Wound Phloem in Transition to Bundle Phloem in Primary Roots of *Pisum sativum* L.

II. The Plasmatic Contact Between Wound-Sieve Tubes and Regular Phloem

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Received March 15, 1985
Accepted July 13, 1985

Summary

Following severance of the root stele mature bundle-sieve tubes show a rapid wound response, plugging their sieve pores and depositing callose. Close to the blocked sieve tubes the predetermined but still immature bundle sieve tubes differentiate and consist of mature sieve elements 48 hours after wounding. Within a serially sectioned area the existence of lateral sieve pores connecting blocked bundle-sieve tubes with those which matured after wounding could be proved. Wound-sieve tubes are initiated close to the latter, linked to them by lateral sieve pores. The wound-sieve tubes elongate bidirectionally, parallel to the interrupted phloem trace, until a first (towards the cortex) deviating member is established on one end and, on the other, the length of the common course with the bundle is sufficient for assimilate transfer. Presumably, both initiation and elongation of wound-sieve tubes are guided by preexisting plasmodesmata, which later give rise to sieve pores. Eventually the deviating wound-sieve tubes are in close plasmatic contact with those bundle-sieve tubes which mature after wounding and hence, indirectly, with blocked sieve tubes.

One precondition to the restitution of translocation within blocked bundle-sieve tubes is a secondary opening of the plugged sieve pores. The reversibility of callose deposition and the structure of functional pores are discussed.

The model of sequential differentiation for channelling auxin in undifferentiated tissue (SACHS 1975) is compared with the sequential differentiation of wound-sieve tubes.

Keywords: Phloem contact; Phloem regeneration; *Pisum*; Sieve pores; Wound phloem.

1. Introduction

During angiosperm development primary tissues initially consisting of homogeneous cells successively differentiate to give rise to protoderm, ground meristem and procambial traces. The latter are precursors of vascular bundles in the shoot and the central cylinder in the root. Within the root stele several vertical tiers of cells, with species-specific radial symmetry, differentiate so as to translocate assimilates and water. Each stelar phloem and xylem pole is established by a few protophloem-sieve tubes resp. protoxylem elements, which develop longitudinally with the youngest member at the apical end. In the elongating part of the root the first developed protophloem-sieve tubes are obliterated and compensated for by metaphloem-sieve tubes. The latitudinal development of metaphloem involves successive initiation of vertical cell tiers to divide and differentiate into sieve tubes and their companion cells, until all such predetermined cell tiers are specialized. Only then does the secondary development of phloem start with cambial activity, enlarging the assimilate translocation capacity by increasing the number of functional sieve tubes. Thus, in a transverse section of the root stele some sieve tubes may have been obliterated (protophloem and early metaphloem), while mature or still differentiating tubes are present (metaphloem), and others may have not yet been initiated (secondary phloem).

A growing root is a sink for assimilates. The transfer
path of assimilates from the source, e.g. the food-storing cotyledons of *Pisum*, to the sink demands not only vertical, but also lateral transfer within the phloem trace, *i.e.* from secondary phloem to functional meta-phloem and, where its sieve tubes are still immature, again centrifugally into protophloem-sieve tubes. In addition lateral flow of assimilates is necessary between stelar and laterally connected phloem. Such connections regularly develop towards lateral roots. As a result of external influences, e.g., bacterial infections, experimental amputation of the root tip or incision into the stele, lateral phloem connections may supply legume root-nodules (Pate et al. 1980), amputation-induced lateral roots, or separated parts of the root. After traversing the remaining cortex bridge wound phloem will reconnect existing phloem bundles (Robbertse and McCully 1979).

A regularly formed lateral root originates in a small population of pericycle cells, which presumably maintain their cell-cycling after emergence from the apical meristem (Blakeley et al. 1982). Phloem induced by external influences, e.g., by wounding, however, must develop from formerly quiescent cortical cells, which are not predetermined to differentiate in this way (Schulz 1986). After severance of the root stele the transport of assimilates has to be laterally deflected towards the cortex and its wound-sieve tubes. Prior to this deviation, vertical flow of assimilates within the wounded bundle-sieve tubes will be blocked, in order to prevent the loss of sugars. This wound reaction occurs very rapidly at the sieve plates. As little as one minute after a first incision into functional phloem the well known artifactual changes (*cf.*, Evert 1982) can be observed.

Several attempts have been made to show sieve tubes and more especially, sieve pores in their functional state, and to minimize fixation-induced artifacts, *e.g.*, by freeze-etching of plant parts (Johnson 1968 and 1973), freeze-fracturing of tissue cultures (Sjolund et al. 1983), rapid fixation by acrolein (Cronshaw and Anderson 1969, Behnke 1971), reduction of the translocation rate (Evert et al. 1973) and fixation of entire plants (Cronshaw and Anderson 1969, Walsh 1980). Few investigations considered intentional artifact development which can show that changes in the sieve plates are variable in extent, according to the time between incision and fixation, and the distance between the incision surface and sieve plate in question (Engleman 1965, Anderson and Cronshaw 1969). The first, very rapid reaction to wounding within sieve tubes is the plugging of sieve pores with P-protein, followed by the production of callose (Evert 1982). The synthesis of callose, which covers sieve plates after their pores have been constricted is not only a wound reaction but also an ordinary feature occurring prior to the later obliteration of non-functional sieve tubes (*"definitive callose"*, EsaU 1969, p. 49). Sieve tubes being obliterated before winter are compensated for by younger ones in spring. In some plants, *e.g.*, *Vitis*, this callose may be decomposed in the spring, in order to allow conduction before the first new sieve tubes have differentiated (EsaU 1965, "dormancy callose", EsaU 1969, p. 49).

In the preceding paper (Schulz 1986) it was shown that wound-sieve tubes are initiated close to pre-existing bundle-sieve tubes and that they run partly parallel to them. This paper will elucidate the role of pre-existing bundle-sieve tubes considering the possible re-uptake of translocation within sieve tubes initially blocked by the incision. Plasmatic contacts between these sieve tubes and those which matured after wounding have been observed. The latter allow lateral assimilate transfer into wound-sieve tubes.

In order to demonstrate the connections of all sieve tubes in a phloem trace following a 48 hour-regeneration period, a reconstruction from serial sections (corresponding to that in the preceding paper) was made. Apart from the course of the depicted sieve tubes the developmental state of their members and the ultrastructure of their plates was also recorded. Combining this data it was possible to evaluate the functional stage of the depicted sieve tubes prior to wounding and also to show the development of this transitional area which facilitates lateral assimilate transfer from bundle- to wound-sieve tubes.

2. Material and Methods

The growth conditions for *Pisum sativum* L. c.v. "Kleine Rheinländerin", the mode of wounding of the primary root stele, fixation and embedding procedures for longitudinal sections, and monitoring of semithin sections for the presence of sieve tubes are as described in the preceding paper (Schulz 1986). The wounded plantlets were allowed up to 48 hours for wound reaction and vascular tissue regeneration. A representative case of the wound phloem (the development of which was described in the preceding paper) to regular phloem transition zone was serially sectioned, omitting only a few semithin sections. All sieve tubes in this area 48 hours after wounding are shown schematically and their ultrastructural state is documented (see Fig. 25). The diagram shows both all bundle-sieve tubes and the incomplete wound-sieve tubes in the depicted phloem trace. Moreover, it includes all plasmatic connections found between the different sieve tubes. In order to ease orientation all depicted sieve tubes within the serially sectioned area are denoted by capital letters. Their sieve-tube