (figure 28). Normally such sieve areas have many sieve pores. The wall portion separating adjacent sieve areas may be homogenous (figures 25, 28) or heterogenous (figures 17, 19–21, 26, 27). Where the sieve areas are close to each other they give a reticulate appearance (figures 29–34) and the wall portion separating the contiguous sieve areas may be homogenous (figures 29–31) or heterogenous (figures 32–35).

All types of sieve area distributions are present in the sieve cells of rachis and rhizome. Intermediate types between solitary, aggregate and reticulate are also observed.

Sieve areas are of different froms and dimensions. Sometimes they extend horizontally through the entire width of the wall (figures 10, 11, 21, 27, 28). They appear circular (figures 14, 15), oval (figures 14, 38), triangular (figures 12, 13, 20, 21, 31), trapezoidal (figures 11, 28) or fusiform (figures 20, 13, 16, 22).

Each sieve area may have one (figures 10, 23, 25, 37) to many (figures 1, 4, 11) sieve pores, usually lined (figures 1, 2, 4, 18, 37, 39, 41) or filled (figures 5,11,16,17,19,34, 42,43) with callose. Sometimes callose deposits of adjacent pores fuse together so that the separate identity of the pores is lost (figure 40). Callose occurs on both sides of the common wall between two contiguous sieve cells (figure 44). Rarely sieve cells in rhizome of *Cyathea* show heavy deposition of callose (figure 45). In *Pteris wallichiana* J G Agardh, *Cyathea* and *Dennstaedtia appendiculata* (Wall.) J Smith sieve areas are sometimes without callose (figures 6, 7, 8, 38).

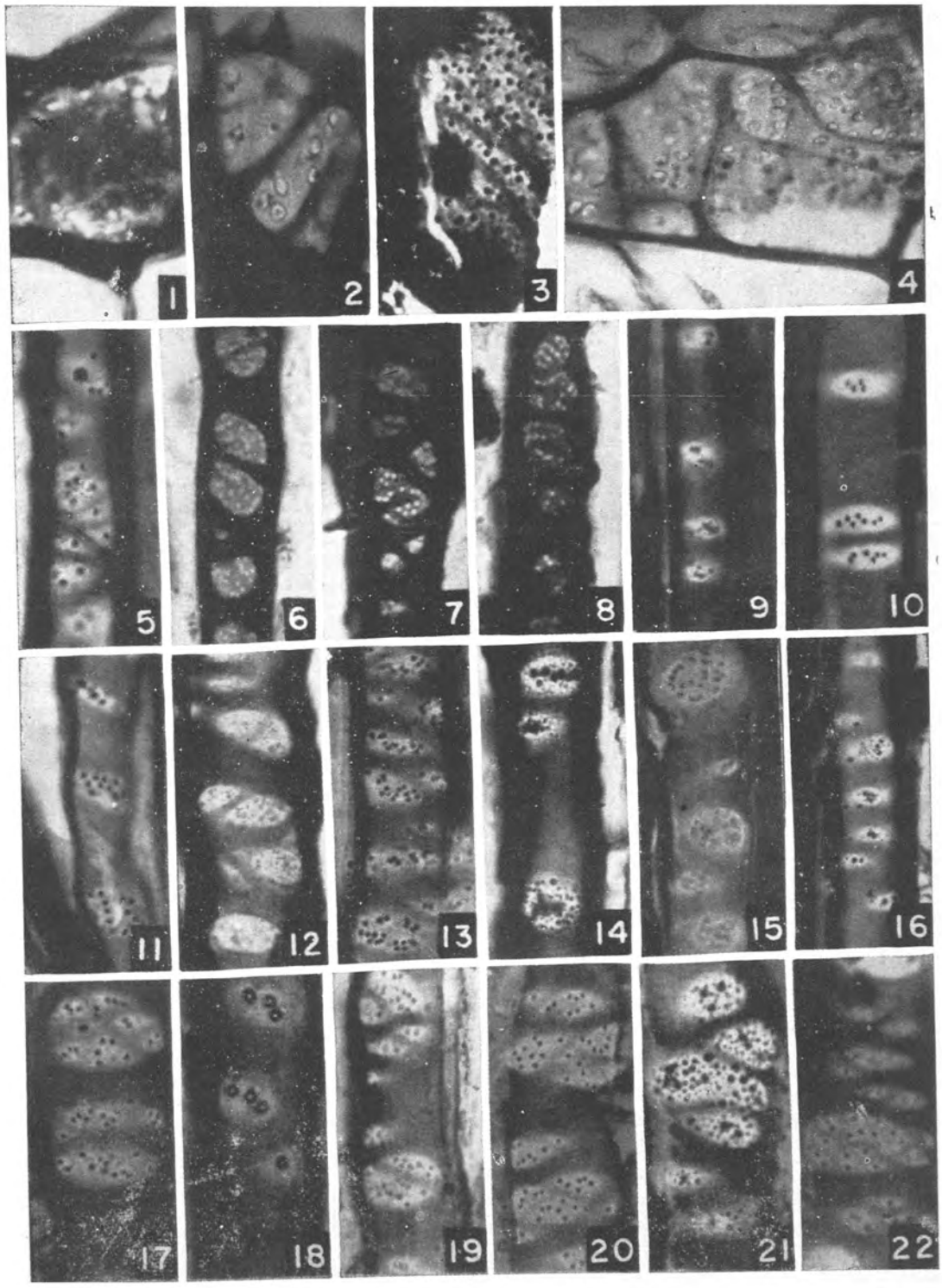
Proteinaceous spherules accumulate near the sieve areas (figure 43) and as in *Cyathea* rhizome sometimes they occlude the sieve pores (figure 36). Table 1 shows the data on the diameter of the pores on the sieve areas of investigated species.

The diameter of the sieve pores on the end walls ranges from 0.4 μm to 1.6 μm in *Dennstaedtia*, 1.2 μm in *Pteridium aquilinum* (L.) Kuhn, 0.8 μm in *Pteris* and from 0.8 μm to 6.4 μm in the rhizome of *Cyathea*. It is less than that present on the lateral walls. The rhizome sieve cells in *Cyathea* have sometimes sieve areas having a single pore of 6.8 μm diameter on the lateral wall (figure 37).

In the rachis of *Cyathea nilgirensis* sieve areas with single sieve pore are frequent (figure 47). The relative frequency of sieve areas with 1–24 pores is shown in figure 47. Sieve pore with 0.8 μm diameter is the most common (figure 46). On an average 24.4% of the lateral wall is occupied by sieve areas and 1.4% of the wall of the sieve cell is occupied by the sieve pores. 5.8% of the tota

Figures 1–22 *1-4* End wall of the sieve cells, showing sieve area (surface view) with open (1,2,4) and closed pores (×964); *3* (×1280); *4* (×914.5). *5–22* Sieve areas on the lateral walls of the sieve cells, with callose (*5,9,22*) and without callose (6, 8). *5,6,8* (×1051); *7* (×1243.5); *9,15,17,19–21* (×914.5); *10,11,18* (×964), *12,16,22* (×777); *13* (×1280); *14* (×857); *1,2,4*, Rhizome of *Cyathea*; *5,10,12,13,14,21,22* Rachis of *Cyathea*; *3,16,18-20*, Rhizome of *Dennstaedita*; *9*, Rachis of *Dennstaedtia*; *15*, Rhizome of *Pteris*; *6,7,8,17*, Rachis of *Pteris*; N K chis of *Woodwardia*

C, callose; P, pore; SA, sieve area; SP, sphercules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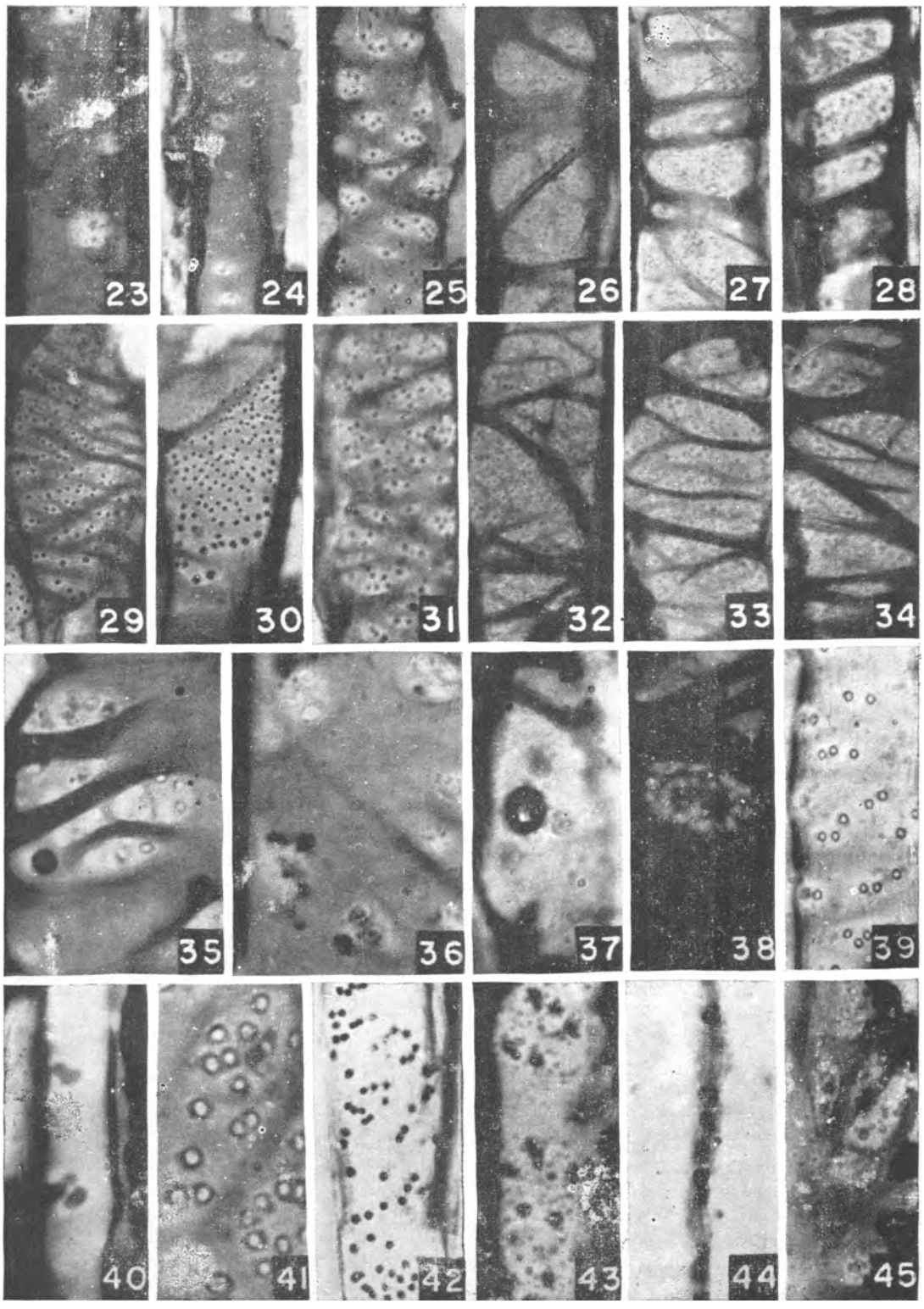
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area of the sieve area is occupied by sieve pores.

It appears that there is no mathematical relationship between the number of pores and average area of the sieve area (figure 48). The x^2 values are greater than the tabulated value for 17 degree of freedom in all possible equations used for analysis. However, when the number of pores increases there is a corresponding increase in the area of the sieve area (figure 48). There is a sharp increase in the area of the sieve area when the number of the pore is 8, 16 and 24 (figure 48).

It is evident that there is a mathematical relation between the number of pores on a sieve area and average diameter of the pores. The calculated x^2 value 0.4076 is far less than the tabulated x^2 value 30.14 for 17 degree of freedom at 95% level of significance. The second degree curve is most fitting (figure 49) because the calculated x^2 values in all relations used are more than that of the quadratic relation.

Discussion

The present investigation shows that the sieve areas on the end wall have not become specialized suggestive of a sieve plate. However, some sieve cells in the rhizome of *Cyathea* have on their end walls, sieve areas with pores having greater diameter than those on the lateral walls. Very few sieve areas have pores with 6.8μm diameter. With respect to the sieve element of *Cyathea* we consider them as sieve cells. We fully agree with Kruatrachue and Evert (1974) that a reconsideration of sieve plate terminology is essential especially after gathering data from lower vascular plants. It may be presumed that the larger diameter of the sieve pores on the end walls of *Cyathea* sieve cells indicates specialization.

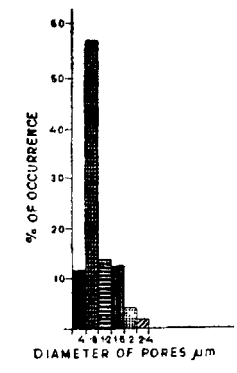
The presence of callose in the sieve area varies with the species. There are sieve areas with and without callose in the same species. It is plausible to believe that callose controls the flow rate and movement of assimilates through sieve pores.

Sieve areas develop from primary pit-fields. Some primary pit-fields change their structure by thickening deposited over the original pit-membrane (Esau 1969). The enlargement and deposition of wall material occur during development of the sieve area. The homogenous and heterogenous cell wall portions or bars between and in the sieve area indicate the above phenomenon. The darkly stained wall portions or bars between sieve areas presumably develop first and hence, they appear to be more thickened because of the additional deposition of wall material during the sieve cell development. On the other hand cell walls which presumably develop later in the pit-field area, or where the deposition of the wall material is not uniform, are stained lighter.

Krautrachue and Evert (1978) observed a single cluster of pores on each end wall and the lateral walls have a few solitary pores in the root sieve cells of *Isoetes*

Figures 23–27, 31–45 Sieve areas on the lateral wall. Sieve areas show solitary (23–24); aggregate (25,28) and reticulate (29–34) distribution. *35*, Heterogenous wall partition between sieve areas; *36*, Sieve pores are occluded with spherules; *37*, Single pored sieve areas; *38*, Sieve area without callose; *39–43*, Thinning of the sieve cell wall. The sieve areas become indistinct. **28–30**, Sieve areas on the oblique end walls; *44*, Sieve area in sectional view; *45*, Heavy deposition of callose in the sieve cell. *23,29,32*, (×731); *24,25,28,30,31, 37* (×964); *26,33,39,43,45* (×777). *27,34* (×914.5) *35*, (×1189); *36*, (×1280); *38*, (×624) *40,44* (×1371); *41*, (×1951), *42*, (1051), *25,29-31,44* Rhizome of *Dennstaedtia*; *23,24,42*, Rachis of *Dennstaedtia*; *28*, Rachis of *Cyathea*; *35,36,37,45*, Rhizome of *Cyathea*; *26,27,32,33,34*, Rhizome of *Peris*; *38*, Rhizome of *Botrychium*; *39,41,43*, Rachis of *Pttridium*; Rachis of *Woodwardia*.

C, callose; P, pore; SA, sieve area; SP, spherules



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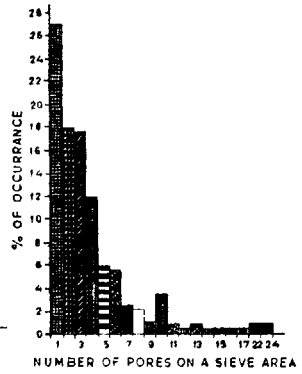

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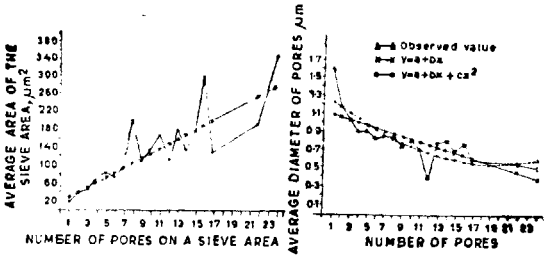
– $y=a+bx$
•–• observed value



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muricata. In general, the distribution of sieve areas, in the species studied is solitary, aggregate or reticulate. It is plausible to believe that the solitary sieve area distribution develops from a single primary pit-field. In aggregate distribution the sieve areas of each group might have been formed from a single primary pit-field but later modified due to deposition of wall material during further growth. The reticulate pattern of sieve area may also result from modification of more than one closely arranged primary pit-fields.

Esau (1969) attempted to establish the relation between the number of sieve areas, length of sieve plate and diameter of the pores in cellulosic part of the wall and diameter of the opening within the callose. Fotedar (1976) calculated the percentage of area of sieve cell wall occupied by the sieve areas in *Dicranopteris dichotoma* and noted that the percentage of lateral wall area occupied by sieve areas and its frequency in narrow and wide sieve cells are not much different. Our present analysis shows that the diameter of pores on a sieve area depends upon the number of pores on that particular sieve area. They show a mathematical relation $Y = 1.36149 + 0.08063148x + 0.00207639x^2$ ($Y = a + bx + cx^2$) where a, b, c are positive constants, x denotes the number of pores and y the diameter of the pores. It was found on checking that if x is increased in one unit the corresponding decrease in y is 0.4554 unit. In other words, when the number of pores on a sieve area increases by 1% there is a corresponding decrease in the diameter of the pore by 0.4554%. A detailed analysis of such data from species belonging to different groups is needed to understand the significance of such mathematical relation in biological systems.

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