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# Calcium deficiency in potato (*Solanum tuberosum* ssp. *tuberosum*) leaves and its effects on the pectic composition of the apoplastic fluid

**Simone Seling[a], Alexander H. Wissemeier[b,*], Pierre Cambier[c] and Pierre Van Cutsem[c]**

[a]*Institute of Plant Nutrition, University of Hannover, Herrenhaeuser Str. 2, 30419 Hannover, Germany*
[b]*BASF Agricultural Center Limburgerhof, P.O. Box 120, 67114 Limburgerhof, Germany*
[c]*Institute of Biotechnology, University of Namur, rue de Bruxelles 55, 5000 Namur, Belgium*
**Corresponding author, e-mail: alexander.wissemeier@basf-ag.de*

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**Potato plants (*Solanum tuberosum* ssp. *tuberosum* cv. Adelheid), multiplied in vitro, were cultivated in growth chambers on nutrient solution at calcium regimes of 1000, 90, 60 or 30 μ*M* Ca. An absolute Ca deficiency, particularly at the low Ca-supply levels of 30 and 60 μ*M* Ca, manifested itself initially in the form of marginal necrosis in younger, but not in the youngest, leaves of the potato plants. Further symptoms were rolling of the leaf lamina, browning of veins and roots, and finally necrosis also of the youngest leaves. Only in an advanced stage of Ca deficiency, the meristem of the shoots died. Ca-deficiency symptoms could be expected at a Ca content in the leaves of less than 5 mg Ca (g dry weight)$^{-1}$. However, there was no close negative correlation between the extent of leaf damage and the total Ca content of the leaves. In order to obtain information about the Ca concentration in the apoplast fluid of the leaves, apoplastic washing fluid was extracted by an infiltration-centrifugation technique. A low Ca supply reduced the Ca concentration both in the apoplast fluid of the leaves and in the cell walls. Up to 60% more diffusible pectin fragments were then found in the apoplast of younger leaves, as compared to the control supplied with an optimum Ca level of 1000 μ*M*. The amount of diffusible pectins accounted for 1–2% of the total pectin content of younger potato leaves. The size of the existing pectin fragments varied depending on the Ca supply. Compared with an optimum Ca supply of 1000 μ*M*, fewer monomers and up to 7 times more diffusible pectin fragments with a degree of polymerization 9–20 were present at the low Ca-supply level (30 μ*M*). In addition, polygalacturonase activity in tissue homogenates increased remarkably with Ca deficiency. Thus it appears that one major effect of Ca deficiency was a stimulation of the activity of polygalacturonase, which could control the breakdown of pectic polysaccharides in the cell wall. Whether the release of potentially biologically active pectic fragments in cell walls might be involved in the occurrence of Ca-deficiency symptoms is discussed.**

## Introduction

On arable soils, plants rarely show signs of absolute Ca deficiency, as Ca is one of the more abundant cations in soil solutions. To a certain extent, however, Ca deficiency may be a problem on acid soils. Also in many vegetables and fruits, physiological Ca disorders may seriously reduce quality (Bangerth 1979), and therefore economic value. Ca deficiency may arise in the whole plant, or in a particular organ of the plant, but relatively little is known about the way in which Ca deficiency causes the observed symptoms. Typically, the symptoms of Ca deficiency, in addition to growth reduction, also comprise browning phenomena and, in severe cases, necrosis of whole areas of plant tissue.

In contrast to other macronutrients, a high proportion of the total Ca in plant tissue is located in the cell wall (apoplast). The main known functions of Ca in the apoplast are to maintain the integrity of the plasmalemma and to stabilize the pectins both intra- and inter-molecularly.

The physiology of Ca shortage in the apoplast, however, is not fully understood. At least in vitro it could be shown that Ca removal from the cell wall by chelating agents promoted the hydrolysis of pectin molecules (Brady et al. 1985). Injection of Ca chelating agents into apples induced symptoms similar to those of bitter pit (Steenkamp et al. 1983), a well known Ca deficiency-related disorder. Similarly, the Ca deficiency-related disorder tipburn in lettuce

*Abbreviations* – AWF, apoplastic washing fluid; CWM, cell-wall material; DP, degree of polymerization; GalUA-eq, galacturonic acid equivalent; PG, polygalacturonase.

could be enhanced by applying Ca chelators such as citric acid (Thibodeau and Minotti 1969). Taken together, these findings may indicate a functional relationship between the level of physiologically active Ca, pectin breakdown in the apoplast, and the occurrence of visible Ca-deficiency symptoms. The observation that injection of polygalacturonase (PG), a pectin hydrolysing enzyme, into plant tissue may cause necrosis (Cervone et al. 1987) also points in this direction. Pectic fragments of a degree of polymerization (DP) of 9–15 are biologically active and elicit browning of cells (Messiaen and Van Cutsem 1993, 1994) and may even lead to cell death (Ryan and Farmer 1991).

We suggest that under Ca deficiency there is a release of pectic fragments into the leaf apoplast, which have been shown to be biologically active (Jin and West 1984) and may be involved in the formation of Ca-deficiency symptoms such as browning and necrosis. We therefore analysed the apoplastic fluid of potato leaves grown at different Ca-supply levels to detect pectic fragments.

## Materials and methods

### Plant culture

In vitro propagated explants of potato plants (*Solanum tuberosum* ssp. *tuberosum* cv. Adelheid) were cultivated in a growth chamber in a nutrient solution of the following composition: 0.5 m*M* $MgSO_4$, 1.5 m*M* $KNO_3$, 0.1 m*M* $KH_2PO_4$, 10 μ*M* $H_3BO_3$, 1 μ*M* $MnSO_4$, 0.2 μ*M* $CuSO_4$, 0.5 μ*M* $ZnSO_4$, 0.01 μ*M* $(NH_4)_6Mo_7O_{24}$, 10 μ*M* FeEDTA, and initially 1000 μ*M* $CaCl_2$. The photon flux density was 180 μmol $m^{-2}$ $s^{-1}$ with a light/dark period of 16/8 h and a day/night temperature of 20/15°C. Relative humidity was in the range of 45–55%. After 10 days the plants were transferred to solutions of 1000 μ*M* (control), 90 μ*M* (experiment 1 only), 60 or 30 μ*M* Ca at pH 5.5. The nutrient solution was constantly aerated and completely renewed every 3 days. After a further 28 days (experiment 1; $n = 10$), 21 days (experiment 2; $n = 6$) or 30 days (experiment 3; $n = 40$), the plants were harvested and fractionated into laminae, petioles, stems and roots (laminae are referred to as leaves in the following). The plant material was dried at 70°C to constant weight before dry matter determination and mineral analysis.

### Extraction of apoplastic washing fluid (AWF)

A technique described by Rohringer et al. (1983) was used. At harvest, younger leaves (experiment 1 and 3: 3 leaves together; experiment 2: 4 leaves separately) were detached, weighed, vacuum-infiltrated with demineralized water, blotted dry and reweighed. The infiltrated leaves were rolled with a polyethylene foil around a 25 ml plastic syringe, which was placed in a 50 ml plastic syringe with a 1.5 ml Eppendorf cup at its tip, and centrifuged for 15 min at 440 *g* to yield the AWF. Cytoplasmic contaminations were in the range of 0.2–0.5%, as determined by the ratio of malate dehydrogenase (MDH; EC 1.1.1.37) activity in the AWF to MDH activity in the total leaf homogenate. MDH activity was determined according to Bergmeyer and Bernt (1974).

### Calcium

Ca in the AWF of leaf tissue (termed diffusible Ca in the apoplast) and isolated cell-wall material (CWM) was measured in 0.25 *M* $HNO_3$ by atomic emission spectrometry (Spectro Flame-EOP), either directly or after dry ashing at 450°C for 8 h.

### Cell-wall preparation

Cell walls were extracted as described by Hu et al. (1996), with the exception that the leaves were first homogenized in ice-cold water and then sequentially washed several times with 80% ethanol, chloroform:methanol mixture (1:1, v/v), and acetone, and then finally dried at room temperature.

### Uronic acid determination

Uronic acid concentration in the AWF was determined after the method of Blumenkrantz and Asboe-Hansen (1973). For this fraction the term diffusible pectins is used. Uronic acid content of CWM was determined as described by Ahmed and Labavitch (1977).

### Analytical HPAEC-PAD of oligogalacturonides

Samples of AWF of 4–6 plants (experiment 3) were pooled to 1 ml solutions, tenfold concentrated by lyophilization, and pectic fragments demethylesterified with 0.15 *M* NaOH. Afterwards the pectic fragments were separated on a CarboPac PA-100 column (Dionex, Sunnyvale, CA, USA) using a linear gradient of 200–800 m*M* NaOAc at 1 ml $min^{-1}$ over 50 min, according to the technique of Rocklin and Pohl (1983). To facilitate the detection of oligogalacturonides and to minimize baseline drift, NaOH (500 m*M*) was added postcolumn at a flow rate of 1 ml $min^{-1}$ using a pressurized reagent delivery system (Dionex). The electrochemical detector was operated at 30 μA (0–10 min) and 0.1 μA (10–50 min) sensitivity in the pulsed amperometric detection. This procedure separates oligogalacturonides within a degree of polymerization (DP) of 1–20.

The DP was determined by comparison of elution time of peaks with that of a mixture of oligogalacturonic acids obtained by hydrolysing a sample (10 g $l^{-1}$) of polygalacturonic acid (Sigma-Aldrich Chemie, Steinheim, Germany; P1879) in HCl by refluxing at 100°C for 45 h at pH 3.2 (Gillet et al. 1992).

### Polygalacturonase (PG) extraction and enzyme assay

PG was extracted from leaves by a method similar to that described by Pressey and Avants (1973) for tomato fruits. Frozen leaves (1 g fresh weight) were homogenized in 4 ml of ice-cold water by using pestle and mortar, and all subsequent manipulations were carried out at 4°C. The slurry was centrifuged for 20 min at 5000 *g*, resuspended in 4 ml of NaCl (1 *M*, adjusted at pH 6 with 0.5 *M* NaOH), and stirred for 3 h before a final centrifugation for 20 min at 5000 *g*. The supernatant was then ready for the assay.

PG activity was determined reductometrically using the cyano-acetamide method according to Honda et al. (1982):

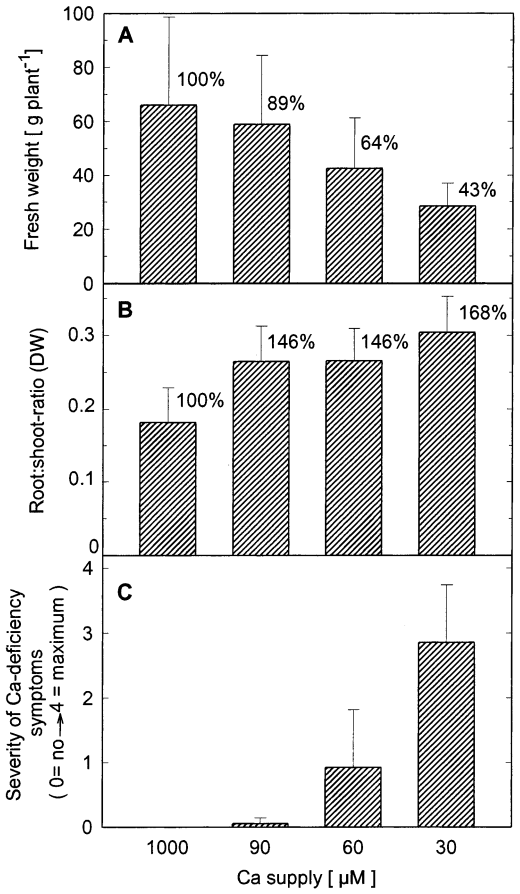


Fig. 1. Fresh weight (A), root:shoot ratio (B) and incidence of Ca-deficiency symptoms of leaves (C) of potato plants after 28 days in nutrient solution at different Ca-supply levels (n = 10, ± SD).

50 µl of supernatant, together with 250 µl of 0.1% (w/v) polygalacturonic acid (Sigma-Aldrich Chemie P1879) were incubated for 2 h at 37°C. The reaction was then stopped by heating to 100°C for 10 min. After addition of 350 µl of 1% (w/v) cyano-acetamide and 700 µl of 0.1 *M* borate buffer (pH 9), the sample was boiled for 10 min at 100°C, and the absorption was measured at $\lambda = 276$ nm. A blank reading was obtained for each sample by substituting the polygalacturonic acid by water.

## Results

### Growth response and Ca-deficiency symptoms

Low Ca-supply levels affected *S. tuberosum* growth and induced Ca-deficiency symptoms (Fig. 1A,C). The symptoms in leaves could already be observed before growth was significantly inhibited. Shoot growth was more seriously affected than root growth. The root:shoot ratio increased with decreasing Ca supply (Fig. 1B).

Ca-deficiency symptoms in shoots of potato plants were first observed as necrosis of leaf margins including the tips. Browning of the veins could also be observed. Ca-deficiency symptoms first appeared in young, expanding leaves only (Fig. 2). In an advanced stage of Ca deficiency, the youngest leaves also began to exhibit necrotic lesions. Isolated necrotic spots were observed in the intercostal area. Together with the occurrence of necrotic lesions, the leaf lamina rolled towards the midrib. An absolute Ca deprivation over a prolonged period of time finally killed the shoot meristem. At a low Ca-supply level, the root growth was reduced and the roots turned brown.

### Calcium content

The Ca content of stems and petioles (data not shown), roots, shoot tips and leaves decreased with decreasing Ca supply. At each Ca-supply level, the Ca content increased from the younger to the older leaves (Fig. 3).

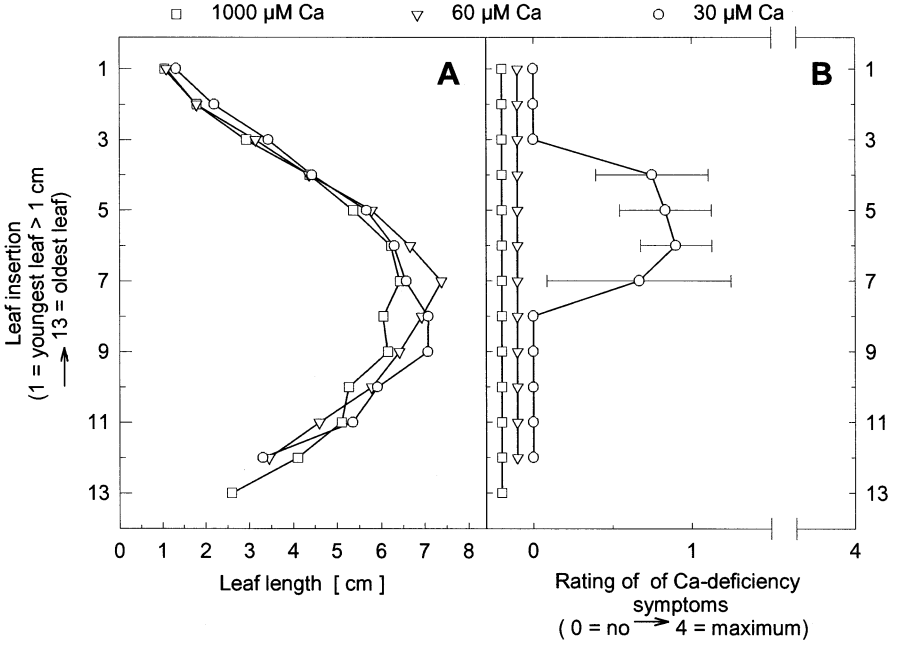


Fig. 2. Leaf length (A) and incidence of Ca-deficiency symptoms (B) of leaves along the plant axis of potato plants grown at different Ca-supply levels for 21 days in nutrient solution (n = 6, differences in A not significant at $P = 0.05$; in B, ± SD).

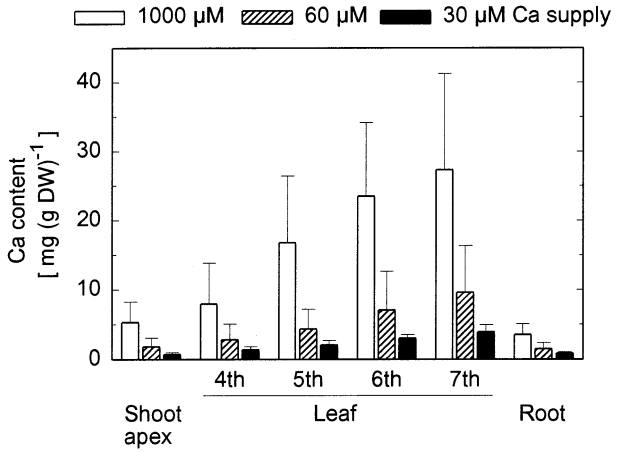


Fig. 3. Ca content of shoot apex, leaves and roots of potato plants grown at different Ca-supply levels for 21 days. The shoot apex includes the shoot apex and the youngest 3 leaves > 1 cm; the other leaves were taken from the 4th to 7th insertion in basipetal direction (n = 6, ± SD).

When Ca-deficiency symptoms (Fig. 1C) were plotted as a function of the Ca content of individual leaves (Fig. 3), an overall relationship could be established (Fig. 4): visual Ca deficiency was universal below 0.9 mg Ca (g dry weight)$^{-1}$ and no Ca-deficiency symptoms occurred at leaf Ca contents over 4.5 mg (g dry weight)$^{-1}$. However, within these limits the Ca content did not correlate with the occurrence of Ca deficiency symptoms.

Not only the total Ca content of the leaves, but also specifically the cell-wall Ca content of the leaves decreased with decreasing Ca supply in the nutrient solution (Fig. 5). The Ca concentration in the AWF was also reduced with a lower Ca supply (Fig. 6). However, this fraction of diffusible Ca was much less reduced than the total Ca content of leaf tissue (Fig. 3). The Ca present in the AWF accounted for 1–2.6% of the total Ca in younger leaves (Fig. 6). There was a trend, however statistically not significant, for the share of

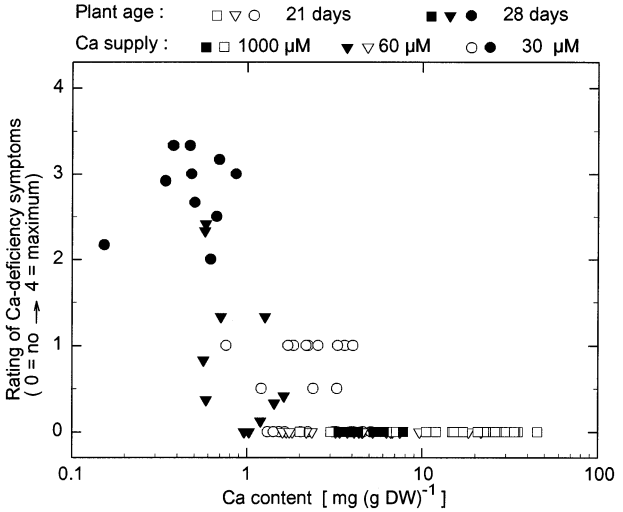


Fig. 4. Incidence of Ca-deficiency symptoms and Ca content of younger leaves of potato plants grown at different Ca-supply levels for 21 or 28 days, respectively.

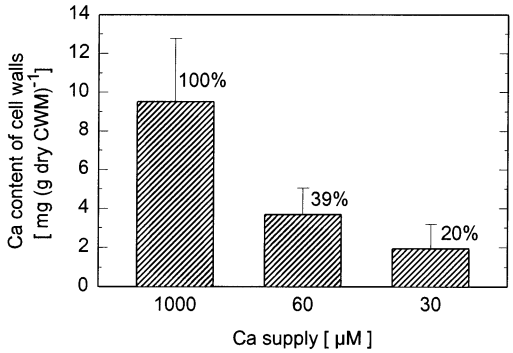


Fig. 5. Ca content of cell walls of younger leaves of potato plants grown at different Ca-supply levels for 28 days in nutrient solution (1000, 60 µ*M*: n = 6; 30 µ*M*: n = 5, ± SD).

AWF-Ca to increase with decreasing Ca supply. As for the total Ca content in the leaves, there was no close relation between AWF-Ca concentration and Ca-deficiency symptoms (data not shown).

## Composition of pectins in the apoplast

Neither the dry weight fraction of the cell wall of young leaves nor its uronic acid content varied as a function of the Ca supply (Table 1). However, with a decreasing Ca supply, more diffusible pectic fragments appeared in the AWF of younger leaves (Fig. 7). The uronic acid concentration of the AWF was approximately two-thirds higher at the lowest Ca-supply level than at an optimum Ca supply. This difference in diffusible uronic acid concentration was equivalent to about 0.6% of the total uronic acid content of younger leaves (Table 1).

Pectic fragments of the AWF were separated by HPLC according to their DP. Fig. 8 shows representative chromatograms of the pectic fragments in the AWF of younger leaves at different Ca-supply levels.

It is conspicuous that by no means all DPs were present, even if it was not possible to assign all of the peaks to a definite DP, since some of the peaks were located exactly between the retention times of two DPs. Possibly, those peaks were caused by oligogalacturonides which carried

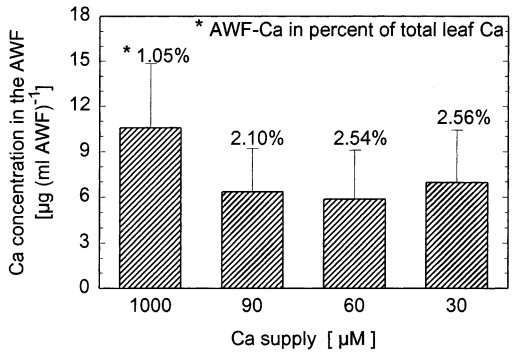


Fig. 6. Ca concentration in the AWF of the 4th to 6th youngest leaf and the percent of AWF-Ca from total leaf Ca as figures above the bars of potato plants grown at different Ca-supply levels for 28 days in nutrient solution (1000 µ*M*: n = 10; 90, 60 µ*M*: n = 9; 30 µ*M*: n = 8, ± SD).

Table 1. Uronic acid content of cell wall and AWF and cell-wall yield in percent of leaf fresh weight of younger leaves of potato plants grown at different Ca-supply levels for 28 days in nutrient solution (n = 6, ± SD).

| Ca supply ($\mu M$) | Uronic acid content in the AWF [µg GalUA-eq (g FW)$^{-1}$] | Uronic acid content of cell walls | | Percent dry cell wall of fresh weight | Uronic acid content in the AWF in percent of total uronic acid content |
|---|---|---|---|---|---|
| | | [mg GalUA-eq (g dry CWM)$^{-1}$] | [mg GalUA-eq (g FW)$^{-1}$] | | |
| 1000 | 51 ± 21 | 141 ± 19 | 3.6 ± 0.5 | 2.6 | 1.4 |
| 60 | 59 ± 25 | 160 ± 12 | 3.8 ± 0.3 | 2.3 | 1.5 |
| 30 | 81 ± 24 | 155 ± 10 | 4.0 ± 0.3 | 2.6 | 2.0 |

galactaric acid instead of galacturonic acid at their reducing end (Spiro et al. 1998).

Fig. 8 also shows that a decreased Ca supply in the nutrient solution caused an increased level of diffusible pectin fragments with a DP of 9 to 16–20 in the apoplast of younger leaves.

An absolute quantification of pectin fragments with specific DP proved to be problematic, because of their very low concentration. However, it was possible to calculate the ratio between the peak areas at a low Ca-supply level and the peak areas at a high Ca supply. Since, as mentioned above, not all samples contained all DPs, the pectin fragments were pooled into 3 classes: DP 1, DP 2–8, and DP 9–20. Fig. 9 shows the peak-area classes of these pectin fragments at low Ca supply relative to high (optimal) Ca supply.

At a Ca supply of 60 $\mu M$, there was only a trend towards a change in the size of the pectin fragments, relative to the control at 1000 $\mu M$ Ca supply. However, clear differences were observed at the lowest Ca level. Compared to the control, substantially less diffusible monomers, but 7 times more oligogalacturonides with a DP of 9–20 were found at a Ca-supply level of 30 $\mu M$ Ca.

### Polygalacturonase activity

The PG activity was increased in leaves at 60 and 30 $\mu M$ Ca supply (Table 2) as compared to the 1000 $\mu M$ control. The average increase was almost twofold at 60 $\mu M$ Ca supply and almost 2.5-fold at 30 $\mu M$.

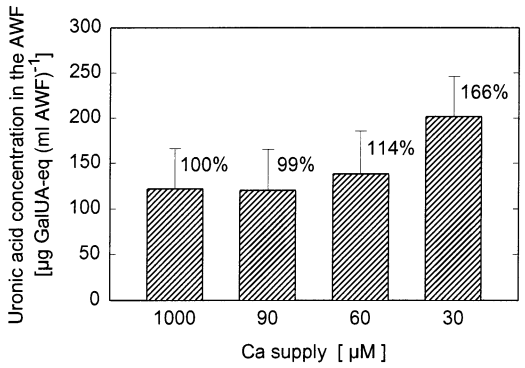


Fig. 7. Uronic acid concentration in the AWF (absolute and in percent of 1000 $\mu M$ Ca supply) of the 4th to 6th youngest leaf of potato plants grown at different Ca-supply levels for 28 days in nutrient solution (n = 9, ± SD).

## Discussion

The first symptom of absolute Ca deficiency in potato plants was marginal necrosis of young leaves. However, the youngest leaves did not show any symptoms, in spite of having the lowest Ca content (Figs. 2 and 3). Also, comparing the data of different cultivars at low Ca supply, there was no correlation between the Ca content of leaves and the occurrence of Ca-deficiency symptoms (Horst et al. 1992). For physiological Ca-deficiency symptoms too, missing relationships between Ca content and symptom development are frequently described (Johnson 1991, Wissemeier 1993). It appears that the physiological activity of Ca on the tissue level is of importance, which is not taken into account by total mineral analysis. In the present case the pectic substance of very young tissue, due to its higher degree of methyl-esterification, may need less Ca to electrostatically compensate the fewer negative charges present, than do older leaves. However, differences in growth rate and there-

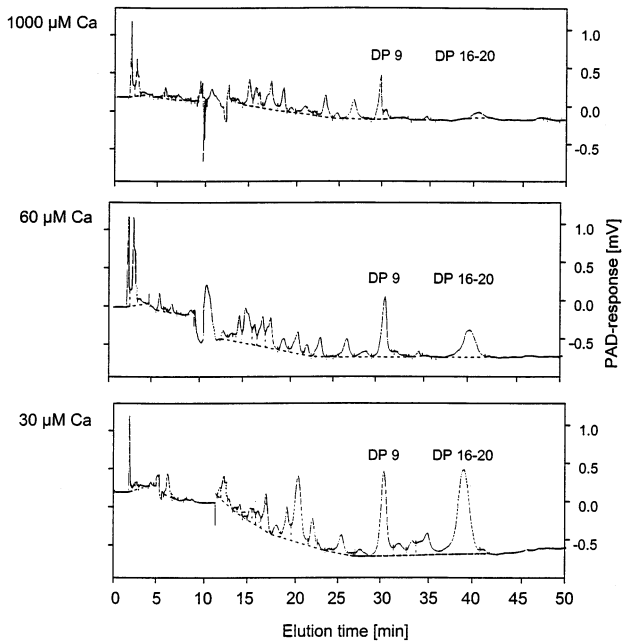


Fig. 8. HPAEC-PAD elution profiles of AWF samples from younger leaves of potato plants grown at different Ca-supply levels for 30 days in nutrient solution.

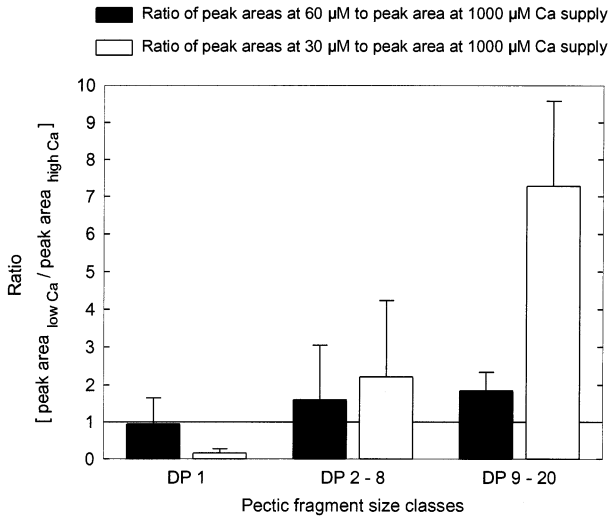


Fig. 9. Ratio of peak areas of pectic fragment-size classes of the pectic fragments in the AWF of younger leaves of potato plants grown at different Ca-supply levels for 30 days in nutrient solution (n = 6, ± SD).

fore in Ca demand among leaves differing in age may also contribute to the poor or even missing relationship between Ca content and Ca-deficiency symptoms.

At a low Ca supply in the nutrient solution, the total Ca content of the leaves (Fig. 3), the Ca content of cell walls (Fig. 5), and to a much lesser extent the concentration of diffusible Ca in the apoplast of leaves is reduced (Fig. 6). Between a Ca supply of 30 and 1000 µ*M*, the Ca concentration in the AWF of young leaves varied only by a factor of less than two and showed hardly any variation between 30 and 90 µ*M* Ca supply (Fig. 6). However, in leaves of the same physiological age, the total Ca content increased by a factor of about 5 between 30 and 1000 µ*M* Ca (Fig. 3). This indicates a rather strong buffering of the diffusible Ca concentration in the leaf apoplast.

The concentration of diffusible pectin fragments, as it can be measured after acid hydrolysis as uronic acid concentration of the AWF, was clearly raised at the lowest Ca level of 30 µ*M* Ca (Fig. 7). The biologically active fragments with a DP of 9–20 increased substantially at the lowest Ca supply only, while at 60 µ*M* Ca the increase was only marginal as compared to the control. The amounts of pectin found in the leaves of young potato plants match data for other plant species: the uronic acid concentration (15% of dry CWM) is comparable to observations for leaves of other dicotyledonous plants (Hu et al. 1996). The content of diffusible oligogalacturonides in the leaf apoplast of younger potato leaves (51–81 µg GalUA-eq [g fresh weight]$^{-1}$; Table 1) is in the range of data reported by Terry and Jones (1981) for pea stalks (77 µg GalUA-eq [g fresh weight]$^{-1}$).

Table 2. Polygalacturonase activity of younger leaves of potato plants (absolute and relative to control) grown at different Ca-supply levels for 28 days in nutrient solution (n = 6, ± SD). One unit of PG activity is defined as the amount of enzyme which causes the release of 1 µmol of galacturonic acid in the reagent mixture (0.1% [w/v] polygalacturonic acid) in 2 h at 37°C.

| Ca supply (µ*M*) | Polygalacturonase activity | |
|---|---|---|
| | [units (g FW)$^{-1}$] | Relative (control = 100%) |
| 1000 | 0.960 ± 0.350 | 100 |
| 60 | 1.757 ± 1.181 | 183 |
| 30 | 2.339 ± 0.428 | 245 |

The uronic acid solubilized in the apoplast under Ca deficiency accounted for about 0.6% of the total wall uronic acid, and was thus within the range of analytical error of this last measurement. Therefore, budget calculations can not help to determine whether the oligogalacturonide increase of the apoplast of Ca-deficient plants originated from enhanced pectin degradation, from inhibited inclusion into the cell wall or from increased oligogalacturonide secretion by the protoplast. However, the increased activity of PG with decreased Ca content of cell walls (Table 2) points to the involvement of pectin degradation.

At 30 µ*M* Ca supply, cell walls contained about half the Ca of 60 µ*M* treated plants (Fig. 5). It has been shown in vitro, that Ca removal from cell walls, e.g. by addition of high concentrations of monovalent cations (Bush and McColl 1987, Gillet et al. 1992, Gillet and Liners 1996), or by chelating agents (Sasaki and Nagahashi 1989, Tu et al. 1992) leads to an increased release of pectic fragments. Besides a purely physico-chemical effect on oligogalacturonate binding, Ca can indirectly affect the wall stability by acting on wall-degrading enzymes. It can modify their concentrations, modulate their activities and/or change the conformation of their substrates. Many wall enzymes can be solubilized by high concentrations of Na or Ca salts, thereby reflecting the electrostatic nature of their binding. On the other hand, it has been shown that enzymes such as the anionic peroxidase bind pectin motifs induced by the presence of Ca (Penel et al. 1999). In other words, Ca could regulate the immobilization-solubilization equilibrium of some wall-degrading enzymes.

One of the first enzymes involved in pectin degradation is PG. PG degrades non-methylated and slightly methylated pectin by hydrolysis (Rexova-Benkova and Markovic 1976). In Ca-deficient potato leaves, the PG activity was more than doubled relative to the control (Table 2). A similar increase of the PG activity was observed by Konno et al. (1984) in Ca-deficient cucumber roots. However, results from Konno (1988) and Pressey and Avants (1976) showed no general negative relationship between PG activity and Ca concentration. The relationship between PG activation and Ca concentration may also be different if exo-PG and endo-PG are compared. No final statement is possible whether in situ PG activity will be changed under conditions of Ca deficiency. It can at least be assumed that a reduced wall Ca concentration induced wall swelling and allowed easier access of the PG to the substrate (Burns and Pressey 1987).

The highest elicitor activity of pectic fragments is frequently reported between DP 9 and 15 (Jin and West 1984), and there is no indication of any effect of the monomer. However, it is evident from a number of studies, that smaller oligogalacturonides with DP 3–8 (Cervone et al. 1987, Forrest and Lyon 1990), as well as larger oligogalacturonides with DP > 15 (Spiro et al. 1998) may exert an eliciting action, albeit at higher concentrations. Physiologically relevant seems the finding that at the lowest Ca supply

of 30 μ*M* which clearly induced Ca-deficiency symptoms, pectic fragments with a DP 9–20 were present in the highest concentrations (Figs. 8 and 9). To the best of our knowledge this has been shown here for the first time for leaves suffering from Ca deficiency. Oligogalacturonides of this size increase the activity of phenylalanine-ammonialyase in cell-suspension culture, so that p-hydroxybenzoic acid accumulates in the cell walls, turning cells brown (Messiaen and Van Cutsem 1993, 1994). Cervone et al. (1987) also report tissue necrosis after application of oligogalacturonides.

Elicitation by oligogalacturonides of DP 9–15 is obtained at concentrations of $10^{-6}$–$10^{-4}$ *M* (Messiaen et al. 1993, Messiaen and Van Cutsem 1993). In the present case the oligogalacturonide concentration in the AWF was converted into concentration in the apoplast water, on the basis of the measured apoplast-water content in younger potato leaves of 8% (indigocarmine method used described by Husted and Schjoerring 1995; data not shown). Therefore, 0.12–12% of uronic acids in the AWF (at 60 μ*M* Ca supply) would correspond to a physiologically-active concentration of $10^{-6}$–$10^{-4}$ *M* of DP 10 oligogalacturonides. The observed uronic acid concentrations in the apoplast water of young Ca-deficient potato leaves thus lie in a range of concentrations that trigger elicitation in other plant systems. In conclusion, Ca deficiency induces both an increase of PG activity and the solubilization of oligogalacturonides with DP 9–20 in the apoplast of younger potato leaves.

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