Vitis vinifera cell wall in response to restriction in major mineral elements

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Grapevine (*Vitis vinifera* L.) is one of the most important crops worldwide from an economic point-of-view, due to a global consumer acceptance. The berry is used as table grape, raisins and, above all, in winemaking industry.

The plant cell wall (CW) is a complex and heterogeneous structure of polysaccharides, glycoproteins and enzymes, which surrounds the protoplast. The deposition and remodeling of CW material plays an essential role during plant growth, determining the cell size and shape, and providing protection against abiotic and biotic stress, enabling the cells to adapt to different physiological and environmental situations.

Nitrogen (N), phosphorus (P) and sulfur (S), are major elements connected to primary plant metabolism - amino acid and nucleotide biosynthesis, protein phosphorylation or protein disulfide bonds. N, P and S deficiency can reduce plant growth and crop yield, nutritional and organoleptic quality of the agronomic product, affecting anatomical and developmental patterns. Despite the diversity in plant species, a generic “stress–induced” response to abiotic stresses including mineral stress can be revealed through the inhibition of cell elongation, localized stimulation of cell division and alterations in cell differentiation status. However, notwithstanding the importance of mineral nutrition in plant development, its influence on the synthesis and modifications of the CW is not fully documented. To address this issue in *V. vinifera*, callus and young shoots, respectively as unorganized and organized tissues, were used as model systems to study CW responses to depletion of each major element (-N,-P or –S).

Using the callus system, changes in CW composition triggered by mineral stress were firstly investigated by Fourier–Transformed Infrared Spectroscopy (FT-IR). The overall results suggested changes in all main CW components. Modifications in the biosynthesis or rearrangements of cellulose microfibers, matrix linked glycans, pectin biochemistry and in the amounts of structural proteins were among the most striking indications. A further

detailed biochemical analysis of the stress responses revealed a significant reduction in cellulose content under –N and –P. In shoots, the primary response of plant CW under mineral deficiency, particularly –N, was also the impairment in CW cellulose. Due to the role of the CW for environment adaptation, plants are equipped with compensatory mechanisms to reinforce their CWs after the biosynthesis and/or deposition of cellulose is impaired. Low levels of cellulose are associated to a decrease in pectin methyl esterification, mainly in the long stretches of the homogalacturonan (HG), as spotted by in situ immunolocalization in both systems. Under mineral stress, this pattern of de-esterification was suggested to reinforce the CW conferring additional stiffening without alterations in CW elastic deformation. Basic PMEs, which promotes the formation of HG Ca^{2+}-linked gel structures and thereby stiffening and reducing CW extensibility, showed compatible gene expression patterns. Xyloglucans (XyG) also play an important role in CW and influence its characteristics. Most drastically in -N shoots, the CW XyG increase was evidenced by immunolocalization techniques. This increase can also be explained as a CW reinforcement mechanism. Simultaneously, -N shoots increased the levels of tightly bonded arabinose to pectic polysaccharides side chains. The observed decrease in cellulose and increase in arabinans may be a general response to mineral stress.

Lower cellulose levels led us to investigate the expression of the cellulose synthase (CesA) gene family. Most of these genes showed an increased transcript accumulation, which conflicts with the observed reduction in cellulose content. Nevertheless, CesA activity is known to be insufficient to produce cellulose, requiring the combined action with members from other families, such as the classes C of the glycosyl hydrolase family 9 (GH9). Supporting this assumption, in –N or –S callus *VvGH9C2* expression was severely down-regulated.

Taking into account the overall results, the impact of -N stress on the CW points to a more pronounced response, supporting the primary role of this major nutrient in plant development and metabolism.