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The Level of Wall Ingrowths Protrusion in Transfer Cells is a Function of the Sink/Source State of the Leaf

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Abstract: The work aimed at studying transfer cell development in *Pisum sativum* L. based ultrastructural observations. Study was carried out using Leaf Plastochron Index (LPI) and observations were related to our earlier report on the sink/source state of leaflets at varying LPI. The results show that the density of wall ingrowths in transfer cells of minor veins increased with LPI. There was an association between the development of wall ingrowths in transfer cells and the transition of the leaflets from sink to source, establishing reports that wall ingrowths help to increase the plasmalemma surface area needed for the uptake of assimilates during export. The onset of wall ingrowth development in leaflets at LPI 0 supports our earlier report that sink-to-source transition commences at LPI 0 in *P. sativum* L. The similarity between wall ingrowths in transfer cells in the upper half of leaflet lamina between LPI 1 and 2 also supported the earlier report that the upper half of leaflets are in full source state by LPI 1.

Key words: Transfer cell, wall ingrowths, leaf plastochron index (LPI), sink, source, transition

INTRODUCTION

The companion cell is regarded by many workers to be a highly specialized parenchymatous cell, which is derived from the same mother cell as its associated phloem sieve tubes and the companion cells have numerous cytoplasmic connections with the sieve tube. The companion cells are distinguished structurally from other parenchymatous cells by their dense, organelle-rich protoplasts and their numerous branched plasmodesmata connecting them to the phloem sieve tubes which are called pore-plasmodesmata. The companion cell remains functionally alive only as long as its phloem sieve tube does (Evert, 1990; Gagnon and Beebe, 1996). The companion cell and sieve element thus form a unit-the sieve element-companion cell complex (SE-CC complex), (Oparka and Turgeon, 1999), which is recognised as the major site of phloem loading in minor veins (Stadler *et al*., 1995; Truernit and Sauer 1995; Gagnon and Beebe, 1996).

Some companion cells become highly modified and included in these are cells with noticeable wall ingrowths. Companion cells with wall ingrowths are called 'transfer cells' (Pate and Gunning, 1969), which are associated with areas where active short-distance transport of solutes takes place (Pate and Gunning, 1972; Esau, 1977; Gamalei, 1989). The development of wall ingrowths in transfer cells begins with the onset of solute flux. Wall ingrowths help to increase the capacity of solute flux

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between the apoplast and the symplast (Folsom and Cass, 1986; Wimmers and Turgeon, 1991; Turgeon *et al*., 2001).

The transfer cells in *P. sativum* have been classified by Gunning and Pate (1969) as 'A' type transfer cells. These are transfer cells that are primarily associated with the phloem and have wall ingrowths along the entire perimeter, except in the portion of the wall abutting the phloem sieve tube, where the ingrowths are relatively few. In most cases, wall ingrowths develop as finger-like projections, averaging 0.2 μm in diameter and up to 2 μm in length (Leopold and Kriedemann, 1975; Wimmers and Turgeon, 1991).

We have previously reported (Ade-Ademilua and Botha, 2007) that the transition from a sink to a source state within developing leaves is synonymous with the maturation processes in leaves. In the report, we showed with the aid of fluorescence microscopy that sink-source transition state correlated with the leaf plastochron index (LPI) of common peas, *Pisum sativum* L. LPI is the physiological age of the leaf as developed by Erickson and Michelini (1957) and the formula had been remodified by Ade-Ademilua and Botha (2005) to suit the peculiar compound leaf of peas. It has been shown that young leaves remain strong sinks until LPI 0 has been reached and then the sink-to-source transition process occurs until the leaf has reached LPI 1.8 and the transition of the leaflets to completely source state is completed by LPI 2.0 (Ade-Ademilua and Botha, 2007).

Reports have shown that transfer cells of minor-vein phloem develop only after export of photoassimilate begins in *P. arvense* (Gunning *et al*., 1968) and *P. sativum* (Peterson and Yeung, 1975) and that wall ingrowths in minor vein transfer cells of *P. sativum* facilitate uptake of photoassimilate by increasing plasmalemma surface area (Wimmers and Turgeon, 1991) as originally proposed by Gunning *et al*. (1968) in their study of transfer cells of *P. arvense*. Thus, we decided that since we have been able to establish with the aid of a fluorescent phloem-mobile tracer that the sink/source state of a leaf is synonymous with its LPI (Ade-Ademilua and Botha, 2007), it is only imperative to study transfer cell development in *P. sativum* in relation to LPI with the hope that the results will not only confirm the conclusion in our Ade-Ademilua and Botha (2007) reports but also those of Gunning *et al*. (1968), Peterson and Yeung (1975) and Wimmers and Turgeon (1991) in terms of the relationship between the wall ingrowths of transfer cells and the sink/source state of a leaf at any LPI.

MATERIALS AND METHODS

Growth pattern and condition is as described by Ade-Ademilua and Botha (2005). *Pisum sativum* var. Greenfeast was grown from seed, under controlled environment in growth chambers (Conviron Model S10H, Controlled Environments Ltd., Winnipeg, Canada) at 25/18°C day/night at 16 h photoperiod with CO_2 maintained at 360 μmol mol^{-1} with fluctuations within ±15 μmol mol^{-1}. CO_2 was monitored using the integrated computer-controlled Horiba APBA-250 indoor CO_2 monitor (Horiba Ltd., Japan). Plants were illuminated using a combination of fluorescent tubes (F48T12.CW/VHO1500, Sylvania, USA) and frosted incandescent 60 W bulbs (Philips, Eindhoven, The Netherlands). Photosynthetic active radiation (PAR at 400-700 nm) was measured at about 250 μmol m^{-2} sec^{-1}, 20 cm above soil level recommended by Olivier and Annandale (1998), with a Li-85A Quantum sensor (Li-Cor Inc., Nebraska, USA). Pot positions were changed every day in a matrix pattern, to avoid any chamber position effect.

Plastochron Analysis

Plastochron index of plants (PI) per day was calculated for each plant based on the Erickson and Michelini (1957)'s formula, as revised by Ade-Ademilua and Botha (2005) where the reference length is 20 mm:

$$PI = n + \frac{\log L_n - \log 20}{\log L_n - \log L_{n+1}} \quad (1)$$

Where:

n = Serial number (counting from the shoot base) of that leaf which just exceeds 20 mm
$\log L_n$ = Natural logarithm of the length of the leaf n
$\log L_{n+1}$ = Natural logarithm of the next or subsequent leaf with a length that is less than 20 mm

Consequently, the Leaf Plastochron Index (LPI) is calculated as:

$$LPI_n = PI - n \quad (2)$$

this is derived by simply subtracting the leaf's serial number from the plant's plastochron age (Erickson and Michelini, 1957).

Transmission Electron Microscopy

Tissues (1×2 mm) from the upper and mid-section of the lamina of leaflets at different plastochron ages with minor veins perpendicular to the longer side of the section, were cut and fixed in a cold solution of 2.5% glutaraldehyde and 0.5% paraformaldehyde in 0.2 m sodium cacodylate buffer for 24 h at 4°C. Buoyant tissues were evacuated by placing vials with fixative in a vacuum desiccator and gradually pulling a slight vacuum (16.93 kPa). Vials were left under vacuum for about 1 h and then returned to the refrigerator. Tissues were then rinsed for 30 min with two changes in 0.2 m sodium cacodylate buffer and the post-fixed in 2% osmium tetroxide solution at 4°C for 4 h, after which tissues were rinsed for 30 min with two changes in cacodylate buffer. Fixed tissues were the dehydrated through a series of 30 (5 min), 50 (5 min) and 70% (overnight) ethanol at 4°C and then further dehydration were carried out at room temperature with 80 (5 min), 90 (5 min) and 100% (10 min with two changes) ethanol and further 30 min with two changes in propylene oxide. Plastic infiltration of the tissue was carried out by putting tissues in series of 75:25 (3 h), 50:50 (overnight), 25:75 (6 h) v:v propylene oxide: ERL-SPURR epoxy resin (Spurr, 1969). Tissues were then left in pure resin overnight. Tissues were then placed in fresh resin in capsules for polymerisation at 60°C for 36 h. Ultrathin (gold-silver) sections were cut using a diamond knife mounted on a RMC MT-7 ultra microtome (Research and Manufacturing Co. Inc., Tucson, Arizona). Sections were collected on Formvar©-coated single-slot copper grids and stained for 30 min in uranyl acetate (2% in H_2O) and 5 min in Reynolds lead citrate (Reynolds, 1963). Sections were viewed and photographed using a Jeol JSM 1210 transmission electron microscope (Tokyo, Japan).

RESULTS AND DISCUSSION

Almost, if not all, transfer cells observed had dense cytoplasm which also contained chloroplasts and mitochondria. It was apparent that the wall ingrowths were more frequent in transfer cells of class IV veins than in those of class V veins. The transfer cells with their characteristic wall ingrowths is typical of that described by Gunning *et al.* (1968) for transfer cells in minor veins of *P. arvense* leaflets. Transfer cells are sites of known or assumed high solute flux (Wimmers and Turgeon, 1991) synonymous with the phloem of the minor veins of source leaves (Gunning and Pate, 1969; Bourquin *et al.*, 1990) and the development of wall ingrowths has been demonstrated to begin with the onset of solute flux (Gunning and Pate, 1969; Folsom and Cass, 1986). Two contrasting specializations which are likely to favour efficient absorption of materials into a cell are: (1) enhancement of symplasmic transfer through the development of abundant plasmodesmata and (2) the promotion of

capacity uptake from the extracellular environment through increase in the cell's surface:volume ratio. The transfer cells of *P. sativum*, reported in this study, like those of their counterpart in *P. arvense* (Gunning *et al*., 1968), are possibly specialized in the latter direction. In the pea plant, the wall ingrowths in transfer cells form part of an irregular layer of wall material deposited secondarily on the primary wall and have loose microfibrillar texture. Wall microfibrils, possibly created by fixation damage, are visible in the space between the plasma membrane and the wall. The plasma membrane appears to have granular or fibrillar material deposited on it and in this is similar to that reported by Gunning *et al.* (1968).

The extent of the wall ingrowths in transfer cells observed in each group of minor veins was also largely dependent on the LPI.

LPI 0

Wall ingrowths are not evident in leaflets until they attain approximately LPI 0. Even at this LPI, the walls of many transfer cells appear to be in the process of forming ingrowths (closed arrows, Fig. 1A). Where present, preformed wall ingrowths are few and not usually pronounce (open arrows, Fig. 1A). The presence of one to two wall ingrowths along with the appearance of new protrusions along the wall of transfer cells of leaflets at LPI 0 is taken as evidence that the emergence of wall ingrowths in transfer cells commences when leaflets are about LPI 0. The onset of the protrusion of wall ingrowths as shown in leaflets at LPI 0 therefore confirms the data from the fluorescence experiments which showed that sink-to-source transition commences at LPI 0 in *P. sativum*. This also confirms the reports of Gunning *et al.* (1968) and Peterson and Yeung (1975) that transfer cells of minor-vein phloem develop only after export of photoassimilate begins in *Pisum* sp. From Pate and Gunning (1969)'s illustration, the development of a transfer cell simply implies the formation of wall ingrowths by companion cells.

LPI 0.5

Wall ingrowths appear denser and more in number in transfer cells at LPI 0.5 (Fig. 1B). From our report in Ade-Ademilua and Botha (2007), we have shown that the process of sink to source transition is at an advanced stage by LPI 0.05. It is therefore not surprising that the formation of wall ingrowths in transfer cells have been more established by this LPI.

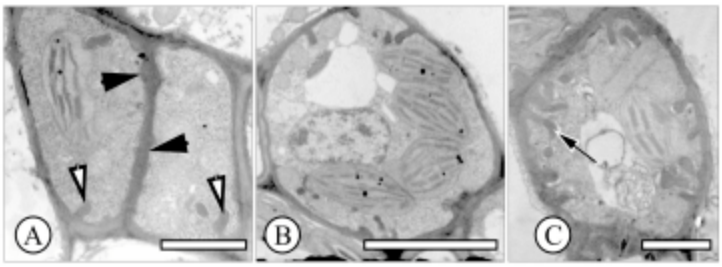

Fig. 1: Transfer cells in the minor veins of *Pisum sativum* L. leaflets at different LPI with characteristic wall ingrowths. Very few wall ingrowths (open arrow heads) are apparent in transfer cells at LPI 0 (A) but numerous small projections of wall tissues are evidence of emergent ingrowths and the wall appear to be in the process of producing more wall ingrowths (closed arrow heads). Note the increase in the number of wall ingrowths visible as tissues progressed from LPI 0.5 (B) to LPI 1 (C). Most of the wall ingrowths in leaflets at LPI 1 are exceptionally long and some form elaborated structures (solid arrow). (A) bar = 1.25 μm; (B) bar = 5 μm; (C) bar = 4 μm

Table 1: Characteristics of wall ingrowths in transfer cells of *Pisum sativum* L. leaflets (upper and mid portion) at certain LPIs

LPI	Wall ingrowth characteristics	Inference in relation to sink/source state
< 0	No wall ingrowths	Sink
0	Just emerging from most transfer cells. Where present, they are few and not pronounced.	Commencement of sink to source transition
0.5	There are more wall ingrowths. They are dense and more pronounced.	Advance stage of sink to source transition
1 - 2	Dense and pronounced wall ingrowths have become elaborate by being bent, with some ingrowths fused with one another	Source state

LPI 1-2

Transfer cells of leaflets at LPI 1 have similar distribution of wall ingrowths as leaflets at LPI 2. Wall ingrowths in transfer cells within leaflets at LPI 1 to 2 are even more numerous compared to those younger leaflets. Also, the ingrowths appear longer and pronounce (Fig. 1C), with some bent (open arrow heads) and some fused (closed arrow head). Leaflets at LPI 1 are typical transition leaflets, half of the lamina is a source (upper) while the other half (lower) is a sink; the transition of rest of the lamina from sink to source continues and is completed by the time the leaflets are at LPI 2 (Ade-Ademilua and Botha, 2007). Therefore the tissues from the upper and mid portion of the lamina used in this study are exporting from LP1 1.0 through LPI 2. The elaborate wall ingrowths observed in leaflets from LPI 1-2 are thus typical of highly exporting mature transfer cells. Table 1 shows a summary of the characteristic features of wall ingrowths in the transfer cells of leaflets at the different LPIs.

It is not surprising that the number of wall ingrowths increase as the leaf matures from LPI 0 to LPI 1 because according to Gunning *et al.* (1968), wall ingrowths increase in number and surface area as the photosynthetic capacity of the leaflet increases, to ensure an increase in plasmalemma surface area needed for higher assimilate transfer across this transfer cell-sieve tube interface and the subsequent export of the assimilate. The elaboration of wall ingrowths in transfer cells of leaflets from LPI 1 was also observed by Wimmers and Turgeon (1991), more especially in minor veins of high-light leaves. These observations along with our earlier report in (Ade-Ademilua and Botha, 2007) therefore shows that the nature of wall ingrowths in transfer cells is an indicator of the sink-source state of a leaf.

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