Callose in the developing pericyclic and phloem fibres

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INTRODUCTION

Owing to the elaboration of new specific methods for callose (β -1,3-glucan) detection, this substance in recent years has again become an object of interest to cytologists.

Callose occurs rather commonly in plants. A detailed review of the literature concerning callose in plant tissues is given in the works of Eschrich (1956) and Currier (1957).

Nevertheless, the role of this substance in the cell remains so far unclarified. In the opinion of most authors callose is a normal component of the cell. The data of some investigators, however, relative to such factors favouring callose formation as change in turgor under the influence of chemical or mechanical stimuli (Eschrich 1963), seem to suggest that this substance is of pathological origin.

The fact of the periodical appearance and disappearance of callose in the development cycle of normal active cells for instance in the development of microsporocytes (Waterkeyn 1964) may perhaps throw a new light on the problem of the role of callose in the cell.

The present report describes another example of periodical appearance of callose in young pericyclic and phloem cells, which seems to point to the physiological role of this substance in plant cells.

MATERIAL AND METHODS

One-year or older shoots of *Salix fragilis* L., *Salix alba* L., *Acer pseudoplatanus* L. and *Tilia cordata* Mill. served as material for investigation.

Serial cross-sections prepared free-hand at various heights of the stem, beginning from the segment just below the apical bud, were investigated.

Callose was revealed by two methods considered as specific for this substance. The first consisted in staining with resorcinol blue (Eschrich 1954) and the second in the fluorescence of callose after application of an aqueous solution of aniline blue (Eschrich and Currier 1964). Both the methods gave consistent results.

For differentiating callose from other components of the cell wall chlor-zinc iodine, phloroglucinol with hydrochloric acid and Mäule's test were used.

The observations were performed by means of a Zeiss Lumipan light microscope and a MUF-3M fluorescence microscope.

RESULTS

In the primary growth of *Salix fragilis* there occur under the endodermis fibres referred to by Metcalfe and Chalk (1950) as pericyclic. In one-year-old shoots of this plant about 30 cm long which were cut in August, the pericyclic fibres are easily distinguishable at a distance of about 2 cm from the apex. They consist of thin-walled (c. $0.5\ \mu$) tetra- or hexahedral cells with a thick cytoplasmic content. Their diameter amounting to $3\text{--}6\ \mu$ is several times smaller than that of the surrounding parenchymatous cells.

At this period of development the cell walls in the fibres contain cellulose. They stain violet with chlor-zinc iodine. No callose was revealed in them, they remained colourless in resorcinol blue and showed no fluorescence in aniline blue.

Fig. 1. Fluorescence of pericyclic fibres in *Salix fragilis*.

Fluorochrome — aniline blue. The arrows indicate fluorescence of callose in the sieve tubes. $520\times$.

On the cross-sections cut at about 10 cm from the shoot tip, light-blue staining of the wall fibres by resorcinol blue and distinct fluorescence could be observed. The middle lamella of these fibres showed a more intense colour than the adjacent layers. The test for cellulose was less pronounced and the tests for lignin gave negative results.

The amount of callose in the walls of the pericyclic fibres increased gradually showing the most intense staining with resorcinol blue and the strongest fluorescence (Fig. 1) at a distance of about 15–18 cm from the shoot apex.

In sections from segments further removed from the shoot tip (20–25 cm), the colour intensity in the cell walls of the pericyclic fibres gradually decreased. The ability to staining with resorcinol blue disappears earliest in the middle lamella and later in the adjacent layers. Within this segment staining with resorcinol blue allows one to observe successively the fibres with very light middle lamellae with a blue inner layer, and further fibres which have lost their ability to staining. In the latter fluorescence in aniline blue also disappears almost completely. Toget-

ther with the fading of the blue staining for callose, lignification of the walls progresses. Lignin appears earliest in the middle lamellae and then in all the remaining wall layers.

In shoots with a smaller annual length increment analogous differences in the chemical characteristics of the walls of the pericyclic fibres occurred nearer each

Fig. 2. Phloem fibres of *Tilia cordata* stained with resorcinol blue: a — layer of fibres staining blue in the preparation. About 400 $\times$.

other. Longer shoots had longer successive segments of fibres with cellulose walls, fibres in which layers of callose appeared and lignified fibres.

At a later vegetation period (September, October) the whole process of chemical changes in the cell walls of the fibres occurs within a relatively short segment of the shoot below its apex.

In sections taken from all the cross-sections the callose of the sieve plates (Fig. 1) and the sheaths of calcium oxalate crystals which occur rather abundantly in primary cortex cells always gave a positive test for callose. The crystal sheaths have been described earlier as callose deposits (Thaler and Weber 1957). The regular

occurrence of callose in these parts of the stem greatly facilitated the evaluation of the results of staining and the fluorescence.

A process of chemical changes analogous to that described in *Salix fragilis* was observed in the cell walls of pericyclic fibres of one-year-old shoots of *Salix alba* and in phloem fibres of *Acer pseudoplatanus* and *Tilia cordata* shoots of the same age.

In shoots in secondary growth there occur in all these plants several layers of phloem fibres arranged alternatively between the remaining elements of phloem. On cross-sections of such shoots all the stages of the chemical changes in the phloem fibre cell walls could be followed. Thus for example in *Tilia cordata* exhibiting at a certain stage three layers of phloem fibres, the youngest ones situated nearest to the cambium gave a positive test for cellulose or, more frequently, for callose. The two layers of older fibres further removed from the cambium had lignified walls (Fig. 2). These layers in contrast to the first one did not stain with resorcinol blue and did not show fluorescence in aniline blue.

On sections from several-years-old *Salix fragilis* shoots stained with resorcinol blue, almost the whole gradation of blue shades could be followed beginning with intensely blue phloem fibres lying close at the cambium, to the oldest colourless fibres most distant from it.

Simultaneously with the fall of the callose content in the phloem fibre cell walls, the amount of lignin in them increased.

Thus callose occurrence precedes the process of lignification of the walls of both pericyclic and phloem fibres. The regularity of this phenomenon seem to point to the physiological meaning of this substance in the process of wall-building. Further investigations may elucidate the nature of this role.

SUMMARY

The presence of callose in the cell walls of pericyclic fibres of *Salix fragilis* and *Salix alba* and of phloem fibres of *Acer pseudoplatanus* and *Tilia cordata* was demonstrated. The studies were carried out by means of light microscopy with resorcinol blue staining and by fluorescence microscopy with the use of aniline blue.

It was proved that callose occurs in the walls of pericyclic and phloem fibres periodically always in the stage preceding the lignification process.

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Kaloza w rozwijających się włóknach perycyclicznych i łykowych

Streszczenie

W ścianach włókien perycyclicznych *Salix fragilis* i *Salix alba* oraz w włóknach łykowych *Acer pseudoplatanus* i *Tilia cordata* wykazano obecność kalozy. Badania przeprowadzono w mikroskopie świetlnym stosując barwienie błękitem rezorcynowym oraz w mikroskopie fluorescencyjnym przy użyciu błękitu anilinowego.

Wykazano, że kaloza w ścianach włókien perycyclicznych i łykowych występuje okresowo, poprzedzając zawsze proces lignifikacji.