The Role of UDP-D-xylose Synthase (UXS) in Alfalfa Cell Wall Synthesis: A Target to Increase Digestibility

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The cell walls from alfalfa stems are rich in polysaccharides such as cellulose, xylan, and pectins, and could be a good source of energy for dairy cows. However, many factors, including lignin and xylan content, contribute to their overall low digestibility. Xylose, the main monosaccharide in the xylan backbone, is formed through the decarboxylation of UDP-D-glucuronic acid (UDP-D-GlcA) by the enzyme UDP-D-xylose synthase (UXS), in a sugar nucleotide interconversion pathway. Arabidopsis mutants with loss-of-function of the cytosolic versions of UXS (AtUXS3, AtUXS5, and AtUXS6) had altered cell wall monosaccharide composition and improved digestibility. Considering this, *uxs* genes are an attractive target for downregulation in alfalfa, as this could decrease xylan levels, improving overall digestibility, while possibly increasing cellulose or pectin levels due to restricted flow of sugar nucleotides through the pathway. Using bioinformatics, we have identified four alfalfa genes belonging to the same clade as *Atuxs3*, *Atuxs5*, and *Atuxs6* (Figure 1A). These genes correspond to two pairs of homologs, and each pair encodes a unique amino acid sequence (named MsUXS2 and MsUXS4). Analysis of published alfalfa RNAseq data showed that the identified genes are more highly expressed in stems than leaves. Assays using recombinant MsUXS2 and MsUXS4 enzymes produced in *E. coli* showed that they have UDP-glucuronic acid decarboxylase activity. We also detected high levels of UXS activity in alfalfa stem extracts. To investigate the role of *uxs* genes in cell wall synthesis, and their impact on digestibility, a hairpin RNAi construct designed to silence all four alfalfa *uxs* genes, and another two constructs for overexpression of a member of each homolog pair were transformed into the Regen-SY27 alfalfa genotype. Cell walls isolated from the stems of silenced lines had reduced xylose content (~35%) compared to control lines (Figure 1B). No significant difference was detected for the cell wall monosaccharide content of overexpression lines. We plan to analyze the silenced lines to assess the impacts on lignin content and *in vitro* digestibility.

Figure 1. *A*. Phylogenetic analysis of UXS from *Medicago truncatula* (Medtr), alfalfa (MSAD), and *Arabidopsis thaliana* (AT). *B*. Xylose content in alfalfa lines with silenced *uxs* genes (purple) compared to empty vector controls (gray).