Abstract

The cell wall is the outer layer of a plant cell, and its structure and the bonds between components are crucial to the function of the whole organism. Arabinogalactan proteins (AGPs) are important components responsible for co-forming the amorphous extracellular matrix. AGPs are glycoproteins belonging to the hydroxyproline-rich glycoprotein family. They consist of a protein domain linked to sugar chains. Disruption of the various stages of AGP biosynthesis is critical to their structure and distribution in cells, which can affect their functional properties during the occurring physiological processes, including fruit ripening.

Accordingly, this thesis proposes the hypothesis that tomato fruit ripening is closely correlated with changes in the distribution and molecular characteristics of AGPs.

The above hypothesis was verified through in situ and ex situ studies, which investigated changes in the structure, content, and localisation of AGPs during the ripening of tomato (Solanum lycopersicum L.) fruit from wild-type plants and lines with modified expression of the SlP4H3 gene. The main objective was to study the role of AGP in the formation of the cell wall structure, with a particular focus on the spatio-temporal distribution of its other components during the fruit ripening process.

The thesis consists of three main research phases. The first phase of the study enabled the identification of AGPs as potential markers indicating the progression of the ripening process. A gradual decrease in AGP content was observed as the fruit ripened. In the early stages of the process, AGP fractions with a molecular weight of 60-120 kDa dominated, while in the later stages, fractions with a molecular weight of 20-25 kDa became more dominant. In addition, bands indicating the presence of AGP with a molecular weight of 30 kDa were observed in the final stages of the process, which can be considered as a marker for the finalisation of the ripening process. Microscopic analysis of fruit tissues confirmed the presence of quantitative changes as well as spatio-temporal modifications of specific AGP epitopes as the ripening process progressed. In fruit at the beginning of ripening, AGP epitopes recognised by JIM13 and LM2 antibodies were mainly localised in the space between the cell wall and the plasma membrane. As ripening progressed, a disruption of the characteristic AGP distribution pattern was observed.

The next stage of the study allowed the determination of changes in the content and

localisation of AGP in the fruit of transgenic lines in which the expression of the SlP4H3 gene had been modified. The study demonstrated correlations between changes in SlP4H3 gene expression and AGP content in fruit during the ripening process. The characteristic AGP fraction, which marks the finalisation of the ripening, was absent, and the layout of molecular masses of the AGP epitopes analysed differed from that observed in wild-type fruit. Anatomical and morphological changes in the fruit of transgenic lines were observed. AGP epitopes in fruit were mainly located in degraded cell wall compartments, in the cytoplasm.

In the final stage of the study, it was possible to determine the direct effect of changes in AGP structure and localisation on cell wall assembly. The amount and spatio-temporal distribution of extensin, xylan, rhamnogalacturonan type I, low and high esterified homogalacturonan were modified. In the fruit of transgenic lines, disruption of polysaccharide and proteoglycan networks in the cell wall was noted as ripening progressed. Additionally, aberrations in cell wall organization led to changes in fruit tissues, observed at the tissue, cellular and subcellular levels.

In conclusion, changes in the structure, content and spatio-temporal distribution of AGPs represent a disruptive factor in fruit ripening. Accordingly, the results obtained in this thesis support the hypothesis that AGPs are crucial for the fruit ripening process.

Keywords: arabinogalactan proteins, cell wall, ripening process, tomato fruit