Planta (1985) 165:191–196

Planta

Plasmatubules in transfer cells of pea (*Pisum sativum* L.)

N. Harris and N.J. Chaffey
Department of Botany, University of Durham, Durham DH1 3LE, UK

Abstract. Plasmatubules are tubular evaginations of the plasmalemma. They have previously been found at sites where high solute flux between apoplast and symplast occurs for a short period and where wall proliferations of the transfer cell type have not been developed (Harris et al. 1982, Planta **156**, 461–465). In this paper we describe the distribution of plasmatubules in transfer cells of the leaf minor veins of *Pisum sativum* L. Transfer cells are found in these veins associated both with phloem sieve elements and with xylem vessels. Plasmatubules were found in both types of transfer cell and it is suggested that the specific distribution of the plasmatubules may reflect further membrane amplification within the transfer cell for uptake of solute from apoplast into symplast.

Key words: Apoplast – Plasmatubule – *Pisum* (plasmatubules) – Symplast – Transfer cell – Vein (minor).

Introduction

The distribution and role of transfer cells in leaf minor veins is well established. Gunning et al. (1968) described their form and distribution in a number of species including *Pisum arvense* L. In an earlier paper Wark (1965) described the ontogeny of wall ingrowths, which she termed 'trabeculae', in companion cells of stem secondary phloem of *P. sativum* L. The distribution and interconnections of the sieve elements, modified companion cells (transfer cells) and parenchyma led her to conclude that the 'likely function of the companion cells might be to regulate movement in and out of the sieve elements'.

From a broad survey of the distribution and function of transfer cells, Gunning and Pate (1969) concluded that they are generally restricted to positions where transport pathways are affected by adverse surface area-volume relationships between donor and recipient, and where solute transport is accompanied by minimal flow of solvent. They subsequently classified transfer cells into four types: type A – modified companion cells with ingrowths on all walls; type B – modified parenchyma cells with ingrowths mainly developed opposite sieve elements and their companion cells; types C and D – associated with xylem parenchyma and bundle-sheath cells, respectively (Pate and Gunning 1972). The main roles of minor-vein phloem transfer cells are the accumulation and passage of photosynthate from the mesophyll to the vascular network, and the recycling of solutes that enter the leaf apoplast in the transpiration stream (Gunning et al. 1974). Solute accumulation is greatly enhanced by the increased surface area of the plasmalemma at sites where active solute uptake from the apoplast to the symplast occurs prior to plasmodesmatal loading of the phloem sieve elements (Gunning and Pate 1974).

Transfer cells are usually found where uptake of solute into the symplast occurs for an extended period of weeks or months. Examination of sites where symplastic uptake occurs for periods limited to a few days revealed the presence of fine tubular evaginations of the plasmalemma (Harris 1981; Harris et al. 1982). Their form and specific distribution led to the suggestion that these 'plasmatubules' are involved in short-term uptake of solutes into the vascular symplast at sites where transfer cells have not developed.

In this paper we show that plasmatubules are present in the transfer cells of the minor veins of

P. sativum leaves. They are distributed in a pattern which indicates that they may act as further amplification of the plasmalemma for uptake of solutes from the apoplast prior to loading into the phloem sieve elements.

Material and methods

Seeds of *Pisum sativum* L. cv. Meteor (Philip Harris, Weston-super-Mare, Avon, UK) were sown and raised in Levington compost under 16-h days at 27° C with a night temperature of 15° C. Young leaflets, approx. 3 cm in length, were harvested and tissue was cut directly into fixative. Tissue was fixed in 2.5% (v/v) glutaraldehyde, 1.5% (v/v) formaldehyde in 0.05 M sodium cacodylate (pH 7.0) for 2 h, washed in 0.05 M cacodylate buffer (pH 7.0) for 2 × 15 min, and post-fixed in 1% aqueous osmium tetroxide for 2 h, all at room temperature. Following dehydration in a graded ethanol series, the leaf pieces were infiltrated and embedded in Spurr resin (Spurr 1969). Sections were mounted on uncoated grids, sequentially stained with ethanolic uranyl acetate and alkaline lead citrate and examined in a Philips EM 400 electron microscope (Philips Industries, Eindhoven, The Netherlands) at either 60 or 80 kV.

Results

Although there was some variation in the proportions of the particular component cell types of the numerous leaf minor veins of *P. sativum*, their general form in transverse section is illustrated in Fig. 1. The vein is a collateral bundle which is surrounded by a uniseriate bundle sheath lying in close contact with the vascular cells. The bundle sheath eliminates direct contact between the vascular cells and the leaf mesophyll air spaces. Phloem sieve elements (SE1 and SE2) are present with adjacent transfer cells (TC1 and TC2) and also a vascular parenchyma cell (PC). The transfer cells contain many mitochondria next to the plasmalemma, starch-free chloroplasts and numerous cytoplasmic ribosomes. The secondary wall is present in both xylem vessels (Xy); retention of some contents in the lumina indicates that the vessels in Fig. 1 are undergoing the final stages of autolysis.

The transfer cell TC1, associated with both sieve elements and the vascular parenchyma cell, shows wall ingrowths predominantly on the walls adjacent to the vascular parenchyma cell. Ingrowths are also seen adjacent to the bundle-sheath cells, particularly near the intercellular junctions. We consider TCl to be a type-A transfer cell. Cell TC2 has walls adjacent to phloem (SE1), vascular parenchyma, xylem vessels and a bundle-sheath cell. The wall ingrowths are most pronounced on the wall adjacent to the vascular parenchyma cell and at intercellular junctions.

Details of the wall of TC2 in the region of its junction with the two xylem vessels (area marked 2 in Fig. 1) is shown in Fig. 2. Aggregations of tubular profiles are seen in both longitudinal and transverse section and in continuity with the plasmalemma. The tubules are most common in regions between the wall ingrowths and opposite to the intercellular junctions (arrows). The form and size of the tubules is similar to plasmatubules previously characterised in barley scutella (Harris et al. 1982).

Figure 3 shows detail of the area marked 3 in Fig. 1 at the junction of TC1, SE2 and the vascular parenchyma cell. Plasmatubules are again localised adjacent to the transfer cell wall at the intercellular junction (arrows). The vascular parenchyma cell contains numerous vesicles; microtubules are seen grouped close to the plasmalemma (darts).

Serial sectioning of the bundle revealed a third transfer cell (TC3), between TC1 and TC2 associated with SE1 at the wall starred in Fig. 1. Figure 4 shows plasmodesmatal connections between this transfer cell and sieve element (SE1). Some plasmatubules are seen in TC2 at the junction between the two transfer cells and SE1. Generally, however, plasmatubules were rarely seen associated with the transfer-cell walls adjacent to sieve elements.

Figures 5 and 6 were taken from other *P. sativum* leaf minor veins where plasmatubules were found in positions equivalent to those described above. In both cases the minor-vein xylem vessels were devoid of contents and thus appeared to be fully functional. Figure 5 illustrates the distribution of plasmatubules in a transfer cell adjacent to two vascular parenchyma cells. Figure 6 shows the intercellular junction between a transfer cell (top) and two bundle-sheath cells. In both cases, plasmatubules are predominantly associated with the region of the transfer cell adjacent to the intercellular junction; few plasmatubules were found in other regions of the transfer cells. Microtubules are seen in a bundle-sheath cell (Fig. 6 darts). Obliquely sectioned plasmodesmata (arrowed) are present in the walls between the transfer cell and the bundle-sheath cells (Fig. 6) although our sections generally revealed relatively few plasmodesmata between the bundle sheath and vascular cells.

Discussion

The role of transfer cells in assimilate accumulation and secretion in leaf vascular bundles is well documented (Pate and Gunning 1972, and references therein). These cells actively accumulate solutes both from the symplast, via plasmodesmatal connections to the bundle sheath and vascular pa-

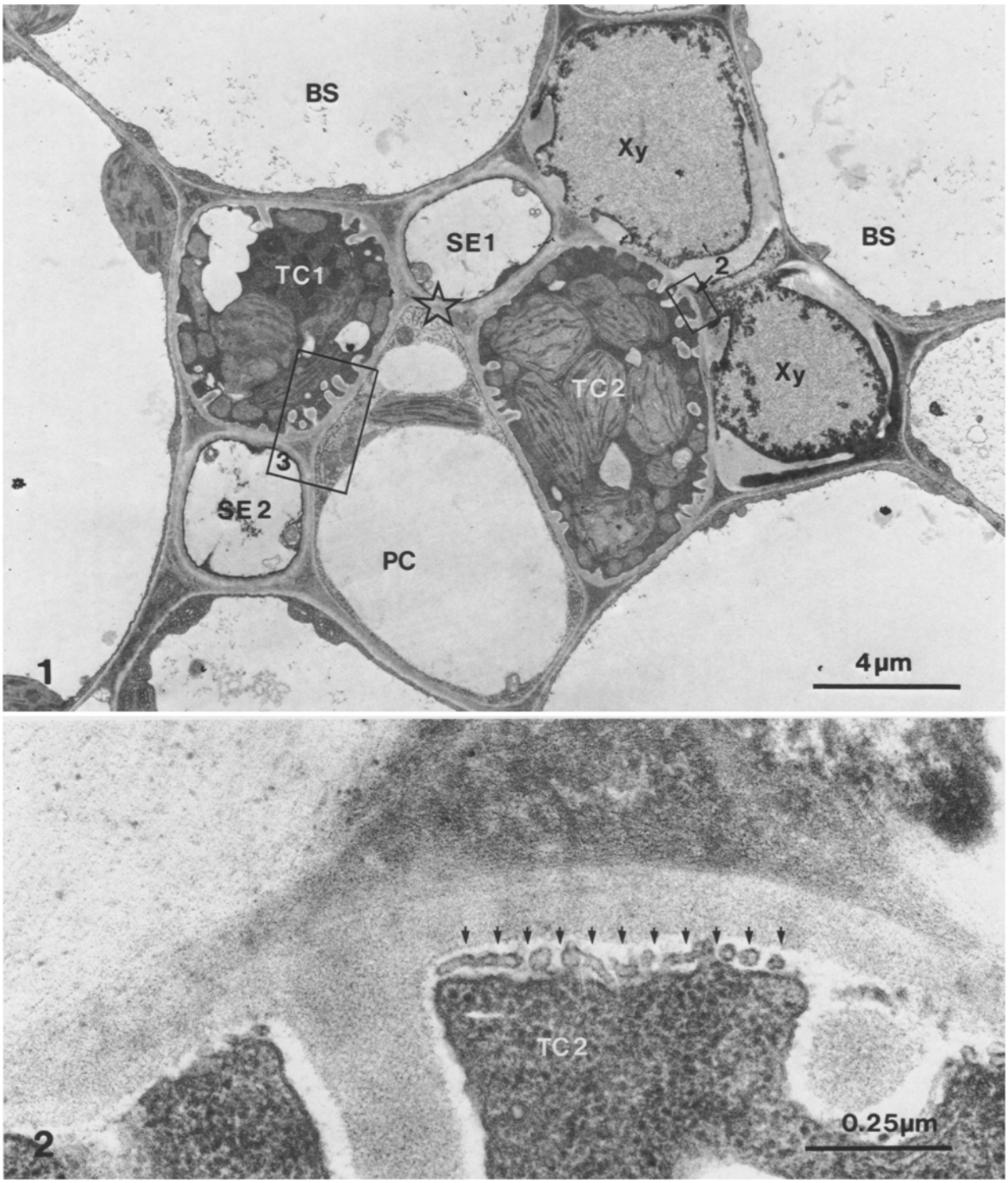

Fig. 1. Transverse section of a leaf minor vein, showing xylem (*Xy*), sieve elements (*SE1*, *SE2*), transfer cells (*TC1*, *TC2*) and a vascular parenchyma cell (*PC*), with bundle sheath (*BS*). ×6000

Fig. 2. Details of junction between TC2 and xylem (marked 2 in Fig. 1); *arrows* indicate plasmatubules. ×96000

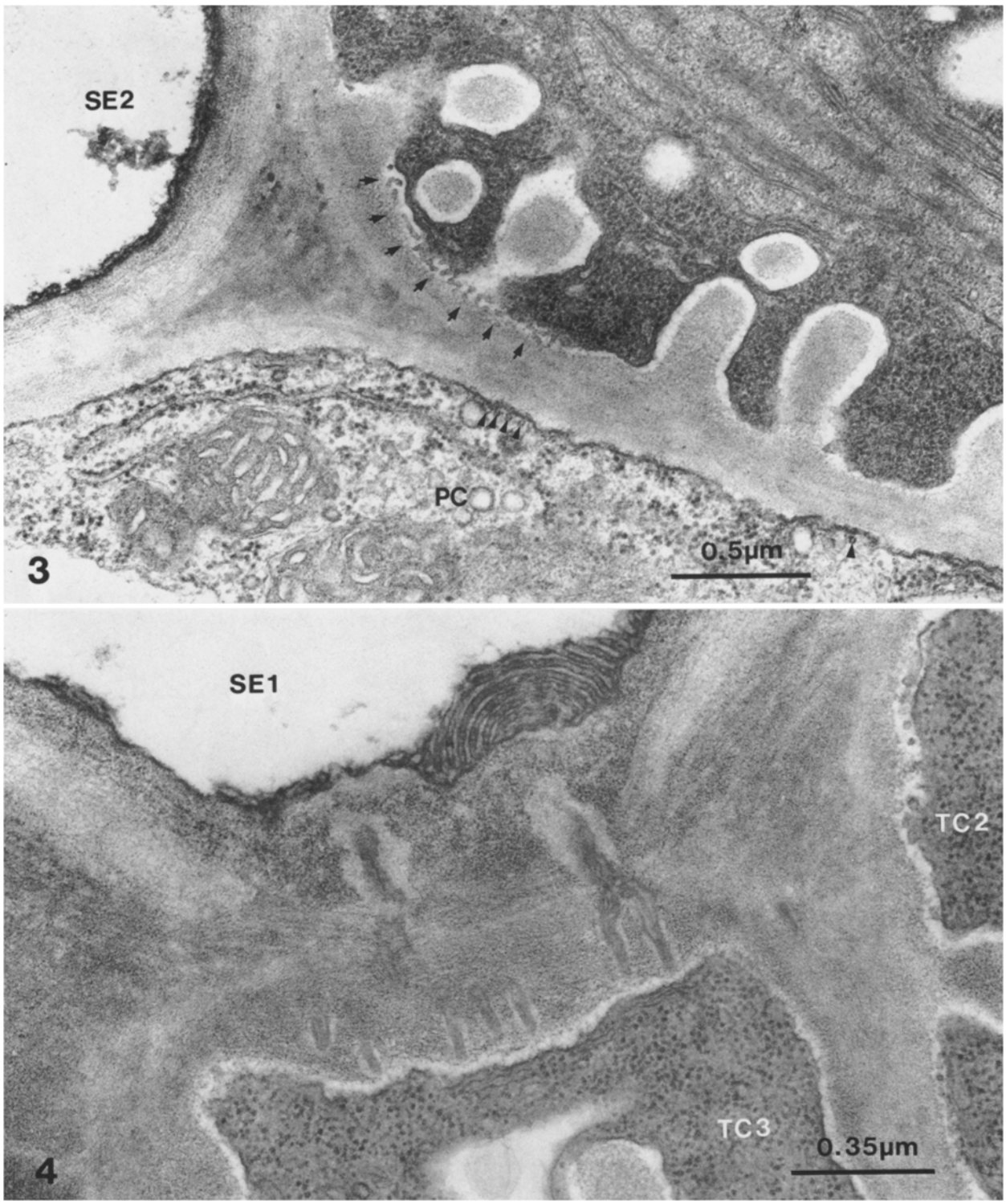

Fig. 3. Detail of junction between TC1, PC and SE2 (marked 3 in Fig. 1); *arrows* indicate plasmatubules, *darts* indicate microtubules. ×46000

Fig. 4. Detail of junction between SE1 and two transfer cells (TC2, TC3) near the *starred* region in Fig. 1. Branched plasmodesmata occur between TC3 and SE1. ×67000

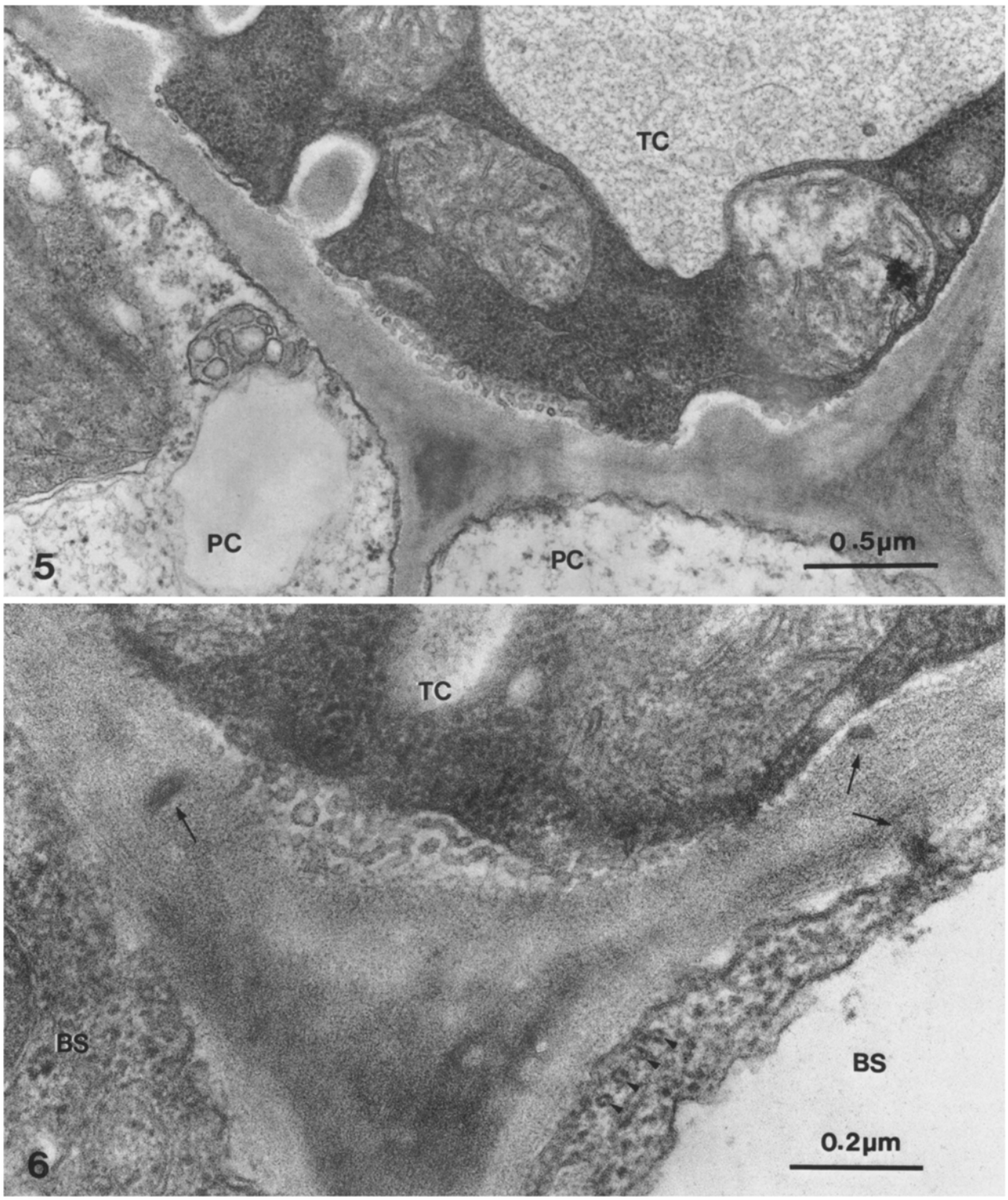

Fig. 5. Details of a transfer cell (*TC*) and two adjacent vascular parenchyma cells (*PC*) showing distribution of TC plasmatubules. ×44000

Fig. 6. Detail of a transfer cell (*TC*) and two adjacent bundle-sheath cells (*BS*) showing association of plasmatubules with the intercellular junction; *darts* indicate microtubules in BS, *arrows* denote plasmodesmata. ×110000

renchyma cells, and from the apoplast by active uptake at the plasmalemma. Close association of the plasmalemma with the numerous wall ingrowths provides a favourable ratio of surface area to volume for plasmalemmal uptake. Transfer of the accumulated solute to the phloem is thought to occur via the numerous complex plasmodesmata which interconnect type-A transfer cells and sieve elements (Gunning 1976).

Our studies revealed relatively few plasmodesmatal connections between bundle-sheath cells and vascular transfer cells in the minor leaf veins of *P. sativum*. The role of the bundle sheath as an intermediary in symplastic uptake and movement of solutes is therefore unlikely to be a major feature of solute transport from mesophyll tissue to the vascular bundle. The bundle-sheath cells do, however, maintain a fairly complete enclosure of the minor veins with little or no direct connection between the minor veins and the mesophyll air spaces. Apoplastic movement of solutes is possible between cells of the mesophyll and the vascular tissue. Two routes can be envisaged: a) the primary walls of bundle-sheath cells, and b) the middle lamella and its accumulations in the intercellular junctions. In contrast to the mesophyll-bundle sheath, the intercellular junctions between the bundle sheath and vascular tissues are not open air spaces but contain loosely-packed material with a form and staining characteristics similar to middle lamellae.

The importance of xylem parenchyma and transfer cells in solute retrieval from the transpiration stream has been emphasised by Pate (1980). The distribution of plasmatubules in developing minor veins indicates a possible role in retrieval of products of xylogenetic autolysis. In mature minor veins it is suggested that the transfer-cell plasmatubules may have a role in retrieval of transpiration-stream solutes. In both cases the transfer-cell plasmatubules could also act in symplastic uptake of mesophyll solutes.

Plasmatubules have previously been found in tissues where there is a high flux of solute from apoplast to symplast for a few days but where transfer cells had not developed (Harris 1981; Harris et al. 1982). These observations led to the suggestion that the tubular evaginations of the plasmalemma might act as a transfer-cell equivalent in increasing the plasmalemma surface area for active uptake into the symplast.

The results reported above show that plasmatubules are also found within transfer cells, although at very specific locations. In type A, phloem-associated transfer cells, plasmatubules were common only at sites adjacent to the intercellular junctions of these cells with a) vascular parenchyma cells, and b) bundle-sheath cells. A similar distribution was found in xylem-associated transfer cells.

It is proposed that plasmatubules may act as an additional structural modification for symplastic uptake into transfer cells at sites where the apoplastic supply of solutes is at its maximum.

We thank Dr. J.A. Pearson for providing the plant material; Mr. P. Sidney for photographic assistance; Mrs. E. Ellis for typing the manuscript. The work was funded by Science and Engineering Research Council grant GR/C47069.

References

Gunning, B.E.S. (1976) The role of plasmodesmata in short distance transport to and from the phloem. In: Intercellular communication in plants, pp. 203–224, Gunning, B.E.S., Robards, A.W., ed. Springer, Berlin Heidelberg New York

Gunning, B.E.S., Pate, J.S. (1969) "Transfer cells" Plant cells with wall ingrowths, specialized in relation to short distance transport of solutes – their occurrence, structure and development. Protoplasma **68**, 107–133

Gunning, B.E.S., Pate, J.S. (1974) Transfer cells. In: Dynamic aspects of plant ultrastructure, pp. 441–481, Robards, A.W., ed. McGraw-Hill, London

Gunning, B.E.S., Pate, J.S., Briarty, L.G. (1968) Specialized "transfer cells" in minor veins of leaves and their possible significance in phloem translocation. J. Cell Biol. **37**, C7–12

Gunning, B.E.S., Pate, J.S., Munchin, F.R., Marks, J. (1974) Quantitative aspects of transfer cell structure in relation to vein loading in leaves and solute transport in legume nodules. In: Transport at the cellular level, pp. 87–126, Sleigh, M.A., Jennings, D.H., eds. Cambridge University Press, Cambridge London New York

Harris, N. (1981) Plasmalemmasomes in cotyledons of germinating *Vigna radiata* L. (mung-bean). Plant Cell Environ. **4**, 169–175

Harris, N., Oparka, K.J., Walker-Smith, D.J. (1982) Plasmatubules: an alternative to transfer cells? Planta **156**, 461–465

Pate, J.S. (1980) Transport and partitioning of nitrogenous solutes. Annu. Rev. Plant Physiol. **31**, 313–340

Pate, J.S., Gunning, B.E.S. (1972) Transfer cells. Annu. Rev. Plant Physiol. **23**, 173–196

Spurr, A.R. (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43

Wark, M.C. (1965) Fine structure of the phloem of *Pisum sativum* II. The companion cell and phloem parenchyma. Aust. J. Bot. **13**, 185–193

Received 13 March 1984; accepted 26 January 1985