SUMMARY

From the beginning of sepal development, the primary plant cell wall on the surface of the abaxial sepal epidermis (i.e. outer periclinal walls of epidermal cells) is the outermost barrier protecting the organ and the flower bud against environmental stresses, pathogens, mechanical damage, and participating in gas exchange. The dynamic remodelling of primary cell walls is essential for their expansion, allowing cells to grow, and for coordination of cell behaviour that is necessary to shape functional organs. While the wall composition has been extensively studied, a comprehensive approach to simultaneously detect all its major components and follow their changes during organ development are limited. In the present study, the primary cell walls on the surface of *Arabidopsis thaliana* sepals were chosen to investigate the primary cell wall composition and cuticular pattern formation in wild type and cell wall mutants during the sepal maturation. Raman microspectroscopy was used in these investigations because it facilitates assessment of overall cell wall composition during single measurements. *In vivo* confocal microscopy imaging of the epidermis surface during sepal maturation provided complementary information about the dynamics of the cell wall structure, with focus on the formation of the cuticular pattern. The objectives of the present investigations were to verify the following hypotheses: (i) Primary cell wall composition of Arabidopsis sepal epidermis changes during the sepal development and is affected by *csi1, mad5, pme32, xyl1* mutations; (ii) Primary cell wall of Arabidopsis sepal epidermis exhibits structural anisotropy resulting from alignment of its components; (iii) Deficiency of a cell wall component in Arabidopsis mutants activates a compensatory mechanism; (iv) The initial pattern of cuticular ridges appearing on the outer periclinal walls of Arabidopsis sepal is influenced by cell growth and geometry at the time of pattern formation; (v) The pattern of cuticular ridges is changing during the cell growth. Raman microspectroscopy measurements revealed ontogenetic changes in primary cell wall composition during maturation of Arabidopsis sepal epidermis, reflected in differences in signal intensity and contribution of two component spectra identified using multivariate curve resolution. These changes indicate compositional remodelling of the cell wall during organ maturation. Raman maps of superficial walls of the sepal enabled to distinguish two regions with assigned specific spectra: one for cell wall located between the cuticular ridges, and another for wall covered by ridges. These two regions showed slight compositional differences, including additional bands in ridge-covered walls. Moreover, qualitative differences between investigated mutants and wild type were revealed. Polarisation-sensitive bands were identified in the Raman spectra. They are a manifestation of structural

anisotropy of the wall. The polarisation-dependent bands were related not only to cellulose but also to the cuticle components. Cellulose was aligned within primary cell walls to the similar extent in wild type and the investigated mutants, except for *mad5*. This *mad5* phenotype is similar to other katanin mutants in which microtubule dynamics is affected. Structural anisotropy of cuticle components of Arabidopsis sepal, similar in the wild type and the investigated mutants, is a novel observation. It suggests that even the thin cuticle of Arabidopsis sepal epidermis exhibits some degree of ordered organisation, although further investigations at higher resolution are needed. Comparison of contribution to the spectra of Raman signals related to pectins, cellulose and hemicellulose facilitated indirect assessment of the relative contribution of three main polysaccharides in individual wall samples of wild type and the investigated mutants. In mutants, a decrease in the signal assigned to one component was accompanied by an increase in others or *vice versa*. This is likely a manifestation of compensatory adjustments mechanism to maintain cell wall structure and mechanical properties despite compositional changes. *In vivo* confocal imaging combined with quantification of sepal surface growth and curvature, showed that cuticular ridges that decorate the abaxial surface of Arabidopsis sepals are formed on an initially smooth outer periclinal walls when the sepal surface is still expanding. The pattern formed by cuticular ridges depends on the anisotropy of the surface expansion and on its geometry. In agreement with models explaining the ridges formation as a mechanical buckling, the orientation of newly formed ridges was most often parallel to the principal direction of maximal growth at both the cellular and subcellular scales. The relation between ridges orientation and directions of surface curvature was much weaker. However, the unique complexity of ridges pattern on the surface of *mad5* sepals seems to be related to the high curvature of mutant epidermal cells. The extent of alignment and morphology of cuticular ridges change during the sepal surface expansion. The anisotropy of the initial ridges patterns either increased or decreased over time. The decrease in anisotropy was often related to the formation of new short ridges that were sometimes perpendicular to the initially formed ones, while the anisotropy increase was associated with the straightening of already existing ridges. The changes in anisotropy of the cuticular pattern during wall expansion are likely a manifestation of stiffness heterogeneity, i.e. the stiff film formed by cuticle proper is covering the relatively soft cuticular layer, in agreement with models of ridges formation that assume buckling.