Altered pectin composition in primary cell walls of *korrigan*, a dwarf mutant of *Arabidopsis* deficient in a membrane-bound endo-1,4-β-glucanase

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Abstract. *Korrigan* (*kor*) is a dwarf mutant of *Arabidopsis thaliana* (L.) Heynh. that is deficient in a membrane-bound endo-1,4-β-glucanase. The effect of the mutation on the pectin network has been studied in *kor* by microscopical techniques associated with various probes specific for different classes of pectic polysaccharides. The localisation of native crystalline cellulose was also examined using the cellulohydrolase I-gold probe. The investigations were focused on the external cell walls of the epidermis, a cell layer that, in a number of plant species, has been shown to be growth limiting. Anionic sites associated with pectic polymers were quantified using the cationic gold probe. Homogalacturonans were quantified using polyenol anti-polygalacturonic acid/rhamnogalacturonan I antibodies recognising polygalacturonic acid, and monoclonal JIM7 and JIM5 antibodies recognising homogalacturonans with a high or low degree of methyl-esterification, respectively. Rhamnogalacturonans were quantified with two monoclonal antibodies, LM5, recognising β-1,4 galactan side chains of rhamnogalacturonan I, and CCRCM2. Our results show a marked increase in homogalacturonan epitoes and a decrease in rhamnogalacturonan epitoes in *kor* compared to the wild type. A substantial decrease in cellulohydrolase I-gold labelling was also observed in the mutant cell walls. These findings demonstrate that a deficiency in an endo-1,4-β-glucanase, which is in principle not directly implicated in pectin metabolism, can induce important changes in pectin composition in the primary cell wall. The changes indicate the existence of feedback mechanisms controlling the synthesis and/or deposition of pectic polysaccharides in primary cell walls.

Key words: *Arabidopsis* (mutant, pectin) – Cellulose – Cell wall – *kor* mutant (*Arabidopsis*) – Pectin (immunocytochemistry) – Polysaccharide

Introduction

The plant cell wall is a complex and dynamic structure which has many crucial functions in plant development and survival (Roberts 1990; Albersheim et al. 1994; McCann and Roberts 1994). Primary cell walls consist of cellulose microfibrils embedded in a heterogeneous matrix of polysaccharides (hemicelluloses and pectins) and proteins. According to the model of McCann and Roberts (1991), the polysaccharides in a typical dicotyledonous primary cell wall are organised into two networks, a cellulose/hemicellulose network and a pectin network. Pectins are principally composed of the three polysaccharides, homogalacturonans, rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II) (O’Neill et al. 1990). Cellulose microfibrils are synthesised by cellulose-synthesising complexes embedded in the plasma membrane (Delmer and Amor 1995), whereas other polysaccharides of the cell wall matrix are assembled in the Golgi apparatus and transported in secretory vesicles to the cell surface (Driouich et al. 1993). The structure, function and biosynthesis of various wall polysaccharides are not fully understood. An important but unsolved question is how the synthesis and the assembly of the different polysaccharide classes are co-ordinated in the expanding primary wall of growing cells. A number of reports illustrate the high plasticity of cell wall polysaccharide biosynthesis in response to varying environmental factors. For instance, changes in cell wall composition have been reported in plants growing under polyethylene glycol (PEG)-induced osmotic stress. Cell walls of tobacco cell cultures grown in the presence of PEG showed a decrease in the percentage of cellulose and an increase in the hemicellulosic fractions, whereas the pectic fractions remained

Abbreviations: CBH-I = cellulohydrolase I; CMC = carboxymethylcellulose; Egase = endo-1,4β-glucanase; *kor* = *korrigan* mutant of *Arabidopsis*; PATAg = periodic acid-thioglycolic acid-silver proteinate; PGA = polygalacturonic acid; RG I = rhamnogalacturonan I

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unchanged (Iraki et al. 1989). In *Cicer arietinum* epicotyls (Muñoz et al. 1993), the PEG-induced inhibition of elongation was correlated with a decrease in hemicellulose and cellulose contents. In contrast, variations in pectin composition, as observed during the different cell growth stages in an elongating epicotyl, were not detected. Recently, Burton et al. (2000) showed that a decrease in cellulose content caused by a virus-induced silencing of a cellulose synthase gene in tobacco was compensated by an increase in homogalacturonans. McCann et al. (1993) reported differences in pectin composition between growing tobacco cells that were either non-adapted or adapted to high NaCl concentrations. Throughout the culture period, pectic polymers of non-adapted cells showed an increase in methyl-esterification during elongation followed by a decrease during the stationary phase. In contrast, the degree of pectin esterification remained unchanged in adapted cells. Several studies have reported that the synthesis and/or deposition of the cell wall components are under control of calcium. For instance, in Norway spruce, high concentrations of calcium in the soil stimulate the deposition of non-cellulosic polysaccharides, especially pectic polymers (Eklund and Eliasson 1990). Calcium also affects the frequency and distribution of anionic sites within the wall of parenchyma cells of apple fruit (Roy et al. 1994). His et al. (1997) observed an increase in acidic pectins, as well as inter-pectin calcium bridges within the hypocotyl cell walls of flax seedlings grown in the presence of calcium. Interestingly, these changes could not be correlated with changes in hypocotyl length.

The first indications for the existence of feedback mechanisms regulating the polysaccharide composition of cell walls came from the study of tomato and tobacco cells adapted to the inhibitor of cellulose synthesis 2,6-dichlorobenzonitrile (DCB). The dramatic reduction of cellulose in these cells was associated with important changes in the composition of non-cellulosic polysaccharides (Shedletsky et al. 1990). The synthesis of xyloglucan was not inhibited, but most of it was secreted in a soluble form into the culture medium. The walls of these cells showed a significant enrichment in pectins, including homogalacturonan and rhamnogalacturonan-like polymers. The integrity of the wall appeared to be maintained by an increase in Ca$^{2+}$-bridged pectates. These findings point to the existence of an independent pectic network in the primary wall, the composition of which is altered in the absence of a cellulose/xyloglucan network. In contrast, walls from DCB-adapted monocot cells did not show increased pectin contents, but normal to elevated amounts of other non-cellulosic material (Shedletsky et al. 1992), confirming the fundamental differences between dicot and monocot cell walls, including the way they compensate for the reduction of their cellulose content. It is not known, however, whether the observed changes in the DCB-adapted cells reflect the plasticity of the machinery involved in cell wall construction, or whether any genetic changes are responsible for these adaptations.

In this report we took advantage of a recently identified dwarf mutant of *Arabidopsis*, kor, to determine how the pectin network is implicated in the plant elongation phenomenon. Plants carrying the recessive nuclear mutation kor show a cell elongation defect in all cell types except for tip-growing cells (Nicol et al. 1998). This growth defect is caused by a T-DNA insertion abolishing the expression of a gene encoding a membrane-bound endo-1,4-β-glucanase (EGase). Although the precise role of the enzyme remains to be determined, all the evidence suggests a function for KOR in the assembly and/or metabolism of the cellulose/hemicellulose network in growing cells (Nicol et al. 1998).

To investigate the interrelation between the synthesis and assembly of the cellulose/hemicellulose network and the pectic network in the primary wall, and knowing the primary defect of the mutant, we compared the composition and distribution of pectic polysaccharides in cell walls of wild-type and mutant hypocotyls. We focused our investigations on the epidermal cell wall known to control the rate of organ elongation in stems and coleoptiles (Kutschera 1992). Using immuno-affinity methods and microscopical techniques, we observed marked modifications in the pectic network. In particular, we show an increase in homogalacturonan epitopes and a decrease in rhamnogalacturonan epitopes in kor compared to the wild type. Our results suggest that a mutation eliminating an enzyme that is not normally directly implicated in pectin metabolism can cause important changes in the composition of pectins within the wall.

**Materials and methods**

**Plant culture conditions**

Seedlings of *Arabidopsis thaliana* (L.) Heynh., ecotype Wassilewskija (seeds provided by K. Feldman, University of Arizona, Tucson, Ariz., USA), and the mutant kor were grown in vitro, in the dark for 7 d as previously described (Nicol et al. 1998).

**Fixation and embedding**

Seven-day-old seedlings were fixed for 90 min in 4% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After post-fixation in 1% osmium tetroxide for 1 h, the samples were dehydrated in a graded aqueous ethanol series and embedded in LR White resin as previously described (His et al. 1997; Nicol et al. 1998). Some *Arabidopsis* samples were submitted to a subtractive treatment with hot water (100 °C) for 2 h to solubilise pectins as described in His et al. (1997).

**Calcofluor staining of cellulose**

Light microscopy was carried out on an Axioskop microscope (Zeiss) equipped with epifluorescence optics. Calcofluor staining was done as described by Mori and Bellani (1996). Briefly, sections (0.5 μm in thickness) mounted on glass slides were incubated with the fluorescent probe (1 mg/ml) for 30 min in the dark. After several washes, the sections were observed with a Zeiss filter set (excitation filter 350-410, barrier filter 470).

**Immunolabelling procedures**

The antibodies used in this study were: (i) the monoclonal antibodies JIM5 and JIM7, specific for pectins with a low and