Phloem in Pinophyta

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The gymnospermous phloem shows major differences from those of the cryptogams on one hand and the angiosperms on the other. These include both the type of conducting cells as well as the cellular composition. Even more important is the degree and nature of functional inter-relationship between the conducting and parenchyma cells. The following evolutionary trends have been suggested:

Increase in the amount of axial parenchyma;
Decrease in the number of albuminous cells in the rays;
Increase in the axial albuminous cells;
Increase in the fibres;
Increase in the regular arrangement of cells.

The work on the fossil taxa does provide variable support to these suggestions. It is generally believed that the cryptogamic sieve element arose from a parenchyma cell and all the phloem produced in the fossil lycopods, Sphenopsida and ferns is primary in origin. Here the phloem consists of either sieve elements only or the sieve elements with scattered parenchyma cells. There is no definite relationship between the conducting elements and the parenchyma cells as seen in the seed plants. Additional parenchyma is often present in the form of a sheath separating the xylem from the narrow phloem tissue.

The typical cryptogamic sieve elements are identical to the elongate parenchyma cells. These are relatively small in diameter, longer than the parenchyma cells but shorter than the gymnospermous sieve cells. In some taxa, the sieve elements are of two sizes: large (up to 600 μm) and small (between 100-1,500 μm). The end walls of the cryptogamous sieve elements are horizontal or slightly oblique and the sieve areas are small and vary markedly in their outline. The sieve pores occur in the sieve areas as well as scattered on the vertical walls. The callose deposition has been found in *Psilotum*, lycopods and the ferns.

Key-words—Evolution, Phloem, Pinophyta, Gymnosperms.

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सारॉंश

पाइनोफ़ाइटी पौधों में पोषवाह

जी० एस० पालीवाल

क्रिप्टोगैमी एव आवृतबीजी पौधों में पाये जाने वाले पोषवाह तथा अनावृतबीजी पौधों के पोषवाह में आपस में असमानताएं हैं। अनावृतबीजीयों में दोनों प्रकार की सवहनी कोशायें एवं कोशिकीय सरचनायें पाई जाती हैं। इनमें निम्नलिखित विकासीय प्रवृत्तियाँ प्रस्तावित की गई हैं:

–अक्षीय मृदूतक में वृद्धि
–किरणो में एल्ब्यूमॅन कोशाओं की संख्या मे कमी
–अक्षीय एल्ब्यूमॅन कोशाओं में वृद्धि
–तन्तुओ में वृद्धि
–कोशाओ के नियमित विन्यास में वृद्धि

अश्मित वर्गको के अन्वेषण मे उक्त प्रस्तावो को विभिन्न प्रकार से समर्थन मिला है। सामान्यत: ऐसा विश्वास है कि क्रिप्टोगैमी छलनी अवयवों का मृदूतक से विकास हुआ है तथा अश्मित लाइकोपोडीयों, स्फीनॉप्सिडो एवं फर्नो में पोषवाह उत्पत्ति की दृष्टि से प्राथमिक है। परन्तु इन पौधों में पोषवाह या तो छलनी अवयव है अथवा छलनी अवयव बिखरी मृदूतक कोशाओं से युक्त हैं। सवहनी अवयवों एवं मृदूतक कोशाओं मे आपस में कोई सम्बन्ध नहीं है। सामान्य क्रिप्टोगैमी छलनी अवयव अनावृतबीजी छलनी कोशाओं से आकार में छोटे हैं। कुछ वर्गकों में छलनी अवयव दो आकारों में मिलते हैं–बड़ा (600 माईक्रॉन तक) तथा छोटा (100 से 1,500 माइक्रॉन तक)। छलनी छिद्र छलनी क्षेत्रो में एवं लम्बवत भित्तियों पर बिखरे-बिखरे पाये जाते हैं। **साइलोटम्**, लाइकोपोड्स एव फर्नो में केलस निक्षेपण प्रेक्षित किया गया है।

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THE sieve elements in gymnosperms have been investigated since the early part of the 18th century. The studies chiefly concern secondary phloem, mostly of Pinatae. Recently, Gnetatae has also been worked out in fairly good details. Kollmann (1964, 1968), Esau (1969), Behnke (1971) and Parthasarathy (1975) have reviewed the phloem structure and ontogeny. Behnke (1974) has provided a survey of the plastid types in the sieve cells of gymnosperms.

In the present article an attempt has been made to bring together the present state of available information on the structure of this complex tissue. It would be seen that more and more investigators have concentrated their attention on the Pinicae (taking both the stem and the needle), and despite its importance as a distinct line of the seed plants, only limited research has been done on the Cycadicae and Gneticae (where the leaves have been the chief object of analysis), although a beginning had been made by Mettenius as early as 1861.

CYCADICAE *

The phloem of cycads is rather poorly known. The genera investigated are listed in Table 1. Available data are based mainly on two species—*Cycas circinalis* Strasburger 1891 and *Microcycas calocoma* Chrysler 1926.

The axial system of the secondary system contains sieve elements, parenchyma cells, and fibres. The sieve elements are relatively long with inclined end walls, and their faces merge with the radial lateral walls. The sieve areas are variable in shape and occur on end walls, and the radial faces of lateral walls.

Parenchyma cells are of two kinds—those containing starch, and sometimes druses of calcium oxalate, and the albuminous cells. In the non-functioning phloem, the starch-containing cells remain intact, and the albuminous cells collapse. Chrysler (1926) also noted pits in the walls between adjacent parenchyma cells and believed them to be identical in shape and distribution to the sieve areas in the sieve elements.

Apparently, in the cycads the primary phloem of the leaf is devoid of fibres. In the older petioles and stems, the proto-phloem is crushed. Metaphloem tissue contains wide sieve elements and narrower parenchyma cells. Parenchyma also separates the tracheids from the sieve elements.

Within the transfusion tissue located on the

Table 1—Phloem in Pinophyta (Gymnosperms)

TAXON/GROUP	SPECIES OR GROUP INVESTIGATED	AUTHORS	OPTICAL	EM	YEAR
A. Cycadicae	*Medulossa* sp.	Smoot, E. L.	+	-	1984
	Cycas circinalis	Strasburger, E.	+	-	1891
	Cycas revoluta	Mettenius, G.	+	-	1861
	Dioon edule	Dippel, L.	+	-	1869
	Microcycas calocoma	Chrysler, M. A.	-	+	1926
	Zamia	Parthasarathy, M. V.	-	+	1975
B. Pinicae					
1. Ginkgoatae	*Ginkgo biloba*	Bertrand, C. E.	+	-	1874
		Seward, A. C. & Gowan, J.	+	-	1900
		Chauvèaud, G.	+	-	1903
		Sprecher, A.	+	-	1907
		Gunckel, J. E. & Wetmore, R. H.	+	-	1946
		Srivastava, L. M.	+	-	1963
		Dute, R. R.	+	+	1983
2. Pinaceae	*Abies conocolor*	Wilson, B. F.			1963
	A. pindrow	Chauveaud, G.	+	-	1902b
	A. procera	Wilcox, H.	+	-	1954
	A. sachalinensis	Shimaji, K.	+	-	1964
	Abies sp.	Grillos & Smith	+	-	1959
	Dacrydium sp.	Ilese, W. & Matte, V.			1962
	Larix laricina	Knudson, L.	+	-	1913
	Picea abies	Timell, T. E.	-	+	1973
	Picea mariana	Brüdarmann, G. & Koran, Z.		-	1973

*Terminology after Cronquist *et al.* (1966).

Contd.

Table 1—Contd.

TAXON/GROUP	SPECIES OR GROUP INVESTIGATED	AUTHORS	OPTICAL	EM	YEAR
	Pinus nigra	Sauter, T T., Dörr, I. & Kollmann, R.	–	+	1976
	P. pinea	Wooding, F. B. P.	–	+	1966
	P. pinea	Wooding, F. B. P.	–	+	1968
	P. pinaster	Carde, J. P.	+	+	1973, 1974
	P. radiata	Mahmood, A.			1965
	P. radiata	Barnett, J. R.	–	+	1971a
	P. radiata	Singh, A. P.			1984a, b
	P. radiata	Barnett, J. R.	+	–	1974
	P. resinosa	Campbell, R.	–	+C	1972
	P. resinosa	Neuberger, D. S.	–	+	1973
	P. resinosa	Neuberger, D. S. & Evert, R. F.	–	+	1974, 1975
	P. resinosa	Neuberger, D. S. & Evert, R. F.	–	+	1976
	P. rigida	Brown, H. P.	+	–C	1912
	P. sabiniana	Alosi, M. C. & Park, R. B.			1983
	P. strobus	Brown, H. P.	+	–	1915
	P. strobus	Abbe, L. B. & Crafts, A. S.	–	+	1939
	P. strobus	Evert, R. F. & Alfieri, F. J.	+	–	1965
	P. strobus	Murmanis, L. & Evert, R. F.	–	+	1966
	P. strobus	Srivastava, L. M. & O'Brien, T. P.	–	+	1966b
	Pinus strobus	Murmanis, L. & Evert, R. F.	–	+	1967
	P. strobus	Srivastava, L. M.	–	+	1969
	P. strobus	Murmanis, L.	–	+	1970, 1972
	P. strobus	Chafe, S. C. & Doohan, M. E.	–	+	1972
	P. strobus	Murmanis, L.	–	+	1974
	P. sylvestris	Hill, A. W.	+	+	1901
	P. sylvestris	Parameswaran, N. & Ilese, W.	–	+	1970
	P. sylvestris	Parameswaran, N.	–	+	1971
		Preston, R. D.	+	–	1963
		Thaine, R. M. C.	+	+	1964
		Parker, B. C.	+	–	1965
	P. sylvestris	Warmbrodt, R. D. & Eschrich, W.	–	–m	1985
	Pinus sp.	Wooding, F. B. P. & Northcote, D. H.	–	+	1965b
	Pinus sp.	Alfieri, F. J. & Evert, R. F.	+	–	1968a, b
	Pinus sp.	Mahmood, A.			1968
	Pinus sp.	Schulz, A., Alosi, A. C., Sabnis, D. B. & Park, R. B.	–	–C	1989
	Pseudotsuga taxifolia	Sterling, C.	+	–	1947
	P. taxifolia	Braun, H. J. & Den Outer, R. W.	+	–	1965
	P. menziesii	Gourret, T. P. & Strullu, D. G.	+	+	1974
	Abietineae	Chrysler, M. A.	+	–	1913
	Pinaceae	Barghoorn, E. S.	+	–	1941
	Pinaceae m-mycorriza	Mühlethaler, K.	–	+	1950
	Pinaceae	Roelofsen, P.	+	–	1959, 1965
	Pinaceae	Srivastava, L. M.	+	–	1963

Contd.

Table 1—Contd.

TAXON/GROUP	SPECIES OR GROUP INVESTIGATED	AUTHORS	OPTICAL	EM	YEAR
	Pinaceae	Harris, W. M.	-	+	1972
3. Taxodiaceae	*Metasequoia glyptostroboides*	Kollmann, R. & Schumacher, W.	-	+	1961
	M. glyptostroboides	Kollmann, R. & Schumacher, W.	-	+	1962a
	M. glyptostroboides	Kollmann, R. & Schumacher, W.	-	+	1962b
	M. glyptostroboides	Kollmann, R. & Schumacher, W.	-	+	1963
	M. glyptostroboides	Bocher, T. W.	+	-	1964
	M. glyptostroboides	Kollmann, R. & Schumacher, W.	-	+	1964
	M. glyptostroboides	Kollmann, R.	-	+	1965
	M. glyptostroboides	Willenbrink, J. & Kollmann, R.			1966
	M. glyptostroboides	Kollmann, R.			1967
	Metasequoia sp.	Schumacher, W.	+	-	1967
	M. glyptostroboides	Hébant, C.	-	-C	1975
	Sequoiadendron giganteum	Hébant, C.	-	-C	1975
	Sequoia sempervirens	Isenberg, I.	+	-	1943
	S. sempervirens	Sterling, C.	+	-	1946
4. Cupressaceae	*Callitris* sp.	Bamber, R. K.	+	-	1959
	Chamaecyparis obtusa	Pesacreta, T. C. & Parthasarathy, M. V.	+	+	1986
	Juniperus communis	Kollmann, R. & Dorr, I.	-	+	1966
	Thuja orientalis	Chauveaud, G.	+	-	1902
	Pinicae	Abbe, L. B. & Crafts, A. S.	+	-	1939
		Huber, B. & Graf, E.	+	-	1955
Gneticae	*Ephedra*	Thompson, W. P.	+	-	1912
		Hepton, C. E. L. & Preston, R. D.	+	-	1960
	E. americana	Alosi, Margaret, C. & Alfieri, F. J.	+	-	1972
	E. californica	Alfieri, F. J. & Mottola, P. M.	+		1983
	Welwitschia bainesii	Evert *et al.*	-	+	1973
	Gnetaceae	Bertrand, E. E.	+	-	1874
	Gnetaceae	Boodle, L. A. & Worsdell, W. C.	+	-	1894
	Gnetaceae	Strasburger, E.	+	-	1891
	Gnetum and angiosperms	Thompson, W. P.	+	-	1919
	Gnetum	Maheshwari, P. & Vasil, Vimla	+	-	1961
	Gnetum gnemon and *Ephedra campylopoda*	Behnke, H. D. & Paliwal, G. S.	-	+	1973
	Gnetum gnemon	Paliwal, G. S. & Behnke, H. D.	+	-	1973
	PINOPHYTA	Bertrand, C. E.	+	-	1874
		Moeller, J.	+	-	1882
		Feustel, H.	+	-	1921
		Holdheide, W.	+	-	1951
		Chang, Y. P.	+	-	1954a, b
		Behnke, H. D.	-	+	1974

C=Chemical analysis; **m**=Mycorriza.

flanks of the bundle, the parenchyma cells next to the phloem are rich in cytoplasmic contents and have rather large nuclei. Strasburger (1891) had suggested that these cells serve as intermediate cells comparable to the albuminous cells.

The ultrastructure of sieve elements of *Zamia*

pseudoparasitica reveals that the ontogeny is identical to that of the Pinaceae (Parthasarthy, 1975). However, unlike the necrotic nuclei reported by various authors, here the nuclei have been observed at various stages of degeneration. P-protein is lacking, as in other gymnosperms. The other cell organelles also exhibit a similar behaviour during differentiation. Aggregates of ER that normally occur on both sides of sieve areas in the mature sieve cells and appear to be interconnected by elements of ER traversing the sieve pores, have also been noticed.

GINKGOATAE

Bertrand (1874) was the first to examine the secondary phloem of *Ginkgo biloba, Salisburia adjantifolia* in which he found the sieve elements (cellules grillagées) to be wider as compared to those of conifers. He distinguished fibres, parenchyma cells, and sieve elements in the phloem of stems and noticed the enlargement of parenchyma cells in the older phloem. Strasburger (1891) discussed certain features of the secondary phloem, especially the albuminous cells in this taxon.

Although comparatively less homogeneous in appearance, the precursory phloem elements in *Ginkgo* are similar to those of the Pinatae. The sieve elements differentiating at a later stage have rather distinct sieve areas. Srivastava (1963a), too found the secondary phloem to be similar to that of Pinatae in being composed of sieve elements, parenchyma strands, and fibres in the axial system and rays in the radial system. He, however, did not confirm the presence of fibres in the secondary phloem of short shoots which had abundant druses as reported by Seward and Gowan (1900), Sprecher (1907), and Gunckel and Wetmore (1946).

The albuminous cells associated with the sieve cells lack starch, and get crushed in the old phloem. These are connected to the sieve cells by one-sided sieve areas. As callose was not present in sufficient quantity, lateral connections between sieve elements and albuminous cells remained untraced. These cells also have plastids but do not store normal, detectable starch.

The fibres are elongated, tapering elements that are flattened tangentially. These are non-septate as observed in the macerated tissue. They have a narrow lumen and thick, distinctly lamellated walls which appear to be composed of cellulose. These do not give positive staining with phloroglucinol and HCl and are strongly birefringent under polarized light.

PINACEAE

Srivastava (1963b) presented a comprehensive study of Pinaceae with regard to different cell types, their distribution, origin of the tissue component in the cambium, and the problem of proper interpretation of the albuminous cells covering 13 species. Evert and Alfieri (1965) studied five species of Pinaceae.

The first detectable change in the cambial derivative, destined to become the sieve cell is the marked increased in cell volume, accompanied primarily by radial expansion. Soon afterwards, it begins to deposit secondary wall thickening which is characteristic to this family and is lamellar in appearance. Older cells have more lamellae in their walls than the younger ones, a fact which indicates that the deposition of the wall material continues for sometime. Throughout these changes, the cytoplasm retains the usual compliments of organelles. Rough ER is evident in the form of cisternae and vesicles and the ground substance is rich in free ribosomes. In some instances, fine fibrillar material can be detected in the vesicles of the rough ER. Mitochondria and dictyosomes are abundant and the cortex of the cell contains microtubules which are usually oriented parallel to the long axis of cell.

The plastids also undergo changes. These accumulate a few electron dense bodies in the stroma, some of which reach a size of 1-2 μm in older sieve lements (Srivastava & O'Brien, 1966; Barnett, 1974). These crystals are thought to be proteinaceous and show weak refrigence under polarized light. Their origin and significance is not known but these may have developed from the osmiophilic intralamellar inclusions in the plastids of cambial initials. Similar inclusions have been seen in the sieve elements of certain dicotyledons also, e.g., *Datura* (Hohl, 1960), *Pisum* (Bouck & Cronshaw, 1965), and *Avena* (O'Brien & Thimann, 1967); although in the last two instances it has not been shown that these bodies have been derived from plastids. It is to be noted here that Wooding (1966) has described in ray plastids the accumulation of granules, comparable in staining to the starch grains.

The second stage of differentiation involves drastic changes in the organelles and membrane systems of the cell. The cisternae of rough ER vesiculate further, lose their ribosomes, and then the vesicles disappear. Simultaneously, a new reticulum is formed, composed of large cisternae which in several instances occur close to the wall. Bouck and Cronshaw (1965) refer it as the 'sieve tube reticulum' (STR), since these also occur in the sieve

cells, whereas Srivastava and O'Brien (1966) have termed it as sieve element reticulum (SER). During these changes in the ER, ribosomes and dictyosomes become infrequent and are no longer detected in the cytoplasm.

Evert and Alfieri (1965) have reported the presence of necrotic nuclei in the mature sieve cells. Muramanis and Evert (1966), Srivastava and O'Brien (1966), Wooding (1966, 1968) and Neuberger (1973) have identified comparable stages of nuclei in the mature sieve elements. In the elements immediately adjacent to the crushed phloem, the vesicular masses, nuclear remnants, and mitochondria associated with longitudinally stranded material persist along the wall, and the lumen of sieve elements appear completely empty. The inclusions of the sieve element plastids disappear by the time it is mature, and they contain only granules resembling starch in their staining behaviour (*Pinus pinea*; Wooding, 1966, Wooding & Northcote, 1965b). In *Pinus radiata* and *P. strobus* the crystalline inclusions change to a fibrillar form.

The mature sieve elements of pine have a thick, lamellated secondary wall, separated from the cytoplasm by a persistent plasmalemma. The peripheral cytoplasm is composed of an elaborate system of smooth ER in the form of long cisternae. Somewhat abnormal mitochondria are often enmeshed between these cisternae. Plastids are present in various stages of breakdown, alongwith the starch grains and protein crystalloids. The ground substance is composed of a fine fibrillar material, derived either from the stroma of degenerated plastids, from the rough ER, or both. Degenerating nuclei are mostly present, but the vacuolar membranes, dictyosomes, and ribosomes are absent. Microtubules may be present at maturity but they are difficult to distinguish with certainty. Plasmalemma seems to be maintained even in the advanced stages of sieve element differentiation, but the initial, three-layered structure is ruled out at this stage (Srivastava & O'Brien, 1966). Barnett (1974) has recorded stacks of hexagonally packed tubules in the lumen of sieve elements of *P. radiata*.

The earliest detectable change in the initiation of the sieve area has been noticed soon after the cell wall formation has begun. The formation of sieve pores, i.e., whether the sieve areas arise from the pit fields in the fusiform initials from which the sieve cells have been derived or whether their origin is *de novo*, needs explaining. Srivastava and O'Brien (1966) explained that some boring of the wall occurs, because a cavitymedian nodule is formed where none existed before. Furthermore, strands from the two sides may join after following a path. The 'pores' in the walls of dead sieve elements are certainly much wider than the plasmodesmata. Barnett (1974) has critically analysed this aspect of the sieve cells of *Pinus radiata*. He did not record any simple pits in the radial walls of the cambium. Gradually, the wall thickness increases in these regions, although these are devoid of the lamellar appearance like the rest of the walls. In an almost mature sieve area, the difference in the structure between the cell wall and the material of which the cell plate is made is more obvious. Usually a sieve plate is homogeneous, staining evenly, but the remaining cell wall is lamellar in structure. This is the stage when communication channels begin to develop between the cells. Later these widen until these have become large enough for entire organelles to pass through them. The pores in the wall are lined by plasmalemma (Srivastava & O'Brien, 1966), but Wooding (1966) observed them to be lined by callose as revealed by fluorescence microscopy.

Callose—Some authors believe that the callose is scanty or lacking in the functional sieve element and that the relatively large amount of callose seen in the sieve element is an artefact of fixation and sectioning (Zimmermann, 1960; Craft & Currier, 1963; Eschrich, 1963; Evert & Derr, 1964). Others, though not denying the earlier postulate yet, maintain that callose does occur naturally and lines the sieve pores (Esau & Cheadle, 1965; Esau *et al.*, 1962; Engleman, 1966a; Srivastava & O'Brien, 1966).

Cell wall—Srivastava (1969) advanced a somewhat unorthodox concept of microfibrillar orientation for the sieve cell walls of white pine. He envisaged a wall composed of lamellae in which microfibrils are aligned at an angle to or at both the horizontal and vertical axis of the cell. This suggests that not only are the microfibrils subtending the horizontal axis at an angle as seen in the surface (tangential) view, but also at an angle to the vertical axis when the wall is viewed in sectional planes.

Chafe and Doohan (1972) demonstrated that, contrary to Srivastava's (1969) concept, the microfibrils are always oriented parallel to the cell wall and they describe 'S' or 'Z' helices around the cell wall. Observations with polarized light suggest that the orientation of most microfibrils is higher than 45° with respect to the cell axis. Oblique sections through the cell wall indicate an orientation of moderately low helical pitch perhaps 50-60° or more from the cell axis. Therefore, these authors held that the observations of Srivastava (1969) may be due to oblique sectioning of the walls.

The microtubules play an important role in the regulation of wall synthesis among the angiospermous taxa (Ledbetter & Porter, 1963; Hepler & Newcomb, 1964; Cronshaw, 1965; Cronshaw & Bouck, 1965). Srivastava (1969) observed microtubules in the sieve cell cortices and their persistence in the later stages of differentiation in *Pinus strobus* also. This led him to conclude that these may serve more than one function in the pines. Wooding (1966) and Barnett (1974), however, could not confirm their association with the process of cell wall formation. On the other hand, in the initial stages of development of the cell, large number of golgi bodies were found to be present together with the microtubules close to the plasmalemma.

Plastids—The sieve cell plastids are among the first organelles to indicate structural changes during cell differentiation. Crystalloids, starch granules, filamentous material or osmiophilic globules may be formed in the plastids depending upon the species. For example, all the four types of inclusions may be found in the sieve cell plastids of *Pinus* species (Srivastava & O'Brien, 1966; Murmanis & Evert, 1966; Wooding, 1966; Parameswaran & Liese, 1970; Parameswaran, 1971; Barnett, 1974; Sauter, 1974) and *Picea abies* (Timell, 1973). As the differentiation of the sieve elements begins, the plastids undergo interesting changes. They accumulate a few electron dense bodies in the stroma. Some of these reach a size of 1-2 μm in the older sieve elements. These have been referred to as 'crystalloids'. It has been suggested that they may arise from the osmiophilic intralamellar inclusions present in the plastids of cambial initials (Srivastava & O'Brien, 1966). Similar structures have been seen in the sieve elements of *Datura* (Hohl, 1960) and *Avena* (O'Brien & Thimann, 1967). Recently, Behnke (1974) has reviewed the organization of sieve element plastids of gymnosperms and concludes that all the Pinaceae examined possess p-type plastids, characterized by a peripheral, ring-shaped bundle of protein filament, an additional protein crystalloid and several starch grains. The latter are mostly club-shaped.

P-protein—Structures referable as 'slime' or 'P-protein' have been observed in the sieve elements of different species of Pinaceae (Srivastava, 1963; Evert & Alfieri, 1965). Abbe and Crafts (1939) commented on their 'transitory' nature. Observations of sieve elements with electron microscope have produced conflicting results with regard to P-protein in *Pinus*. However, Srivastava and O'Brien (1966) and Barnett (1974) documented the origin of this fibrillar protein from the plastid matrix and doubted its resemblance to the slime. Neuberger (1973) and later Neuberger and Evert (1974) indicated that P-protein is absent in *Pinus*. Interestingly enough, Thaine (1964) had reported discrete strands running in the sieve elements, between the sieve pores in which material is translocated.

Parenchyma Cells—It has been pointed out that the organelle component of the parenchyma cells and the other cells are similar. Srivastava and O'Brien (1977) found the cytoplasm of the albuminous cells of *Pinus strobus* to be rich in mitochondria and rough ER. Traditionally, the companion cells of the angiosperms have been implicated as a likely centre of energy supply for at least some of the processes involved in the transport of solutes.

TAXODIACEAE

Moeller (1882) described the arrangement, types of crystals, and their distribution in a few species, such as *Cunninghamia sinensis, Podocarpus thunbergii, Sequoia gigantea, Taxodium distichum* and *Taxus baccata*. Strasburger (1891) reported the presence of albuminous cells in Taxodiaceae. Holdheide (1951) gave a description of the phloem of *Taxus baccata*.

Sieve cell protoplast and sieve plate differentiation

Kollmann and Schumacher (1964) observed in *Metasequoia* a remarkable though temporary, increase and expansion of the endoplasmic reticulum throughout the cell lumen of the sieve elements during development. Plastids also begin differentiation simultaneously.

Nuclear disintegration in *Metasequoia* sieve cells appears to be preceded by a coarsening of the internal structure of the swollen nucleus which is plainly visible under the electron microscope. The nuclear substance is particularly conspicuous (Kollmann & Schumacher, 1961). Later, there is a general loosening of internal structure of the nucleus accompanied by remarkable changes in the nuclear envelope (Kollmann, 1961a). The endoplasmic reticulum may play a part in the subsequent resorption of the nuclear substance (Kollmann, 1960). The nucleus in *Metasequoia* (though of a changed shape) is seen in sieve elements near the cambium with well-developed plasmatic connections (Kollmann,1961a; Kollmann & Schumacher, 1961). This is in contrast to the reports in *Cucurbita maxima*, where the nuclei disintegrate in the differentiating sieve elements before the sieve pores develop (Esau *et al.*, 1962).

Sieve areas in presumably active sieve cells have an extensive median nodule which is about 3 μm in diameter and is represented by a lens-shaped cavity in the region of the middle lamella and the primary walls of the sieve area containing dense cytoplasmic structures. From this median nodule, many protoplasmic strands, about 50-80 (max. 350) μm in diameter, pass to the protoplast of both adjacent sieve cells.

Sieve areas in the sieve cells are localized mainly on the radial tapering end walls, each measuring 4-5 square micron and containing about 7 sieve pores per square micron. The diameter of the sieve pores vary between 50-500 μm. Connecting strands of about 270 μm are observed with sufficient frequency (Kollmann & Schumacher, 1962).

A comparative study of the fine structure of cytoplasmic strands in the sieve areas and pit-fields give best support to the common concept that the connecting strands are highly differentiated plasmodesmata. Kollmann (1964) postulated that the sieve elements should be considered as functional from the moment the differentiated connecting strands join the adjacent protoplast, which are established in the very early stage of sieve element development. In that case all the changes which take place later in the cytoplasmic fine structure and which ultimately lead to the degeneration of the sieve cell, might be considered not to be a prerequisite to, but rather a result of the physiological functions peculiar to the conducting element.

Well-differentiated plastids occur in sieve cells whose protoplast is still intact. These have an internal matrix with numerous vesicles, occasional electron dense tubules and a few stroma membranes which are continuous with the layer of limiting membrane. During spring and summer, the plastids form many starch grains within the stroma. In the dormant phloem, the starch is only rarely found but in later stages the internal structure of the plastid is greatly reduced and many starch grains are stored. Finally when the sieve element has become fully differentiated, the plastids have poorly organized internal matrices and are almost completely filled with starch grains of different shapes and sizes. Their matrices become more and more electron transparent as cell differentiation progresses. Behnke (1974) reports only S-type plastids in the sieve elements of other members of Taxodiaceae, *viz., Cryptomeria japonica, Sequoiadendron giganteum* and *Taxodium distichum.*

P-protein is absent in the sieve elements of *Metasequoia glyptostroboides* (Kollmann, 1964). Scanty information is available on the parenchyma cells of Taxodiaceae. Although albuminous cells are present in the phloem, not all of them are connected with the sieve cells (Schumacher, 1967).

CUPRESSACEAE

Differentiation of the sieve cells is similar to that of the Pinaceae although in contrast to the latter the sieve elements do not have secondary walls but possess numerous small crystals in the middle lamella of the radial walls of all the cell types. Such crystals are absent at the sieve areas and the primary pit fields (Evert & Alfieri, 1965). Perhaps the crystals encountered in this study are similar to the granules, Chang (1954a, b) had reported for this plant. Slime bodies were described in young sieve elements. Internal strands traversing from one cell to another, through the sieve area pores, have also been seen in both fresh and PAA-fixed tissue. Srivastava (1963) interpreted these as 'slime'. The plastids (Behnke, 1974) of *Calocedrus decurrens, Chamaecyparis nootkatensis, Juniperus fragrans,* and *Thuja plicata* are of the P-type.

GNETICAE

Maheshwari and Vasil (1961) compiled information on the phloem of *Gnetum.* During the past few years the phloem of this taxon together with the other two genera has been investigated both with the light as well as electron microscopes.

EPHEDRACEAE

The secondary phloem of the genus *Ephedra* comprises sieve elements, two kinds of axial parenchyma cells, and multiseriate parenchymatic rays, reported to be absent in Gnetaceae. The older phloem contains fibre-sclereids as a real cell type. Sieve elements bear sieve areas on the radial walls and on the walls common with the albuminous cells. Within the sieve area, pores are generally combined in groups; the latter have been termed as sieve fields (Russow, 1882; Strasburger, 1887; Hill 1901; Evert & Alfieri, 1965). Each pore, whether in a lateral sieve or on the overlapping end wall, measures approximately 0.8 μm. Sieve areas have not been recorded on the transverse walls between sister cells, whether they be two sieve cells or a sieve cell and an albuminous cell (Alosi & Alfieri, 1972). Callose occurs as only a narrow layer of the sieve elements. The starch-containing-parenchyma cells may maintain their integrity or develop secondary walls and become fibre-sclereids. Strasburger (1891) stated that albuminous cells are found in the axial

system and form continuous files or are dispersed in the same row with starch-containing cells. These are generally half the length of sieve cells (Alosi & Alfieri, 1971).

Ray cells accumulate calcium oxalate in the middle lamella in the form of rod-shaped crystals, similar to the crystals seen in the phloem cell walls of *Juniperus* (Evert & Alfieri, 1965).

Ontogeny of the secondary sieve element

The ontogeny of pholem in *Ephedra californica* and *E. viridis* has been observed by Alosi and Alfieri (1972). Behnke and Paliwal (1973) have recorded some features of the sieve cells and parenchyma cells of *E. campylopoda*.

Phloem initials look very similar to the cambial initials from which these have been derived. They possess long, thin, granular nuclei with several nucleoli, delicate cell walls with primary pit fields, small radial diameter, very oblique end walls, and lightly staining cellular contents. However, an ovoid body appears in the differentiating cell. It is often located near the nucleus and resembles in shape and staining the largest nucleolus observed. Some of the fusiform phloem derivatives undergo a transverse division before developing into shorter sieve cells (Alosi & Alfieri, 1973). These divisions are similar to those described by Esau and Cheadle (1955) and Zahur (1959) in some dicotyledonous taxa. This shortening of sieve elements has been considered as an advanced character.

The nucleus flattens and in some cells appears to curl around the periphery of the cell in a bracelet-like fashion. It may be irregular in outline, but maintains its granular character and chromaticity. No evidence of the degeneration of the nuclear membrane has been observed. Necrotic nuclei, previously described for Pinatae, are also frequent in the mature sieve cells of *Ephedra*.

Smaller and less distinct slime bodies have been recorded to appear later than the larger slime bodies (Alosi & Alfieri, 1972). These authors further inferred that the smaller slime bodies anastomose and then disperse. The dispersed slime may become deposited at the site of the future sieve area. Finally, it forms a thin parietal film and may become collected near the differentiating sieve areas.

Differentiation of the sieve area begins very early and the progress is rapid in developing sieve elements, as in *Cucurbita* (Frey-Wyssling & Mühlethaler, 1957; Buvat, 1963). The time of complete perforation of sieve areas could not be discerned but it is complete at the time the element becomes functional (Alosi & Alfieri, 1972; see also Evert *et al.*, 1970).

Mature sieve elements have blunt to tapering ends. Their walls remain thin with no nacreous or secondary thickening. The sieve elements fall into two categories based on their length: those about 400 μm long and other measuring only 220 μm. The former are the direct derivatives of the fusiform initials whereas the latter arise after a transverse division in their precursors. Alosi and Alfieri (1972) have described the stranded and thread-like composition of the slime-body and have shown intracellular sieve area connections through these thread-like structures. However, this information needs further confirmation, especially because the occurrence of P-protein has been contradicted by Behnke and Paliwal (1973).

Albuminous cells have connections with the small sieve areas of adjoining cells. An ovoid body, about 3 μm in diameter appears just after transverse divisions of the phloem mother cells and lies near the newly constituted daughter nucleus. Because of its positive reaction with Ponceau S, this body has been referred to as slime-body as in the angiospermous sieve elements. Ultrastructural studies by Behnke and Paliwal (1973), however, failed to confirm this inference. The albuminous cells have dense protoplasm, rich in elongated mitochondria, plastids, granular ER, free ribosomes and a prominent nucleus, and are connected to the sieve cells by branched plasmodesmata on their side, fusing with a sieve pore on the sieve cell side.

The two daughter cells formed after a transverse division in the sieve element precursor may not necessarily differentiate into sieve elements, but one of these may differentiate into the sieve cell whereas the other may form the parenchymatous albuminous cell (Alosi & Alfieri, 1972). Cells that appear to be transitional between sieve cells and albuminous cells have been observed in *Ephedra*. The fact that an albuminous cell of *Ephedra* is formed directly from a single division of a fusiform mother cell distinguishes it from the albuminous cells of most other gymnosperms and correlates the two groups with specialized parenchyma cells, including companion cells of higher plants.

WELWITSCHIACEAE

Strasburger (1891) found the vascular bundles in the leaf of *Welwitschia mirabilis* to be similar to those in *Gnetum*, with the sieve elements somewhat narrower and appearing to have swollen walls.

Electron microscopic observations indicate that most of the xylem and phloem elements in *Welwitschia* are arranged in the radial rows or files in both primary as well as the secondary phloem

(Evert *et al.*, 1973). Two types of cells have been described in the phloem of this taxon, sieve cells and parenchyma cells. The structure of the former is similar to that of *Gnetum* and *Ephedra*. In a manner similar to what has been observed in Pinatae and *Gnetum*, the plastids in *Welwitschia* sieve cells also undergo remarkable changes such as the formation of starch grains, proliferation of internal membranes, and decrease in the density of the matrix. Osmiophilic globules continue to be present in the plastids but disappear from the cytoplasm. These plastids differ from those of *Pinus* in that they lack crystalline inclusions and fibrillar material at all stages of differentiation. The starch grains in them are also club-shaped like those in *Gnetum*.

In contrast to the spatial relationship between pyconotic nucleus and endoplasmic reticulum reported in some angiosperms and pteridophytes (Bouck & Cronshaw, 1965; Northcote & Wooding, 1966; Liberman-Maxe, 1966; Esau & Gill, 1971; Esau, 1972), *Pinus strobus* (Murmanis & Evert, 1966) and *Gnetum gnemon* (Behnke & Paliwal, 1973), only a resemblance of this has been reported between the nuclear envelope and mitochondria in *Welwitschia mirabilis* (Evert *et al.*, 1973). Portions of the inner membrane of the nuclear coat develop protrusions into the nuclear matrix, so that in some profiles part of the nucleus appears to be perforated.

Sieve cells of *Welwitschia* have much in common with those of Pinatae. In addition to nucleus, mitochondria and ER, the mature, plasmalemma lined sieve cell contains plastids with starch granules. In contrast to plastids of immature sieve cells, those of mature sieve cells lack internal membranes and their matrices are hyaline (very clear) in appearance. The mitochondria undergo no apparent structural modification. The sieve cells lack ribosomes, dictyosomes, and microtubules. The vacuoles of young sieve cells and lumen of mature ones sometimes contain a coarse fibrous substance, similar in appearance to that found in the vacuoles of parenchymatous cells of the leaf. The nature of this substance has been determined but it should not be confused with slime or P-protein which is totally absent. During maturation the tonoplast, which delimits the vacuolar contents from the cytoplasm in young cells, ceases to be identifiable and the sieve cell then appears to contain one large central cavity.

GNETACEAE

The first information on the phloem tissue in this group is perhaps that of Strasburger (1891) Here the phloem contains sieve elements with sieve areas on the lateral walls and thus the adjacent parenchyma cells (usually referred to as 'companion cells') are in direct association with the former, occasionally the laticifers (which occur in some species of *Gnetum*, e.g., *G. gnemon* and are in close association with the sieve elements) and the fibre sclereids invariably develop secondary walls after the sieve elements cease to function. In the early secondary phloem the sieve elements tend to form homogeneous radial files alternating with radial rows of parenchyma cells. Thompson (1919) depicted such a tissue at a level where parenchyma cells appear narrow and occupy the corners delimited by four sieve cells. Although, he considered that the sieve cells, and the parenchyma cells are not sister cells, he chose to call the latter as companion cells. The recent study of Paliwal and Behnke (1973), however, indicates that although ontogenetically unrelated these are placid close to each other. Moreover, they could demonstrate that the sieve cells and phloem parenchyma cells do not originate from a common mother cell by radial divisions, as is true for angiosperms. Evidently, therefore, the two types of cells have an independent origin. Thus, by virtue of their independent origin, plasmatic connections, and densely staining contents, the phloem-parenchyma cells of *Gnetum* fit the definition of albuminous cells originally provided by Strasburger (1891). Coupled with this are the findings of ontogenetically related parenchyma cells, but not the companion cells in members of the Calycanthaceae (Cheadle & Esau, 1958), and in *Luffa cylindrica* (Shah & Jacob, 1969). Srivastava's (1969) work on the secondary phloem of *Austrobaileya scandens*, a recognizedly primitive angiosperm (reported to lack companion cells by Bailey & Swamy, 1949), led him to post the idea that a clear distinction between all these different cells that are cytologically and physiologically related to the sieve elements is difficult. Further, in view of the great similarity between the companion cells of angiosperms and the albuminous cells of gymnosperms including their being the same cell type and in having originated through the adaptation of some parenchyma cells to assist the sieve elements in long distance transport, Srivastava also proposed to abandon the term albuminous cells and retain only companion cells both for gymnosperms and angiosperms (*see also* Behnke, 1986). Behnke and Paliwal (1973) have indicated that a similar situation occurs in *Gnetum gnemon* and hence the term parenchyma cells should be used for describing these albuminous cells.

In the crushed condition the primary phloem gets delimited by a massive accumulation of the wall

material. The ray cells have thick walls except those few that differentiate into sclereids. Ray cells or sclereids contain starch and crystals. In contrast to the ray cells of *Ephedra*, those of *Gnetum* accumulate calcium oxalate in the lumen not in the wall. Strasburger found it remarkable that the ray cells containing crystals had no less starch than the crystal-free cells. In the active phloem, the parenchyma cells are filled with starch but in the older phloem these become empty and crushed like the sieve elements.

Internally their structure undergoes simplication, the thylakoids become disorganized and the matrix materials also decrease. Starch is present in them from the earliest stage of sieve cell differentiation, the number of starch grains increasing gradually. Some of these are always club-shaped, a feature that seems to be characteristic of *Gnetum* and some members of the Taxodiaceae (Behnke, 1974).

The changes in the ER during sieve cell differentiation are more significant. The granular ER, present in the young sieve cells tends to be modified by a swelling of interacisternal spaces. After the loss of ribosomes, the ER assumes a more tubular structure; tubules spreading throughout the cell lumen but have a tendency to form aggregates. Within the aggregates, the ER tubules lie parallel to each other but may also become twisted altogether. Frequently the ER complexes are associated with the degenerating nuclei or with the plastids. Various opinions have been expressed for this association of ER (most of all close to the sieve areas), and its functional significance, if any. Dictyosomes and microtubules also disappear. Finally ER, plastids, and mitochondria represent the only organelles left in the living protoplast, an arrangement which is perhaps the characteristic condition of the mature sieve cell in gymnosperms. The central cell sap cavity sometimes contains fibrillar material that mixes with and disappears within the protoplast after tonoplast degeneration. P-protein has not been detected either by light or electron-microscopy in young or mature sieve elements (Paliwal & Behnke, 1973).

Parenchyma cell (of all the types including those associated with the sieve cells) differentiation, revealed features which are quite similar to those of the companion cells in angiosperms. The most remarkable change in the cambial initial destined to become a parenchyma cell is that it becomes rich in cellular organelles. The ribosomes become abundantly distributed in the cytoplasm and are deposited on the ER. Microtubules, dictyosomes and mitochondria, with well-defined cisternae are also present. Plastids may have ovoid, hemispherical, or elongated profiles. These contain thylakoids, a dense matrix, and accumulate varying quantities of starch. The nucleus persists for the entire life of these cells. Cell sap is retained in several small vacuoles, which generally fuse to form a central cavity. They are devoid of proteinaceous substance in contrast to the reports of Strasburger (1891), but are identical to those described by Liberman-Maxe (1971) in *Polypodium vulgare* in this respect (*see* Maxe, 1966).

The above description would lead us to conclude that the studies on the phloem tissue in Pinophyta had begun in the last century and have continued to invite the attention of a host of researchers till date. Of course, with the availability of the new tools and techniques now, more and more details have come to light pertaining to the structure of the sieve cells/elements and the nature of the connections between these and the associated parenchyma/albuminous cells. For instance, we now know that a differentiating sieve element in *Gnetum gnemon* is discernible from its neighbouring parenchymatous cell by its very long nucleus with densely stained patches of chromatin, partly attached to the inside of the nuclear envelope by mitochondria often in close vicinity to the outside of the nuclear envelope, by starch-containing plastids, and a less dense cytoplasmic matrix, containing some ER aggregates. On the other hand, the nucleus of the parenchyma cell extends partly into a cytoplasmic bridge, passing through the vacuole. Moreover, the albuminous cells of the three genera of the Gneticae (as well as the parenchyma cells) are interconnected through plasmodesmata occurring in groups and united in the region of the middle lamella by the median cavities. The connections to the sieve elements are intermediate between a sieve area and a plasmodesmatal field. This also shows the growth of our knowledge since the publications of de Bary (1884) or even Coulter and Chamberlain (1917; Behnke & Sjolund, 1990).

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