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Sieve-element plastids in some species of Rutaceae *

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Riassunto

I plastidi presenti nei tubi cribrosi a seconda del loro contenuto rispettivamente proteico o amilifero, sono stati classificati in due tipi: P-type e/o S-type. Essi hanno valore tassonomico per la classificazione delle piante a seme. La famiglia delle Rutaceae presenta nei tubi cribrosi plastidi di tipo S.

Noi abbiamo esaminato alcuni generi appartenenti alle due sottofamiglie: Rutoideae e Aurantioideae per apportare ulteriori contributi allo studio delle Rutaceae.

I plastidi riscontrati da noi nei tubi cribrosi sono di tipo 'S' in *Citrus limon, Citrus volkameriana, Citrus sinensis, Citrus paradisi*, di tipo 'P' in *Ruta chalepensis, Calodendrum capensis, Pilocarpus pennatifolius.*

Sulla base delle suddette osservazioni proponiamo una revisione sistematica delle Rutaceae.

INTRODUCTION

Sieve-element plastids were classified by BEHNKE (1981) and by BEHNKE and BARTHLOTT (1983) into two types according to their protein and/or starch contents i.e. into P-type and S-type respectively. He studied the above mentioned plastids in relation to Angiosperm systematics (1972). Already ESAU and GILL (1973) described sieve-element plastids in *Allium cepa*. A previous study on development of sieve elements in *Ruta chalepensis* (MATARESE PALMIERI *et al.*, 1982) reported the occurrence of a typical plastid-type.

Key words: Plastids, Sieve-element, Rutaceae.

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Plastid-types containing protein crystals, protein filaments and starch grains were proposed to be interpreted as primitive, while plastid-forms lacking any of these contents were said to be derived from.

In our opinion, this implies that S and P-plastids (Behnke, 1981) when lacking contents at all should be regarded as more evoluted forms.

The species belonging to the family of Rutaceae, as investigated by Behnke are: *Correa pulchella* Mackay, *Choisya terrata* Kunth, *Citrus limon* (L.) Burm., *Dictamnus albus* L., *Evodia hupehensis* Dode, *Eriostemon myoporoides* DC., *Orixa japonica* Thunb., *Phellodendron amurense* Rupr., *Ptelea trifoliata* L., *Ruta graveolens* L., *Skimmia japonica* Thbg., *Xantoxylum simulans* Planch.

Behnke (1976) described the sieve-element plastids of the above mentioned species as of S-type, he also quoted the genus *Rhabdodendron* Gil. & Pilg. with P-type plastids, wich Prance (1968) moved to the family Rhabdodendraceae.

Hoefert (1979, 1980), in his paper on *Thlaspi arvense*, suggested that changes in plastid content during sieve-element differentiation are probably caused by digestion of starch. This, in turn, would cause starch grains to appear as granulous especially in the peripheral part of plastids.

We described in *Ruta chalepensis* (Matarese Palmieri *et al.*, 1982), sieve-element plastids as belonging to P-type. Therefore, we examined more species of the same family so as to establish wich sieve-element plastids-types differentiate during their sieve-element ontogeny. On the basis of such sieve-tubes characters and of our previous work on ontogeny of vascular tissue in the ovary, fruit (Tomasello *et al.*, 1977a, 1977b), (Matarese Palmieri *et al.*, 1979) (Tomasello *et al.*, 1981a), and bark in the secondary stem of Rutaceae (Tomasello *et al.*, 1981b; Lo Giudice *et al.*, 1982), we reconsidered Rutaceae systematics.

Materials and methods

Young leaves and leaves at in intermediate stage of development, petioles and flower pedicels of the following species, were collected in the Botanical Gardens of Messina (Sicily):

Aurantioideae: *Citrus sinensis* (L.) Osbeck, *Citrus limon* (L.) Burm., *Citrus volkameriana* Pasq., *Citrus paradisi* Macfayden;

Rutoideae: *Calodendrum capensis* Thunb., *Pilocarpus pennatifolius* Lem., *Ruta chalepensis* L.

The samples were fixed in cold glutaraldehyde 4%, 5%, 6% buffered with 0.2 M phosphate buffer at pH 7.2; all the samples have been treated for 30', 2h and 4h. After this, they were postfixed in 1% osmium tetroxide in a 0.2M phosphate buffer at pH 7.2 for 2h at 4°C. The samples were dehydrated in ethanol and embedded in a Epon-araldite mixture (Mollenhauer, 1964). Pieces were then sectioned with an ultrotome LKB V. Ultrathin sections were stained with uranile acetate and lead citrate (Raynolds, 1963) and observed with a Siemens Elmiskop 102A electron microscope.

Observations

Ruta chalepensis

Differentiating sieve-elements show periferic cytoplasm with vesicles, ribosomes and the presence of plastids, in fact they have a double membrane with rare thylakoids and with a protein body inside (fig. 1).

These plastids appear similar to those ones described by Behnke (1981) and ascribed by us to P-type.

In companion cells we observed cytoplasm, nucleus, mitochondria, proplastids and plastid P-type (fig. 7). In mature sieve-elements circular profiles of endoplasmic reticulum have been found, but no plastids were observed (fig. 8, II).

Calodendrum capensis

In immature sieve-elements we noted cytoplasm with vesicles, lomasomi, mitochondria and plastids with circular thylakoidal profiles (fig. 13). The wall shows connection in the form of branched plasmodesmata between sieve-element and companion cell (fig. 13).

In mature sieve-elements we observed and ascribed to P-type plastids, in fact these have periferic filamentous proteins and starch grains (fig. 2). Moreover in a cell near to sieve-element we observed a fibrillar P-protein body (fig.12).

Pilocarpus pennatifolius

In immature sieve-elements we noted plastids with few thylakoids, vesicles, ribosomes, vacuoles and nucleus (fig. 15).

In mature sieve-elements we observed plastids and ascribed then to P-type, in fact they have starch grains and protein matrix (fig. 3).

In sieve-elements vesiculous bodies are present with electron-dense material (fig. 4).

Citrus limon

In mature sieve-elements we observed the typical plastid-types. These were classified of S-type as they appeared to contain numerous starch grains, located near the sieve plate (fig. 5).

These sieve-elements were rich in fibrillar P-protein (fig. 6).

Citrus volkameriana

In mature sieve-tubes were observed and described plastids of S-type, as they contained starch grains (fig. 4). Also proplastids with osmiophilic globuli and electron-dense matrix as well as plastids with rare thylakoids in parenchimatous cell were observed. As in *Pilocarpus*, also in *C. volkameriana* vesicles can be found with electron-dense material inside.

Citrus sinensis

In mature sieve-elements, plastids contained an electron-dense matrix and starch grains. Therefore they can be ascribed to the S-type.

Citrus paradisi

Plastids which can be described of S-type were noted, since they appeared to contain starch grains (fig. 5).

In all species studied, immature sieve-elements contained cytoplasm with polyribosomes, organelles and rough endoplasmic reticulum. Also in differentiating sieve-elements of *Calodendrum* (fig. 12) and *Ruta* (fig. 9) were noted fibrillar P-protein bodies and tubular P-protein changes in the endoplasmic reticulum. In fact, circular profiles of endoplasmic reticulum indicate that its cisternae are tubular in shape (fig. 8, 9, 11), P-protein appeared to change in its appearance in a similar way as described by HOEFERT (1980) i.e. changing from a tubular to an extended form.

In *Calodendrum* some parietal mitochondria, lomasomes and vesicles were observed (fig. 13, 16).

DISCUSSION

Ultrastructural observations were performed on differentiating sieve-elements in some genera of the subfamilies, Rutoideae and Aurantioideae, with the purpose of comparing our observations with those reported by ESAU & GILL (1973) and BEHNKE (1981) and BEHNKE & BARTHLOTT (1983) on plastids by CRONSHAW & ESAU (1968 a,b) on P-protein by OPARKA & JOHNSON (1978) and ESAU & GILL (1971) on changes of endoplasmic reticulum, by HOEFERT on differentiating sieve-elements (1979, 1980).

During their ontogeny, sieve-elements showed to contain plastids P-type or S-type according to BEHNKE (1981). In particular, he stated that all plastids occuring in sieve-elements of the genera of Rutaceae studied, have to be classified of S-type.

Our results generally agree with those of BEHNKE (1981) with regard to the subfamily of Aurantioideae, since we observed S-type plastids in all species studied.

As to the subfamily of Rutoideae, plastids were ascribed to P-type even though starch was sometimes found.

During their early stages of development, the immature sieve-elements show to contain a cytoplasm rich in organelles, ribosomes, small vacuoles, sometimes lobed nuclei, tubular and fibrillar P-protein.

The granular P-protein in sieve-tubes of *Thlaspi arvense* as identified by HOEFERT (1979), was not observed in all species we studied. In Hoefert's opinion, coated vesicles are involved in the formation of the granular P-protein; we observed vesicles and lomasomes in about all species studied and especially in *Calodendrum*, no granular P-protein could be found. In mature sieve-elements, a gradual loss of ribosomes was detected as well as a degeneration of nuclei and changes in the structure of endoplasmic reticulum. These last changes were especially observed in *Ruta*, according to OPARKA and JOHNSON (1978) and to ESAU and GILL (1971, 1972). The observations of OPARKA and JOHNSON (1978) on the protophloem in *Nymphoides peltata*, indicate that convoluted endoplasmic reticulum is gradually converted into stacked endoplasmic reticulum during its ontogeny.

We observed tubular crystalline P-protein in *Ruta* (fig, 9), filamentous bodies in *Calodendrum*, granular P-protein in *Citrus*. Filaments appeared to go through the sieve-plate in *Citrus* and *Ruta*.

In regard to the development of P-protein bodies in sieve-elements of *Cucurbita* and *Nicotiana*, CRONSHAW and ESAU (1968 a,b; 1967) proposed two different type of P-protein bodies to occur: one forming fine fibrils and another one forming groups of tubules. These two types could indicate that a sequence exists in the development of tubules starting from the protein fibrils.

Furthermore, CRONSHAW and ESAU (1968 a,b) argued that dispersed or not dispersed P-protein bodies can depend on the studied part of the plant (stem or petiole).

In mature sieve-elements, we have observed peripheral endoplasmic reticulum and P-protein in filaments going through the sieve-plate. The present paper mainly deals with the plastid-types occurring in the sieve-elements of the species studied. These plastid-types, observed by ESAU and GILL (1973) and BEHNKE (1972), were described as of phylogenetic significance for the classification and phylogeny of seed plants and provide more information to the classification of the family of Rutaceae.

With respect to Behnke's above data, we studied different genera of the family of Rutaceae and found plastid-forms somewhat different from each other. Besides we observed Behnke's

(1981) plastids can be found in companion cells; according to our previous observations, this is probably due to the heterogeneous nature of this family. On this basis, we would like to propose the revision of the two subfamilies Aurantioideae and Rutoideae and to attribute the family rank to them.

The numerous different anatomical and ultrastructural features and the different plastid-types, character considered of diagnostic significance (Behnke, 1972; Behnke and Dahlgren, 1976; Takhtajan, 1980), could suggest this revision be reasonable.

Summary

Sieve-element plastids have been classified into two types according to their protein and/or starch content i.e. P-type and S-type. They were considered as a significant character for taxonomy of Angiospermes. The family Rutaceae has the sieve-elements plastids S-type. We examined some genera in the subfamilies Rutoideae and Aurantioideae under this respect to provide further informations about the family Rutaceae. S-type plastids have been observed in *Citrus limon, Citrus volkameriana, Citrus sinensis* and *Citrus paradisi* whereas *Ruta chalepensis, Calodendrum capensis* and *Pilocarpus pennatifolius* show P-type plastids. On the basis of the present observations we suggest to reconsider systematics of Rutaceae.

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REFERENCES

BEHNKE H. D., 1972. *Sieve-tube plastids in relation to angiosperm systematics. An attempt towards a classification by ultrastructural analysis.* Bot. Rev., **38**: 155-197.

BEHNKE H. D., 1976. *Sieve element plastids of* Fouquieria, Franchenia *(Tamaricales) and* Rabdodendron *(Rutaceae), taxa sometimes allied with Centrospermeae (Caryophyllales).* Taxon, **25**: 265-268.

BEHNKE H. D. and DAHLGREN, 1976. *The distribution of characters within an angiosperm system. 2 Sieve-element plastids.* Bot. Notiser., **129**: 287-295.

BEHNKE H. D., 1981. *Sieve-element characters.* Nord. J. Bot., **1**: 381-400.

BEHNKE H. D. and BARTHLOTT W., 1983. *New evidence from the ultrastructural and micromorphological fields in angiosperm classification.* Nord. J. Bot., **3**: 43-66.

CRONSHAW J. and ESAU K., 1968a. *P-Protein in the phloem of* Cucurbita. *I. The development of P-Protein bodies.* The J. of Cell. Biology, **38**: 25-39.

CRONSHAW J. and ESAU K., 1968b. *P-Protein in the phloem of* Cucurbita. *II. The P-Protein of mature sieve-elements.* The J. of Cell. Biology, **38**: 292-303.

CRONSHAW J. and ESAU K., 1967. *Tubular and fibrillar components of mature and differentiating sieve-elements.* J. Cell. Biology, **34**: 801.

ESAU K. and GILL R. H., 1971. *Aggregation of Endoplasmic Reticulum and its relation to the nucleus in a differentiating sieve element.* J. Ultrastr. Research, **34**: 144-158.

ESAU K. and GILL R. H., 1972. *Nucleus and endoplasmic reticulum in differentiating root protophloem of* Nicotiana tabacum. J. Ultrastr. Research, **41**: 160-175.

ESAU K. and GILL R. H., 1973. *Correlations in differentiation of protophloem sieve elements of* Allium cepa *root.* J. Ultr. Research, **44**: 310-328.

HOEFERT L. L., 1979. *Ultrastructure of developing sieve elements of* Thlaspi arvense L. *I. The immature state.* Amer. J. Bot., **66**: 925-932.

HEFERT, L. L., 1980. *Ultrastructure of developing sieve elements in* Thlaspi arvense L. *II. Maturation.* Amer. J. Bot., **67** (2): 194-201.

LO GIUDICE L., MATARESE PALMIERI L., TOMASELLO D., 1982. *Anatomia comparata del cilindro corticale del fusto secondario in alcune Aurantioideae e Rutoideae.* Riv. di Biol. Norm. e Patol., **8**: 17-31.

MATARESE PALMIERI R., TOMASELLO D., LO GIUDICE L., 1979. *Vascular bundles in the ovaries and fruits of* Citrus volkameriana Pasq. Biologie et Ecol. Médit., tome VI: 19-26.

MATARESE PALMIERI R., LO GIUDICE L. and TOMASELLO D., 1982. *Observations on the developing sieve elements in* Ruta chalapensis L. Caryologia, **35**: 391-393.

MOLLENHAUER H. H., 1964. *Plastic embedding mixtures for use in electron microscopy.* Stain Technol., **39**: 111-114.

Oparka K. J. and Johnson R. P. C., 1978. *Endoplasmic Reticulum and crystalline fibrils in the root protophloem of* Nymphoides peltata. — Planta, **143**: 21-27.

Prance G. T., 1968. *The systematic position of* Rhabdodendron Gil. et Pilg. Bull. Jard. Bot. Natl. Belg., **38**: 127-146.

Reynolds E. S., 1963. *The use of lead citrate at a high pH as an electron opaque stain in electron microscopy.* J. Cell. Biol., **17**: 208-212.

Taktajan A., 1980. *Outline of the classification of flowering plants (Magnoliophyta)* The Bot. Review, **46**: 225-359.

Tomasello D. and Matarese Palmieri R., 1977a. *An electron microscopy study of the ovary and fruit of* Volkamer lemon (Citrus volkameriana Pasq.) Caryologia, 30.

Tomasello D., Matarese Palmieri R., 1977b. *Ricerche ultrastrutturali sull'ovario e sul frutto di* Citrus volkameriana Pasq. Atti Soc. pel. di Sc. F.M.N., **23**: 61-79.

Tomasello D., Matarese Palmieri R., Lo Giudice L., 1981a. *Vascular bundles in the ovaries and fruits of the Rutaceae.* Biol. Médit., **8**: 23-30.

Tomasello D., Matarese Palmieri R. e Lo Giudice L., 1981b. *Osservazioni preliminari sulle caratteristiche strutturali del floema in alcune Rutaceae.* Gior. Bot. Ital., **115**: 425-426.

Tab. 1, fig. 1-7 Sieve element plastids.

Fig. 1 — Cross section of *Ruta chalepensis*. Enucleate sieve-tube (St) containing P-type plastids (p) with thylakoids (t) and filamentous protein body (Fp). × 44000

Fig. 2 — Cross section of *Calocendrum capensis*. P-type plastids (p) containing filamentous periferic protein bodies (Fp) and grains starch (s). × 28000

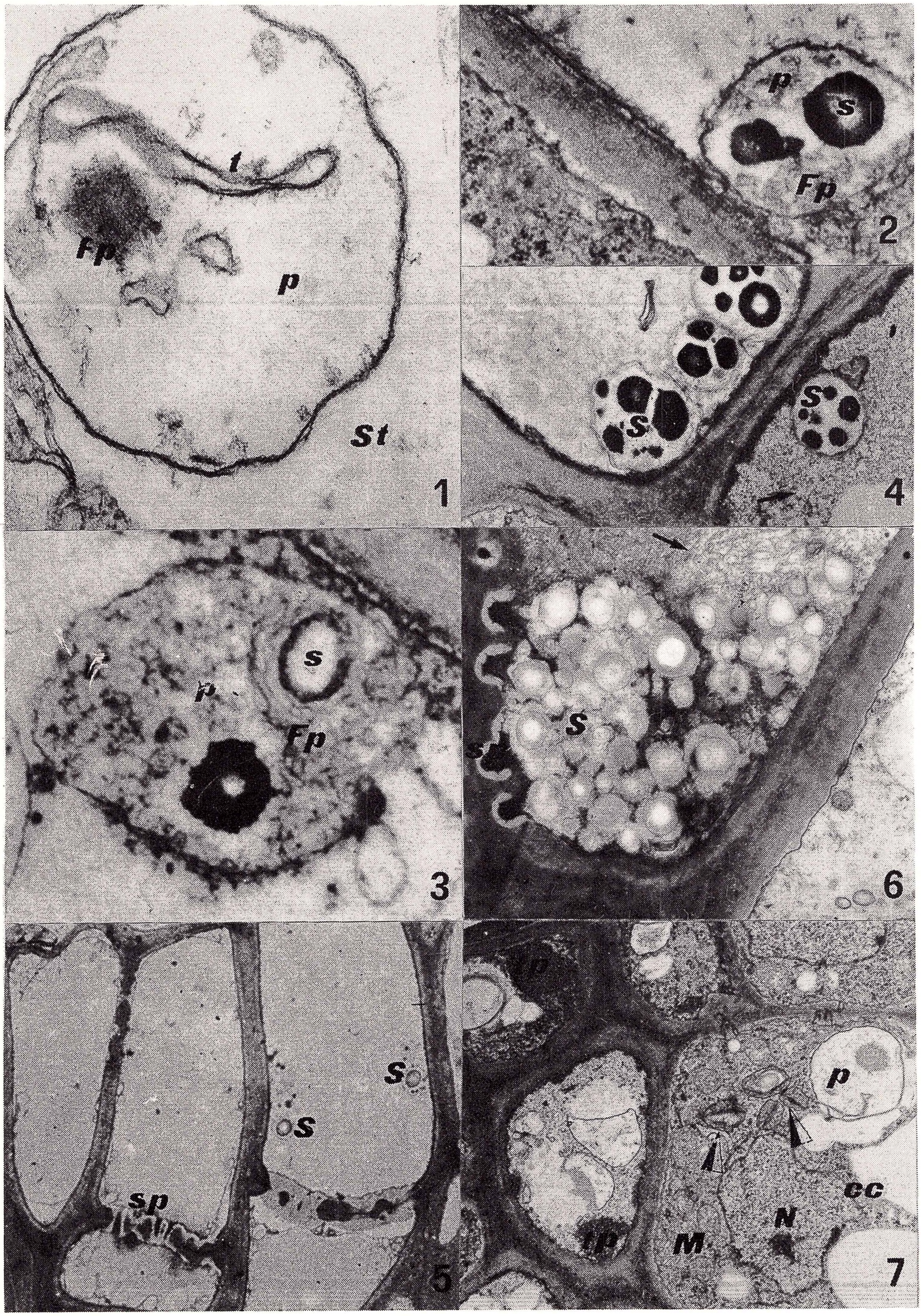
Fig. 3 — Cross section of *Pilocarpus pennatifolius*. P-type plastids (p) with filamentous (Fp) and stacked protein bodies (Sp) and starch (s). × 60000

Fig. 4 — Cross section of *Citrus volkameriana* with S-type plastids (S) and P-protein (⟶). × 15000

Fig. 5 — Longitudinal section of *Citrus paradisi*. S-type plastids (S) and sieve-plate (sp). × 8000

Fig. 6 — Longitudinal section of *Citrus limon*, S-type plastids (S), filamentous P-protein (⟶) and sieve-plate (sp). × 8000

Fig. 7 — Cross section of *Ruta chalepensis*. P-type plastids (p), proplastids (▶), nucleus (N), mitochondria (M) in the companion call (cc). P-protein in sieve-elements (tp). × 6000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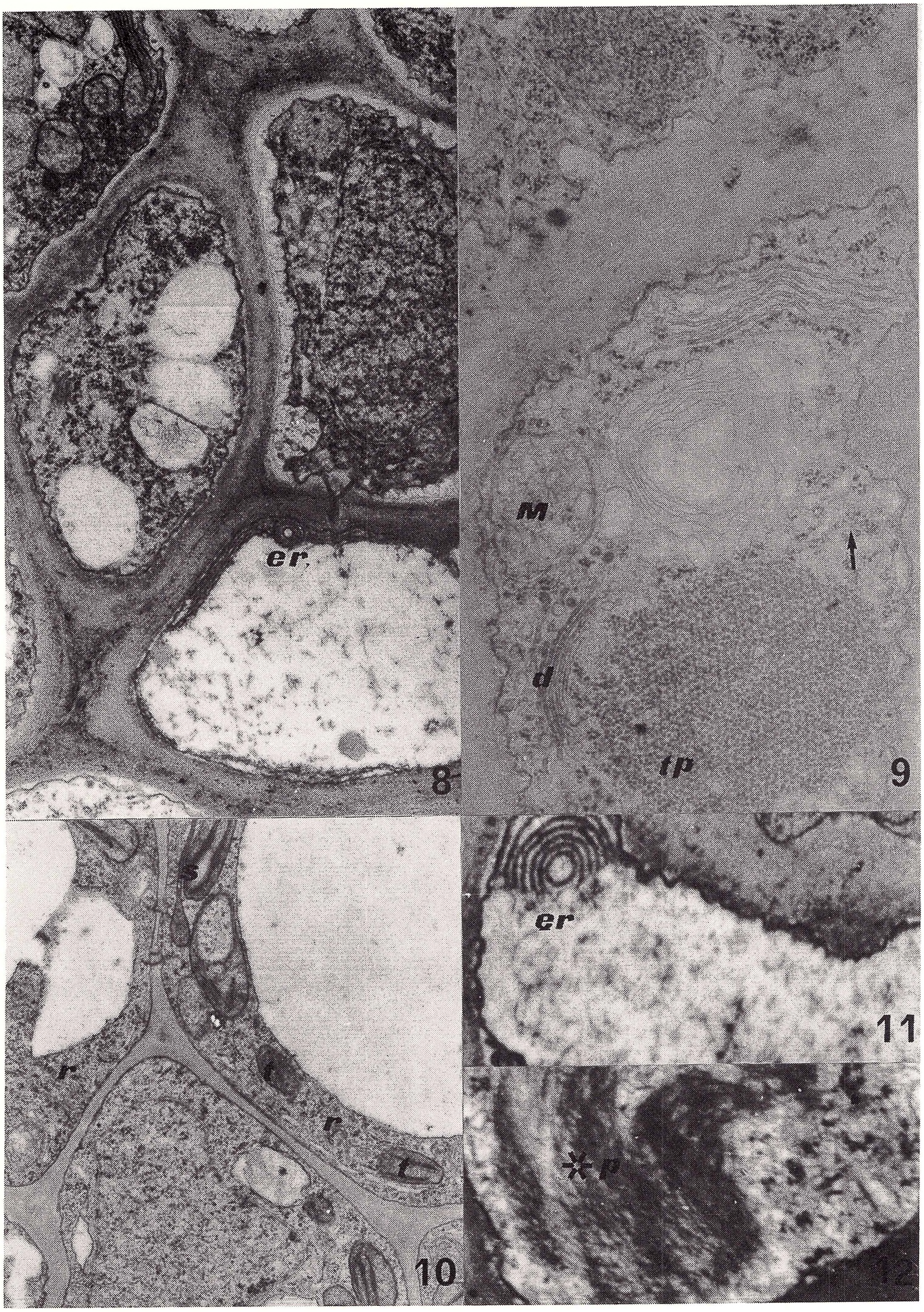
Tab. 2, fig. 8-12 Differentiating sieve-elements.

Fig. 8-11 — Cross sections of *Ruta chalepensis*. Changes in endoplasmic reticulum with circular profiles (er). × 16000, × 30000

Fig. 9 — Cross section of *Ruta chalepensis*. Changes in endoplasmic reticulum. Presence of tubular P-protein (tp), dictiosomes (d), mitochondria (M) and polisomes. (—▸) × 32000

Fig. 10 — Cross section of *Ruta chalepensis*. Differentiating phloem with proplastids containing primary thylakoids (t) and grains starch (s). Cytoplasmic ribosomes (r). × 16500

Fig. 12 — Cross section of *Calodendrum capensis* with fibrillar P-protein bodies. (☼ p) × 20000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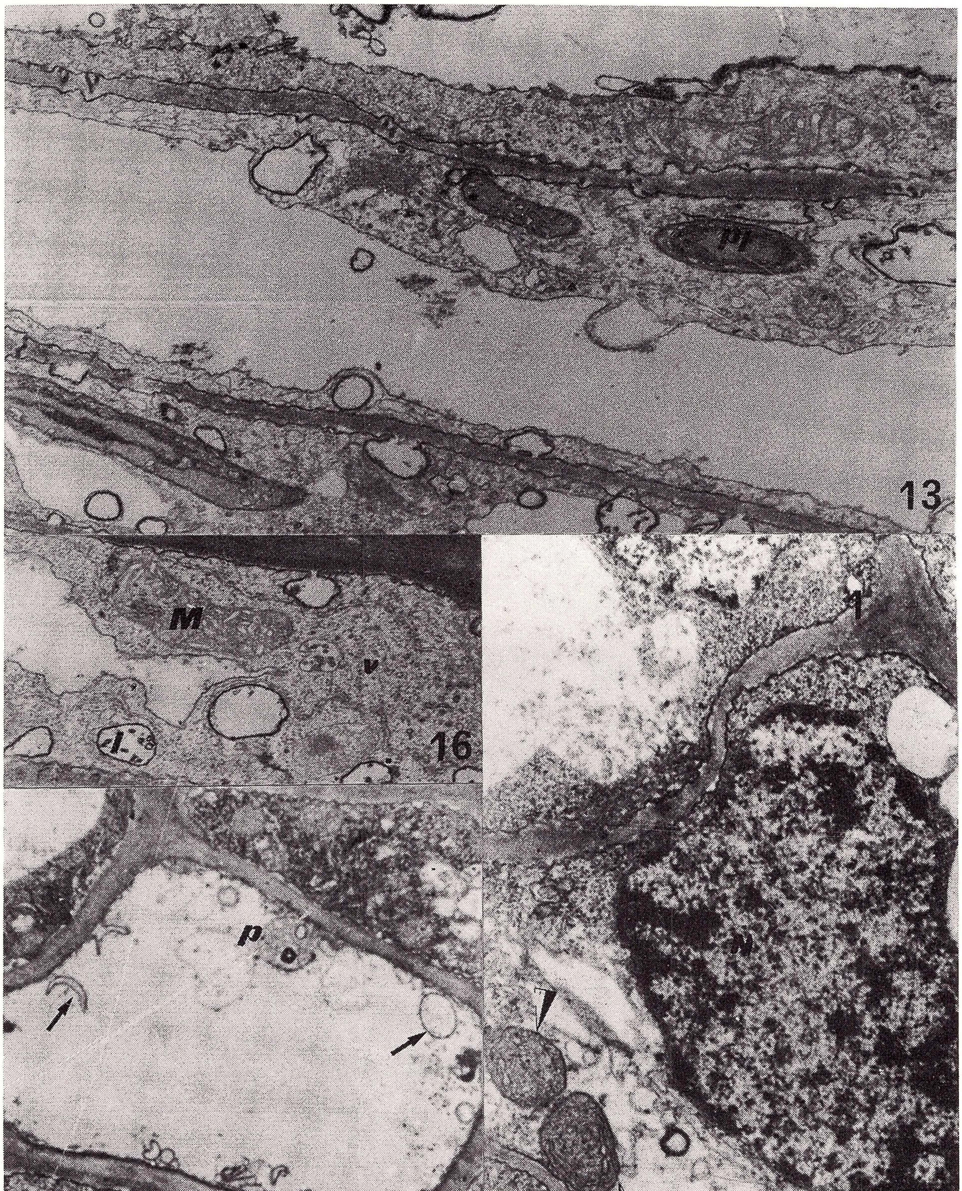
Tab. 3, fig. 13-16 differentiating sieve elements

Fig. 13 — Longitudinal section of *Calodendrum capensis*. Presence of plastid (pl) with convoluted thylakoids, plastoglobuli and vesicles. × 18000

Fig. 14 — Cross section of *Pilocarpus pennatifolius*. Sieva-tuba with P-type plastids (p), vesiculosus bodies (⟶) and companion cells. × 7500

Fig. 15 — Cross section of *Pilocarpus pennatifolius*. Nucleate sieve-tube (N), proplastids (▶) with few circular thylakoidal profiles. × 14000

Fig. 16 — Longitudinal section of *Calodendrum capensis*. Presence of vesicles, moyochondria (M), lomasomy (l). × 18000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