



## The role of UXS in alfalfa cell wall synthesis: A target to increase digestibility

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# Alfalfa: Queen of forages

Very important forage

High nutritional value

Minerals



Protein

Vitamins

# What about the stems?

LEAVES



Protein, minerals



STEMS



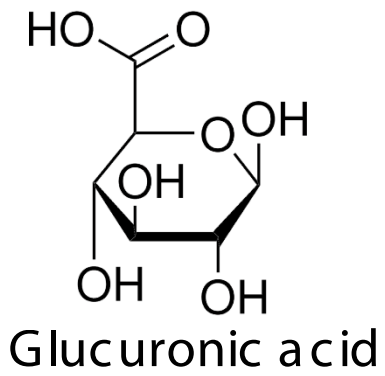
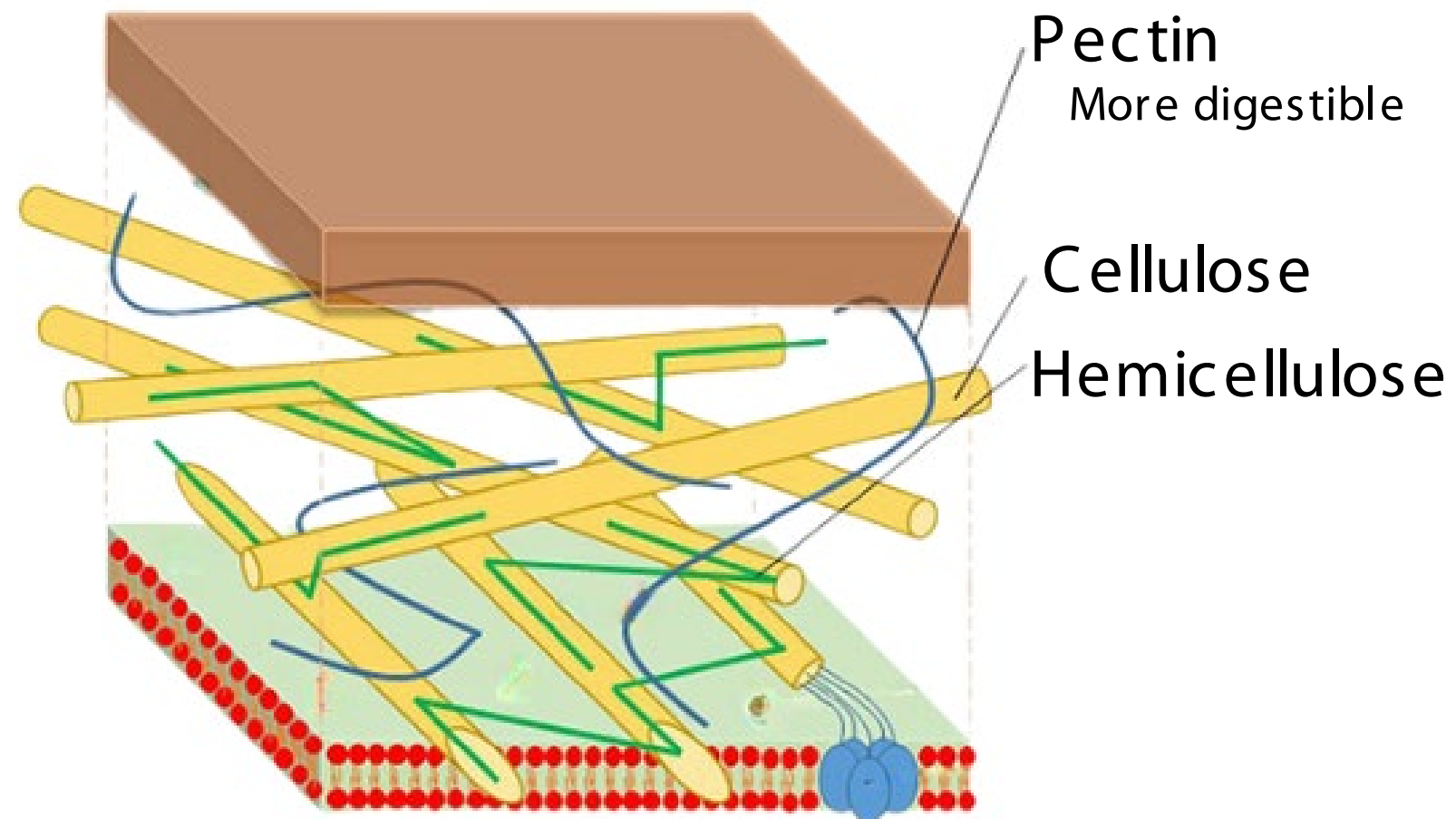
Higher concentration of cell walls (fibers) with lower digestibility

Source of energy!

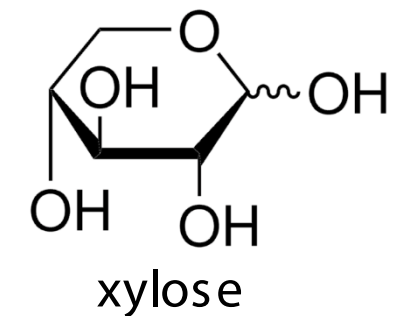
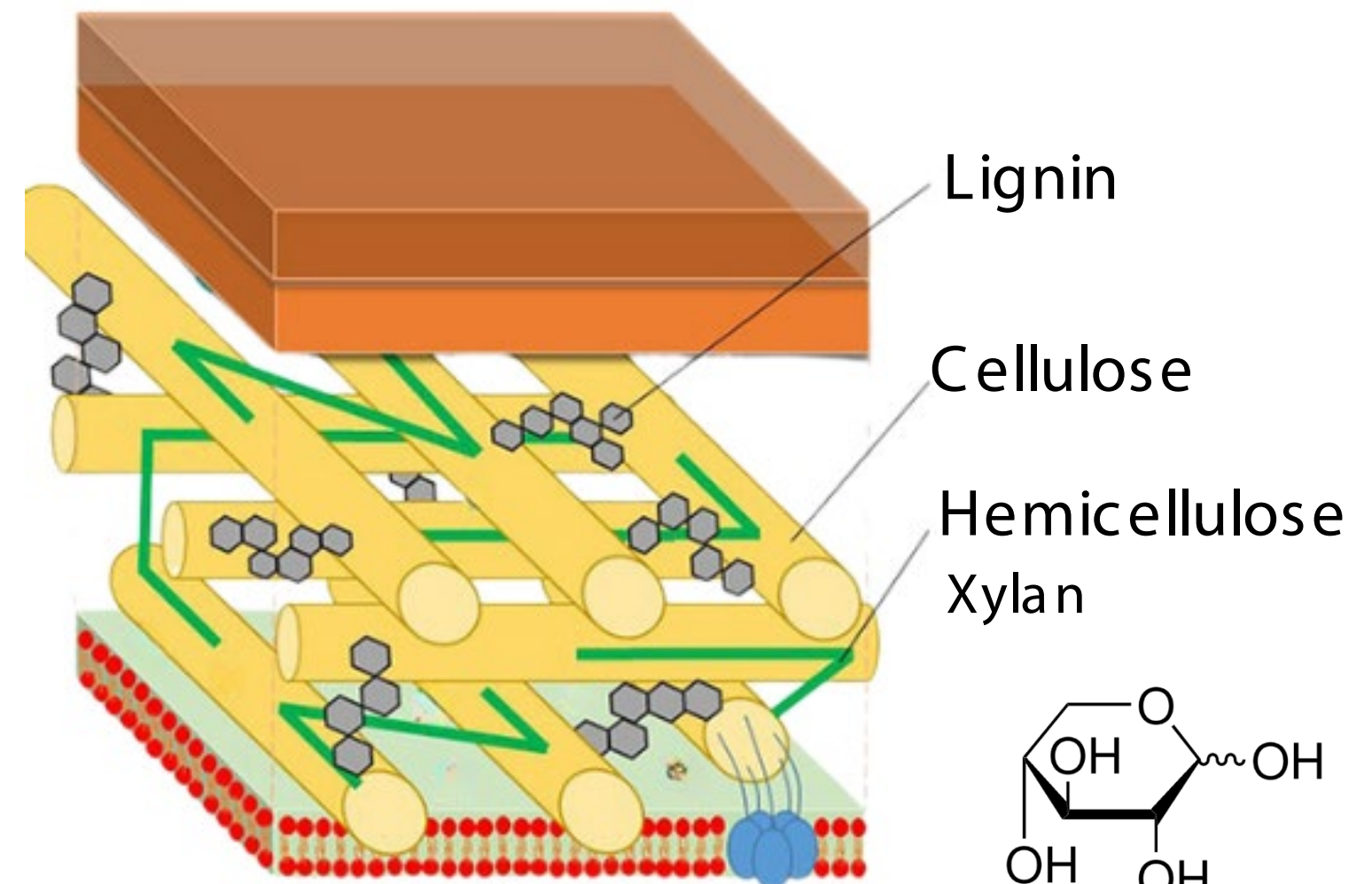
Improve digestibility

# The cell wall

## Primary wall



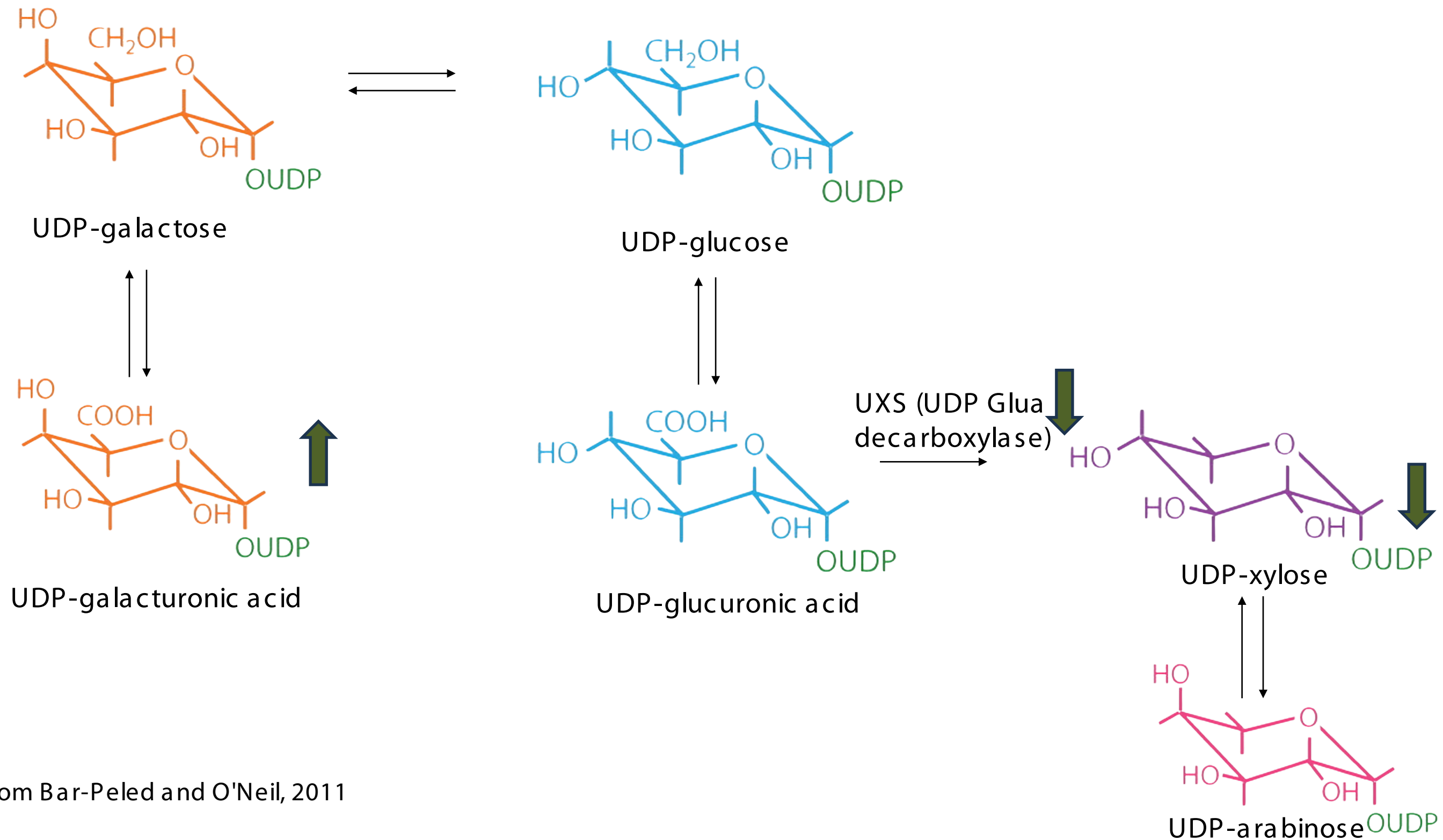
## Secondary wall



Source: Adapted from Loux et al., Front. in Plant Science, 2017

Use genetic engineering to manipulate the components of the cell wall

# Nucleotide sugar pathway



Adapted from Bar-Peled and O'Neil, 2011

# Objectives



Identify *UXS* genes in the alfalfa genome



Produce recombinant alfalfa *UXS* enzymes (*E. coli*) and characterization *in vitro*

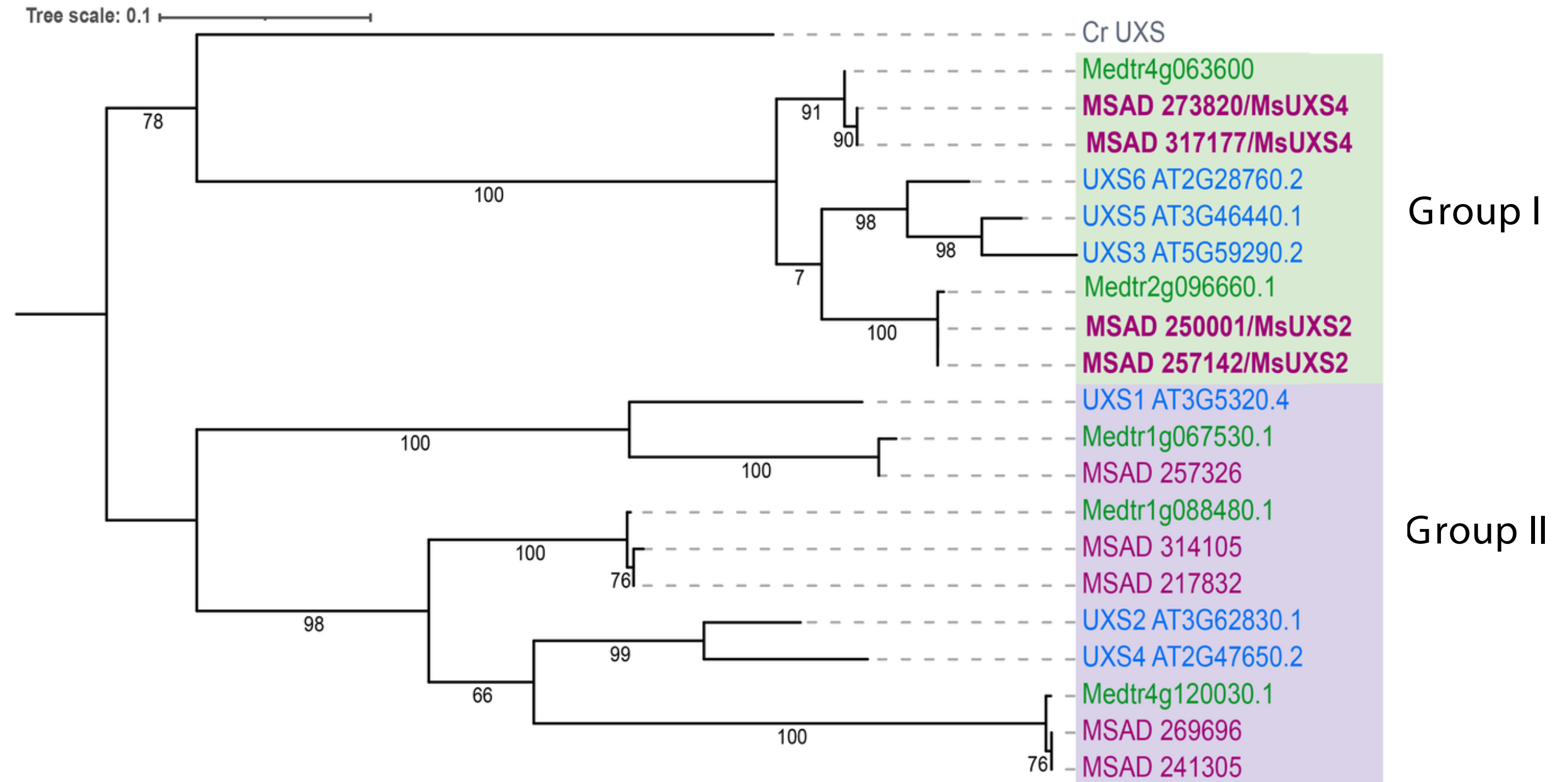


Functional characterization (silencing/overexpressing)

# There are four *UXS* candidates in alfalfa genome

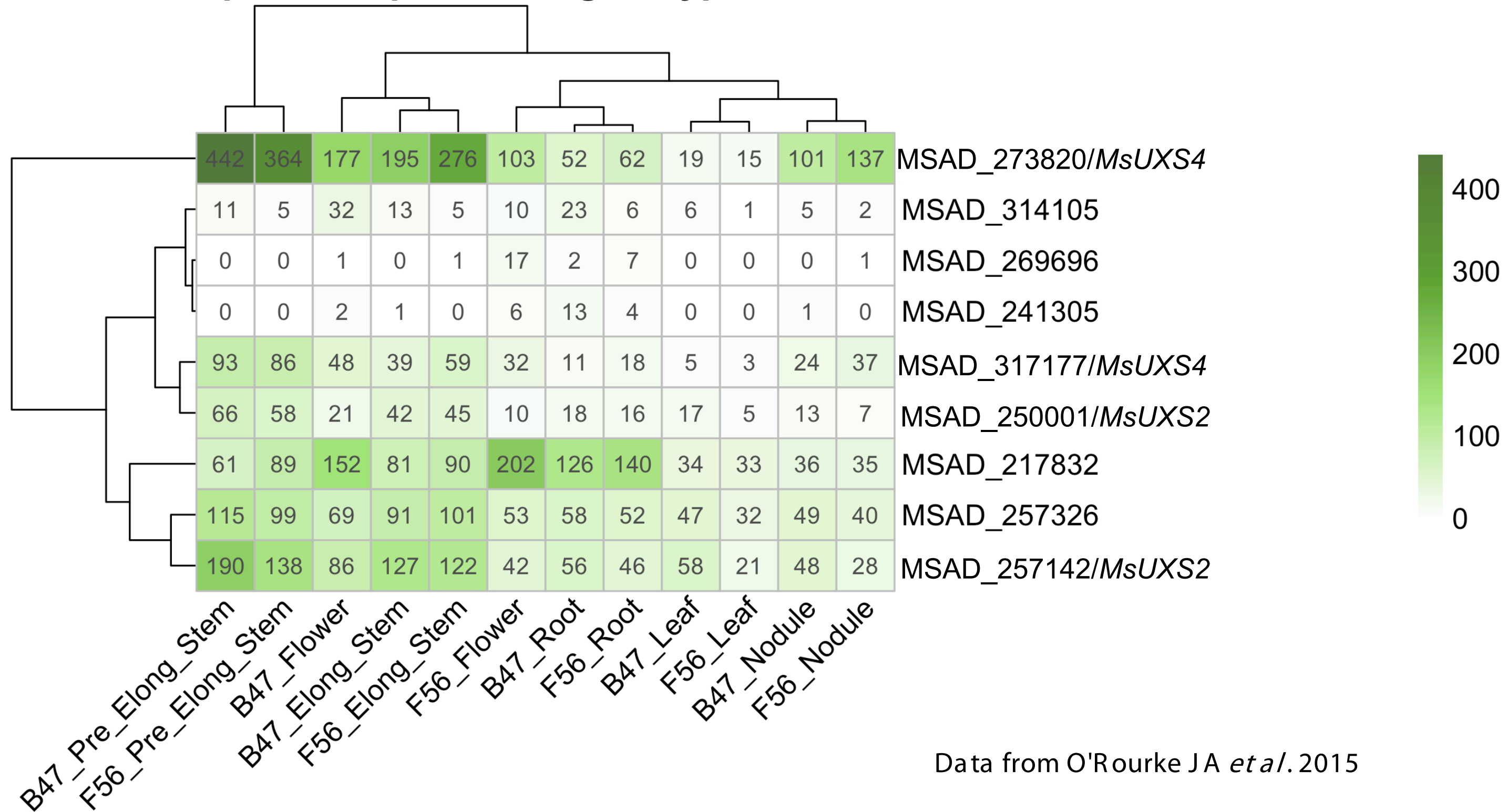
Annotated sequences in the alfalfa genome similar to *uxs* in *Medicago truncatula*

Phylogeny: **Alfa lfa**, *Medicago truncatula*, *Arabidopsis thaliana*



# Expression of *MsUXS* is higher on the stems

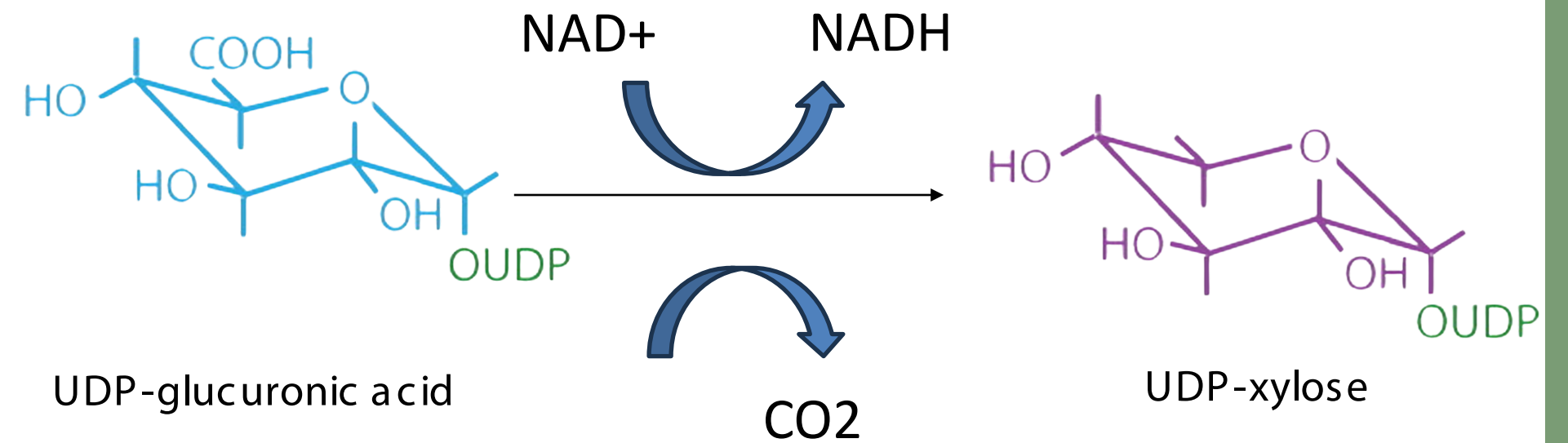
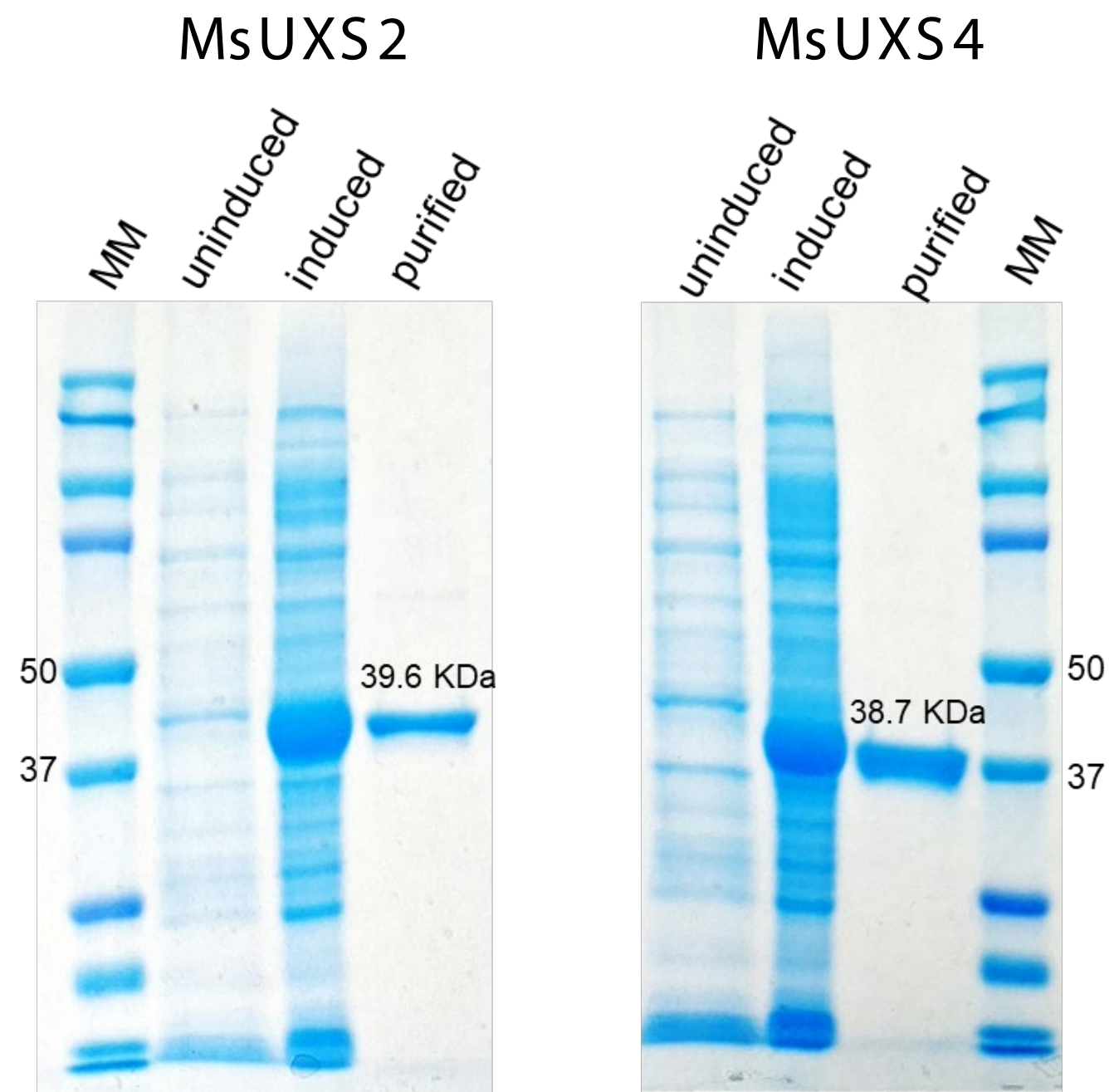
UXS expression profile for genotypes B47 and F56



Data from O'Rourke JA *et al.* 2015



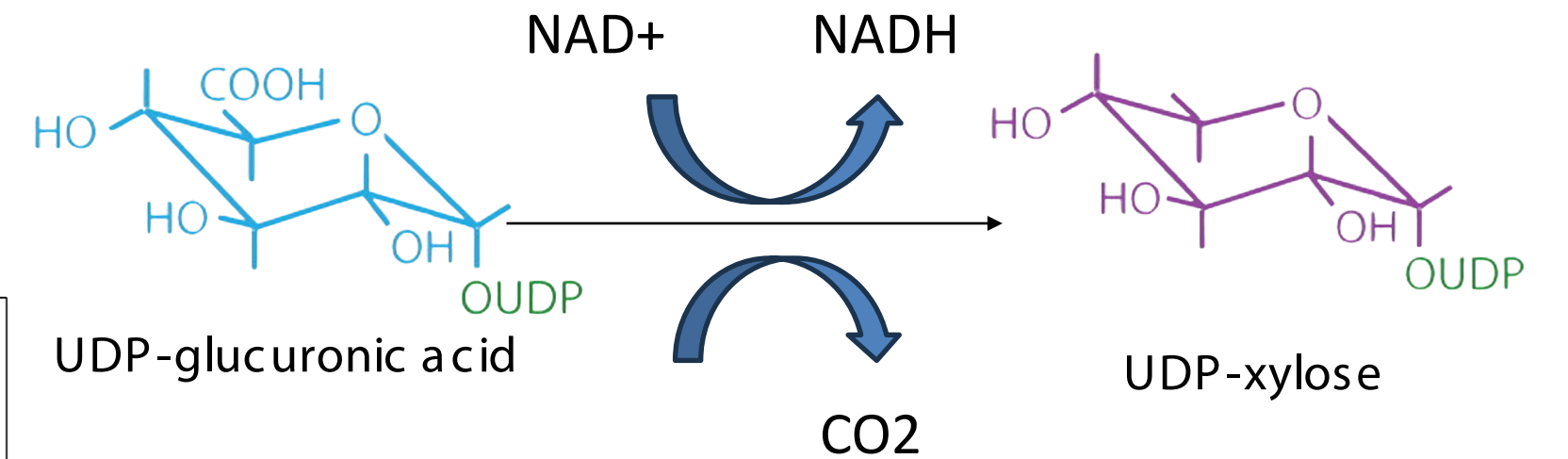
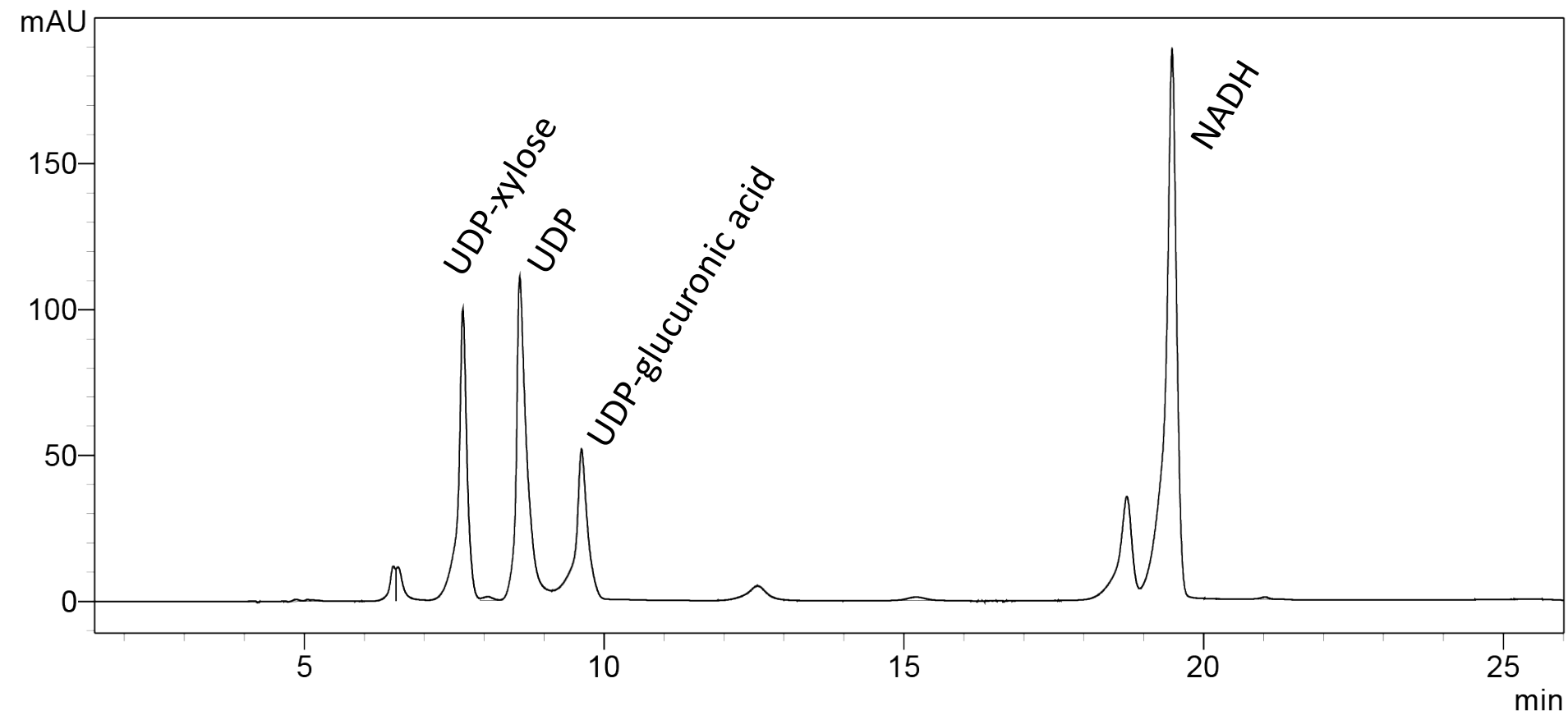
# UXS expression in *E. coli* and activity assays



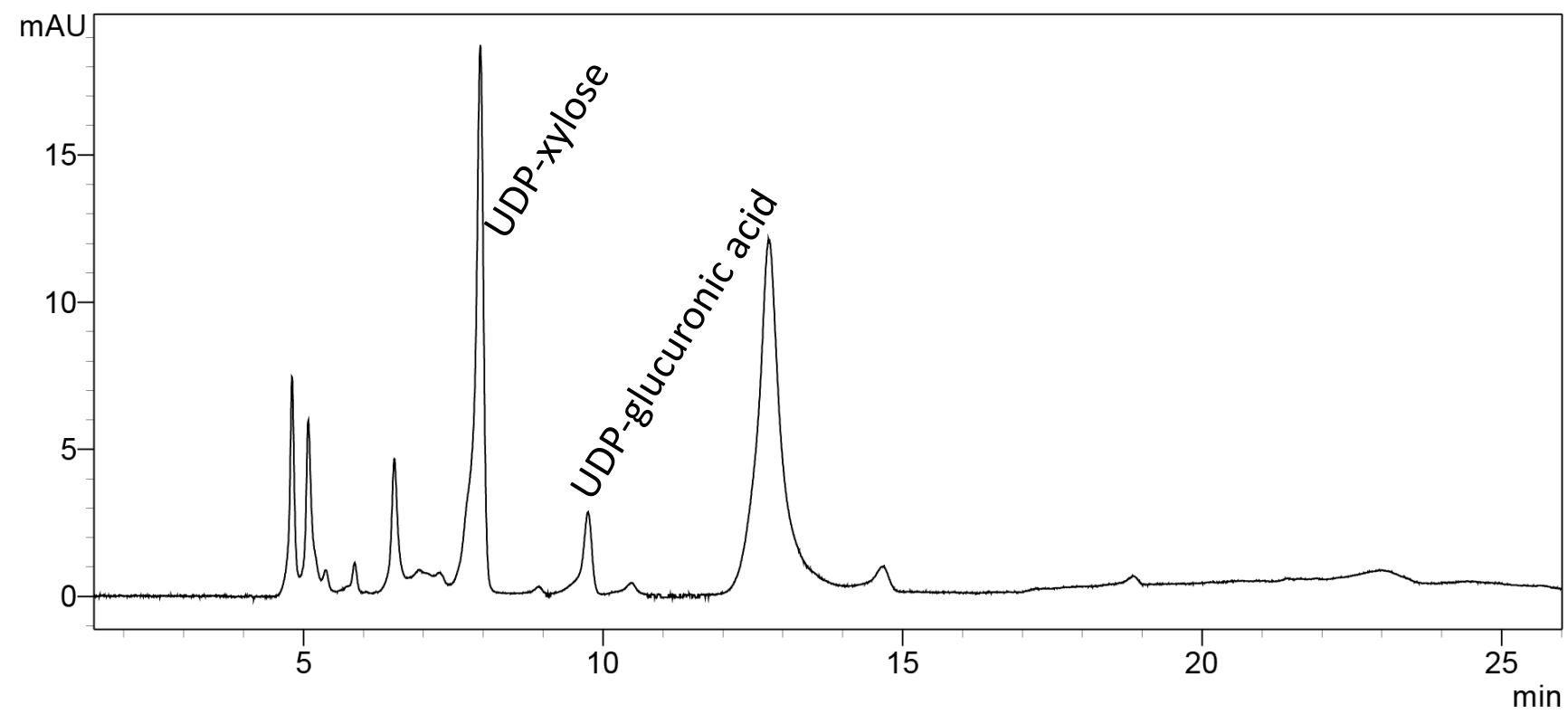
Quantification using HPLC  
PDA detector

Expression and purification

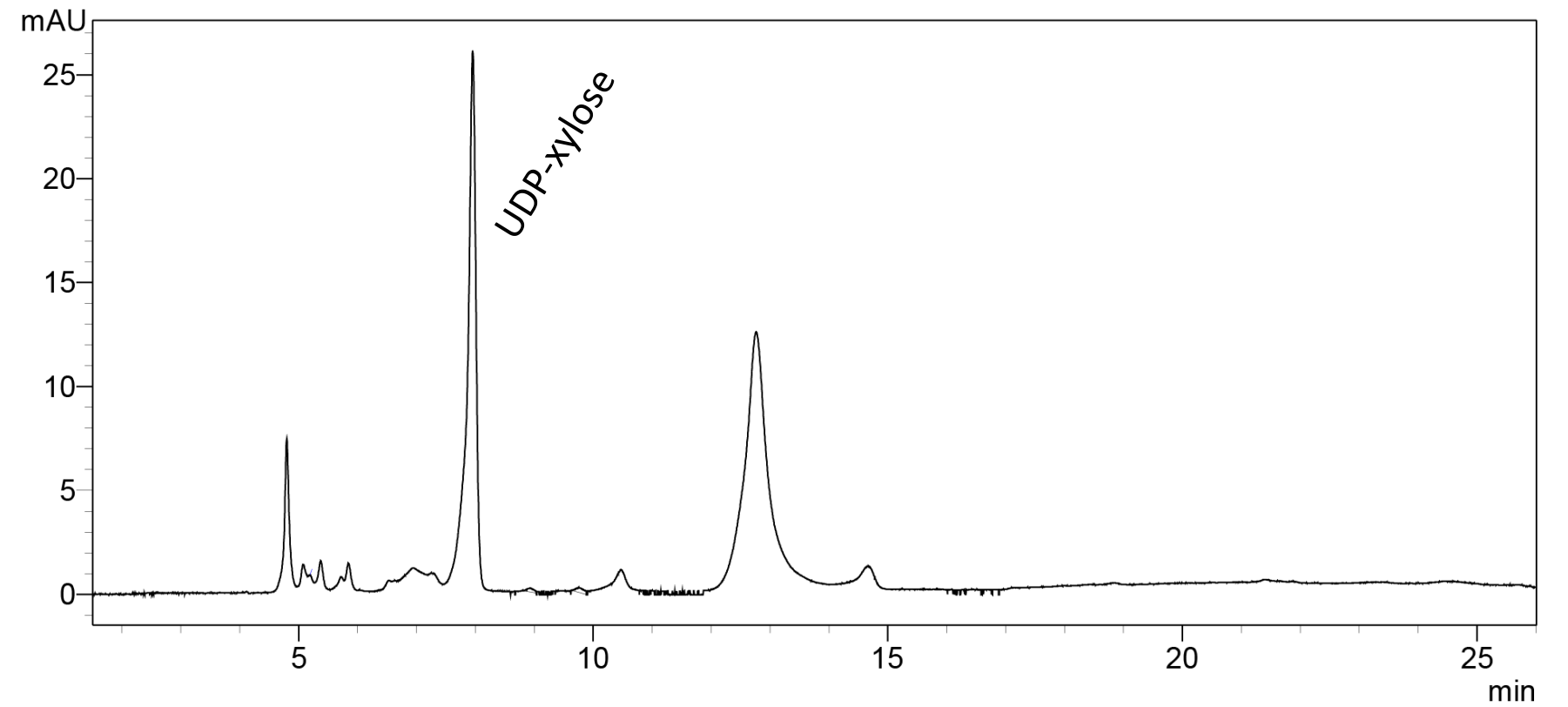
# MsUXS 2 and MsUXS 4 have UDP-glucuronic acid decarboxylase activity



# UXS activity in alfalfa stems



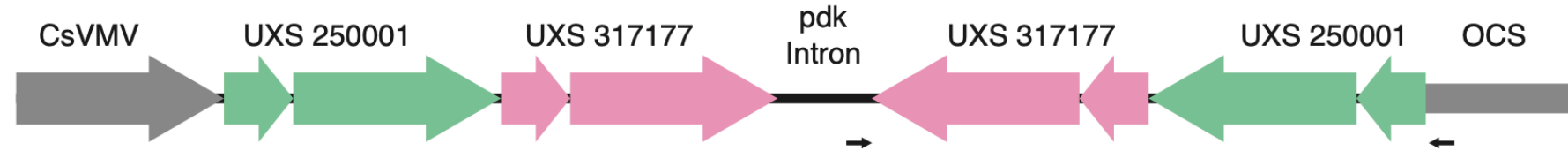
1 hour



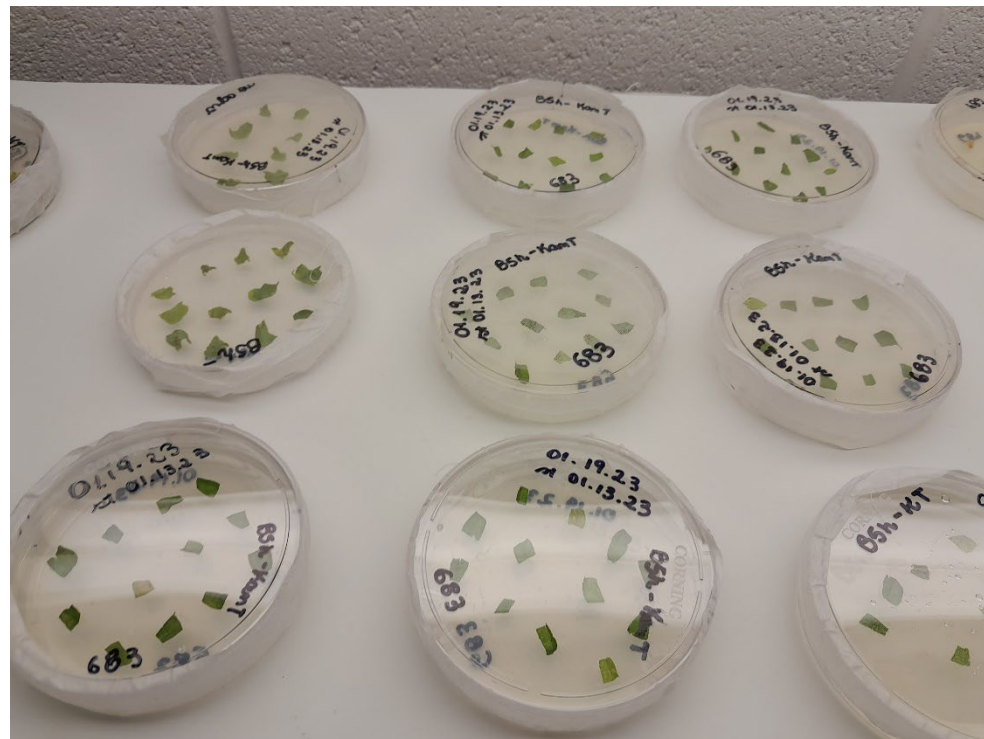
3 hour

# Generation of alfalfa transgenic lines

## Silencing



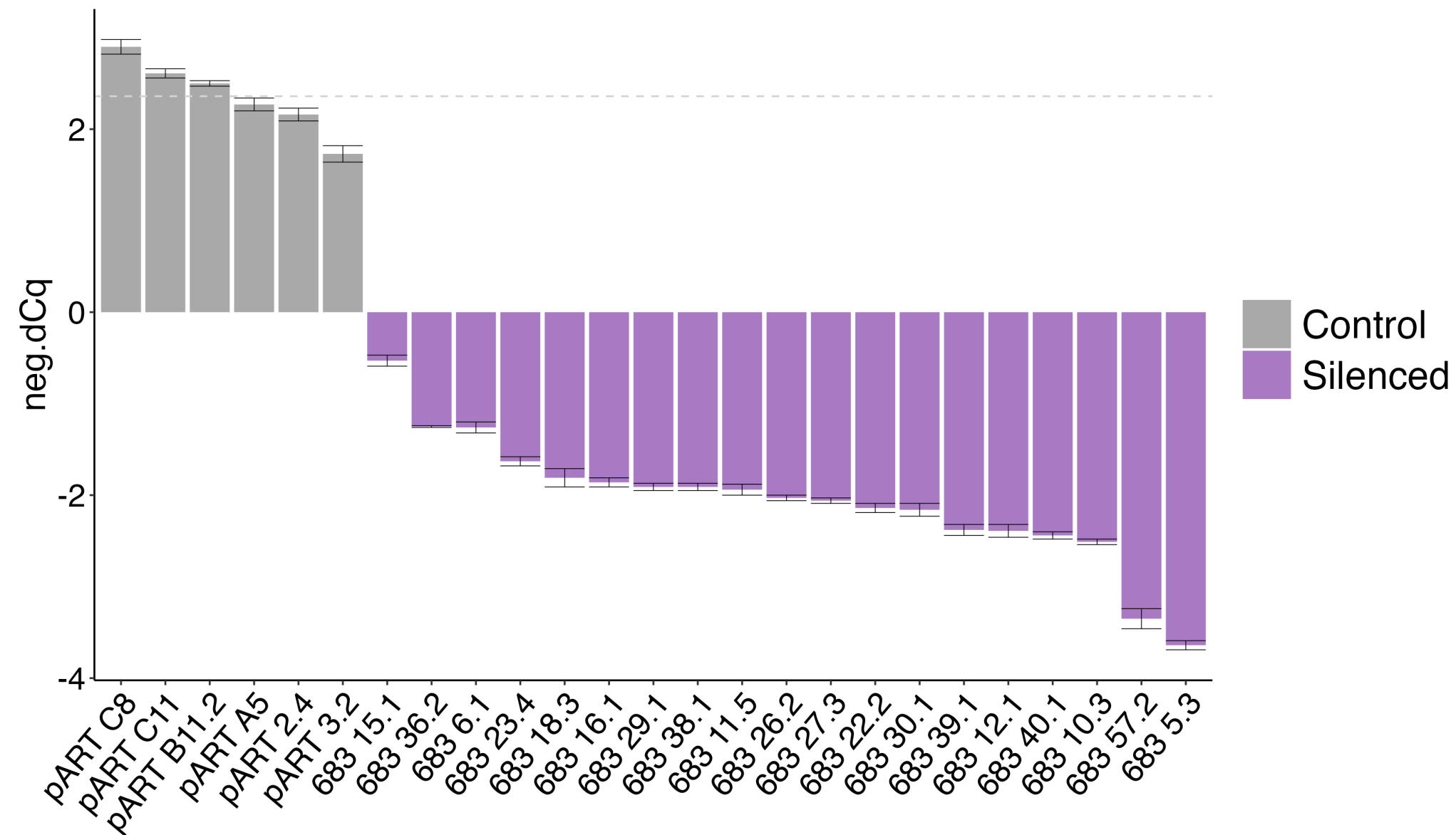
## Overexpression



Silenced,  
Overexpressed and  
control lines  
confirmed by PCR

# Expression levels by RT-qPCR

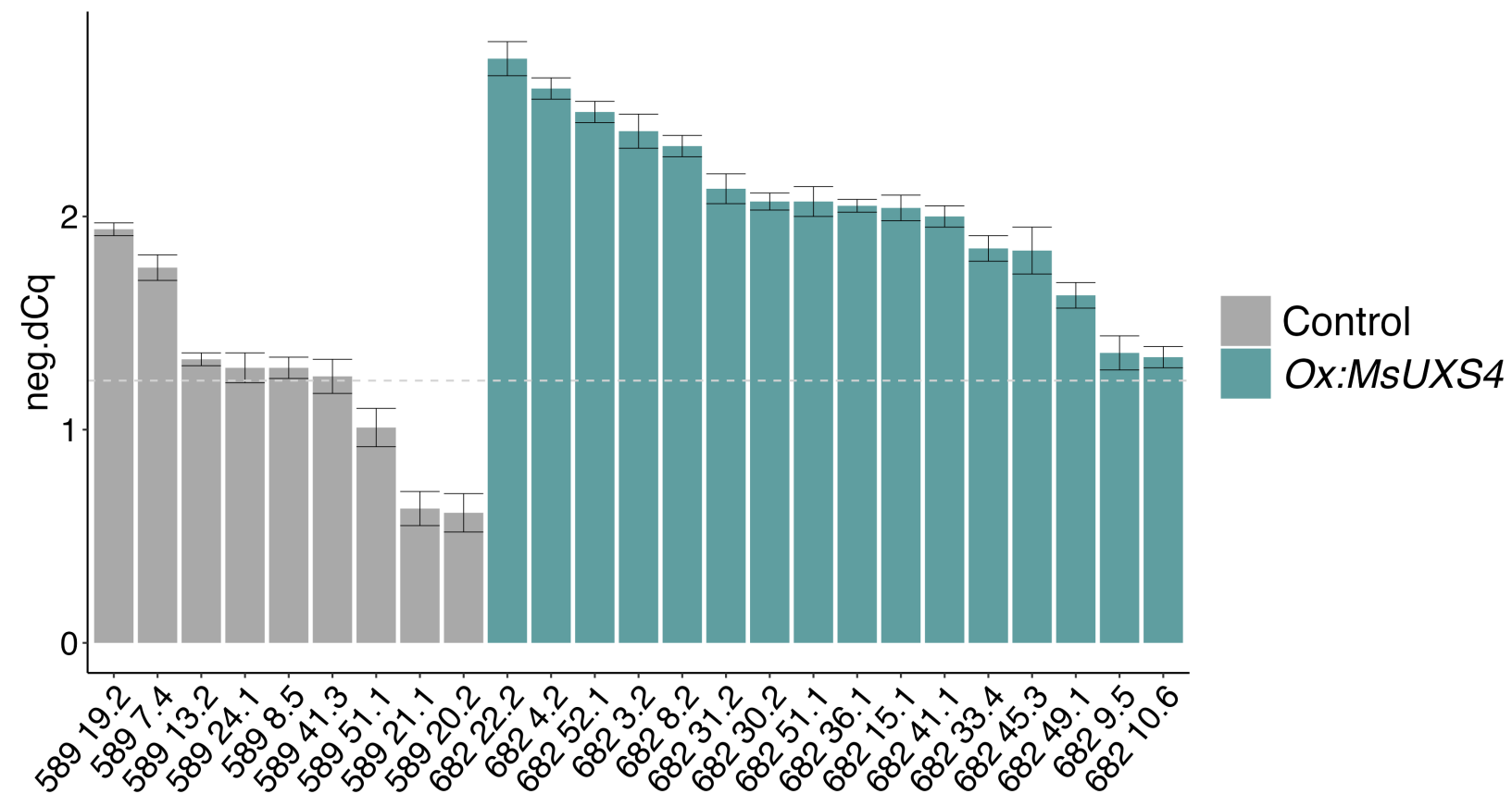
Silenced



Wilcox test significant  
 $p = 0.0001618$

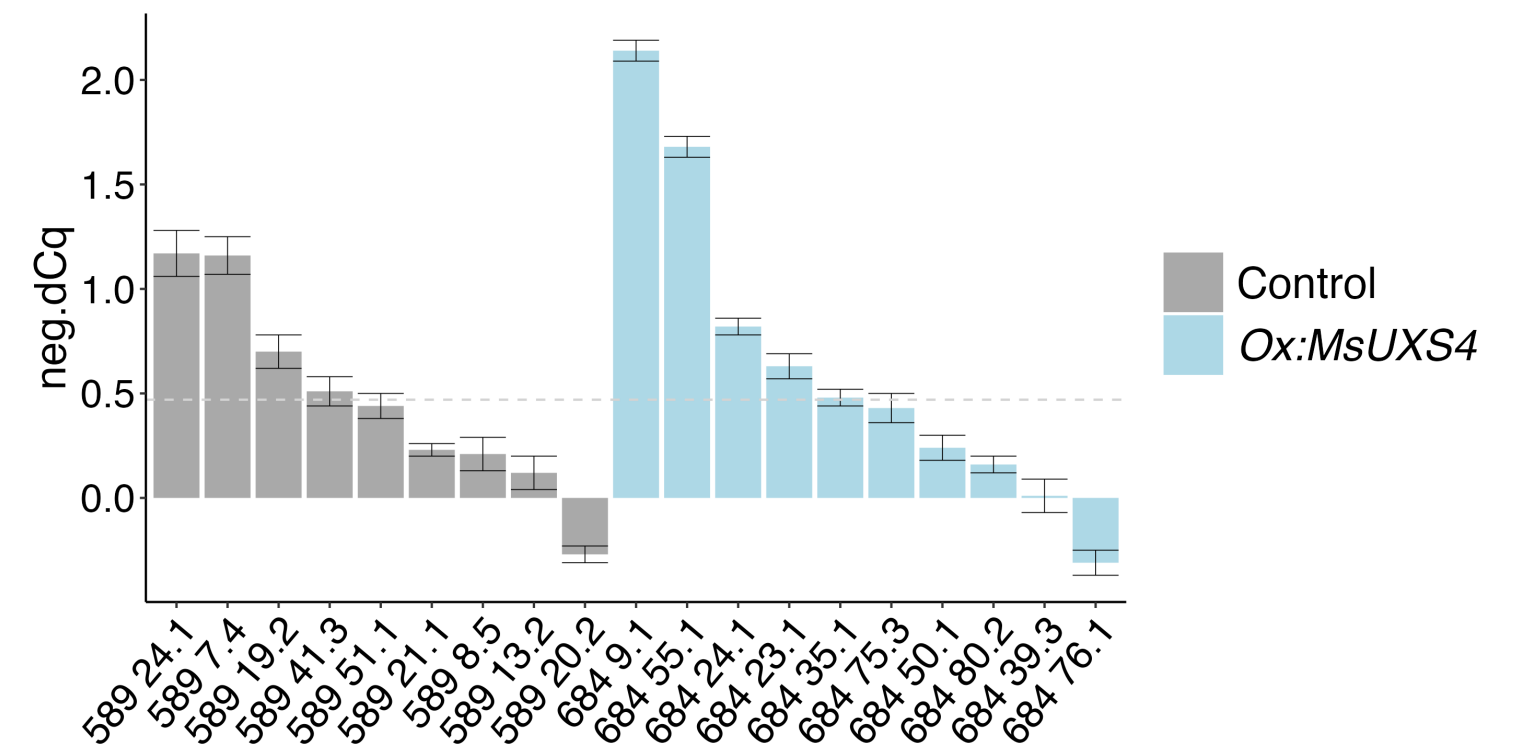
# Expression levels by RT-qPCR

*Ox:MsUXS4*



Wilcox test significant  
 $p = 0.0001613$

*Ox:MsUXS2*



Wilcox test not significant  
 $p = 0.4524$

# Uxs silenced lines have a stunted phenotype



589 21.1

589 51.1

PART27 C11

683 5.3

683 57.2

683 39.1

683 22.2

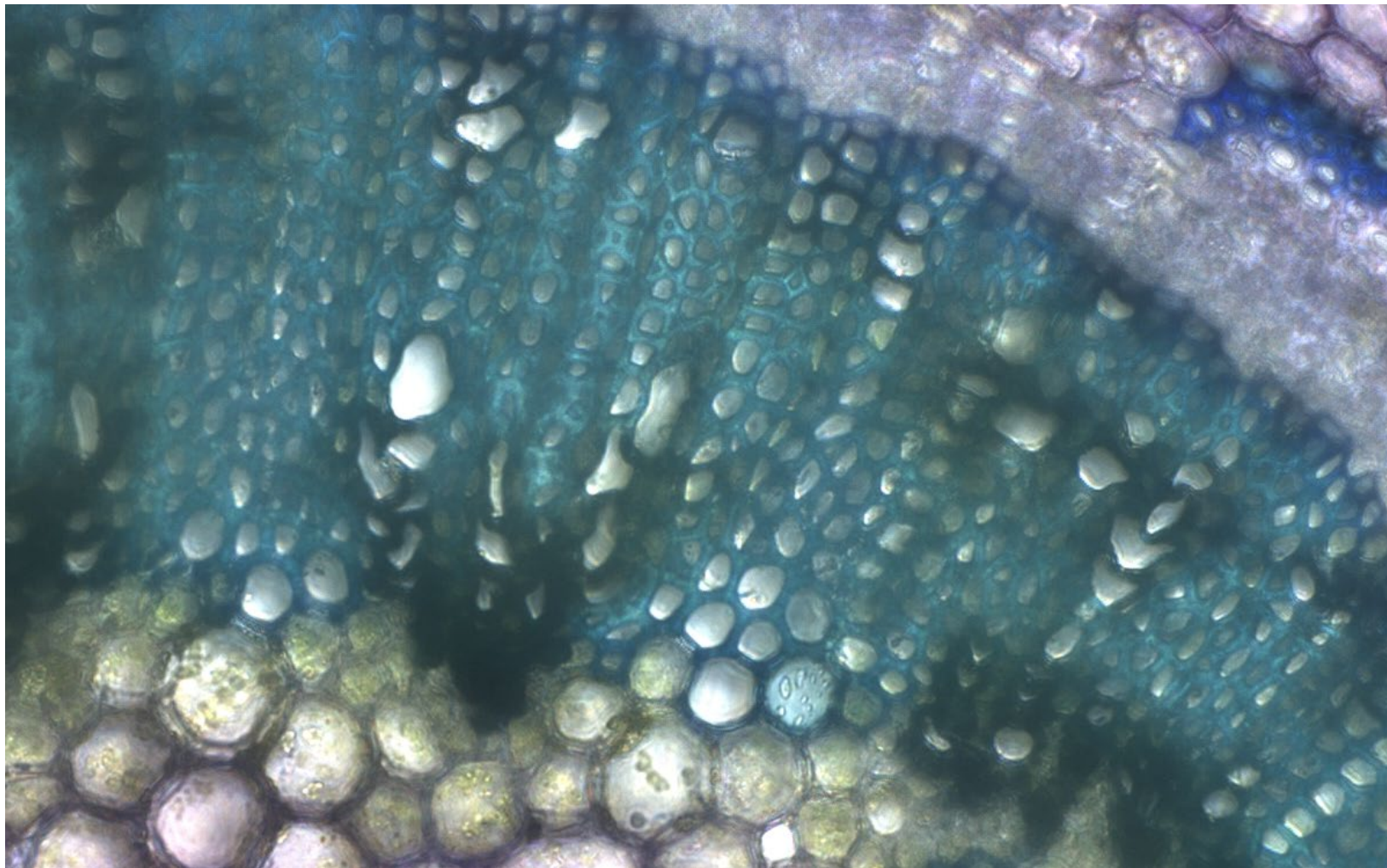
683 12.1

683 10.3

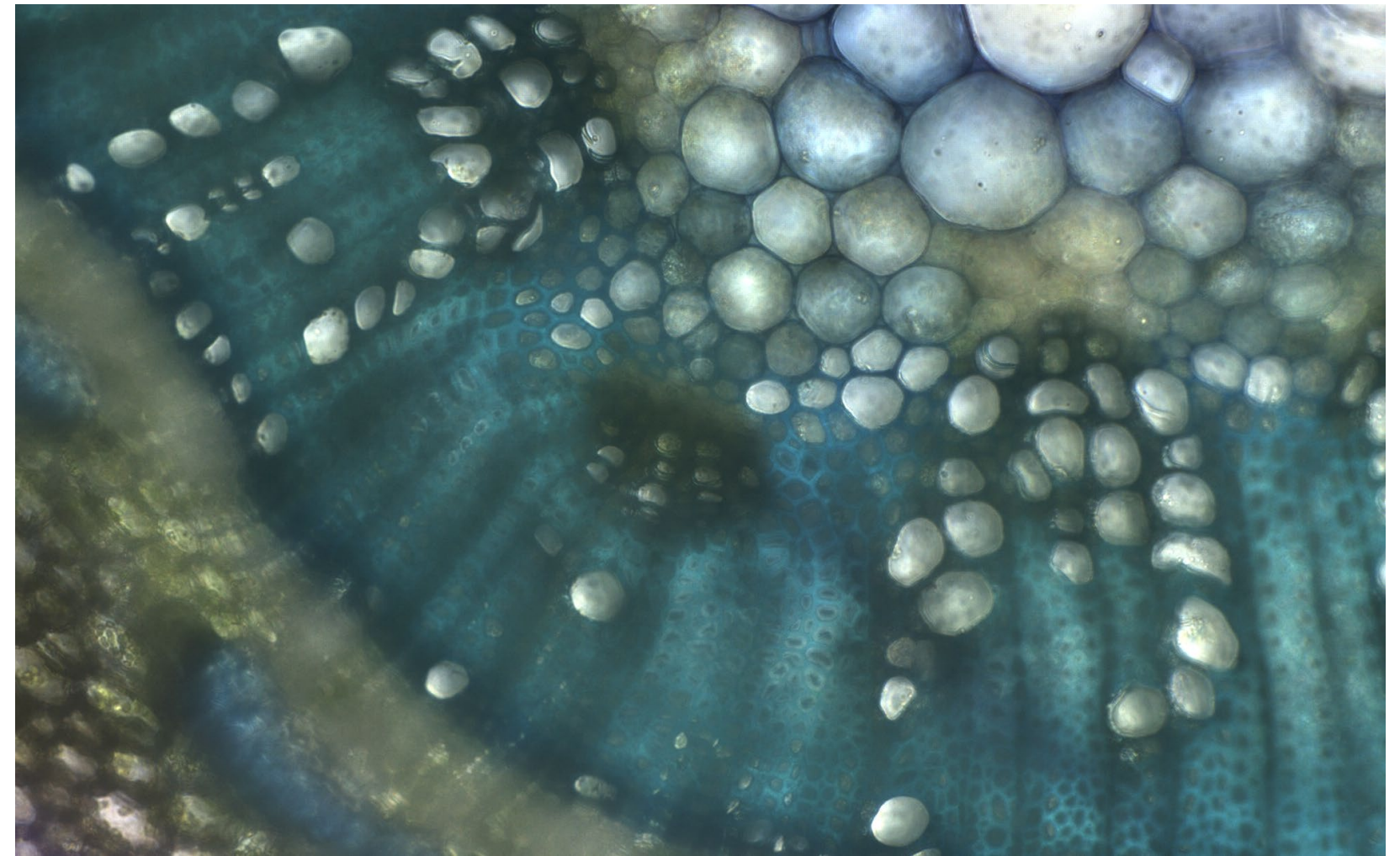
Control

Silenced

# Uxs silenced lines have misshapen vessels



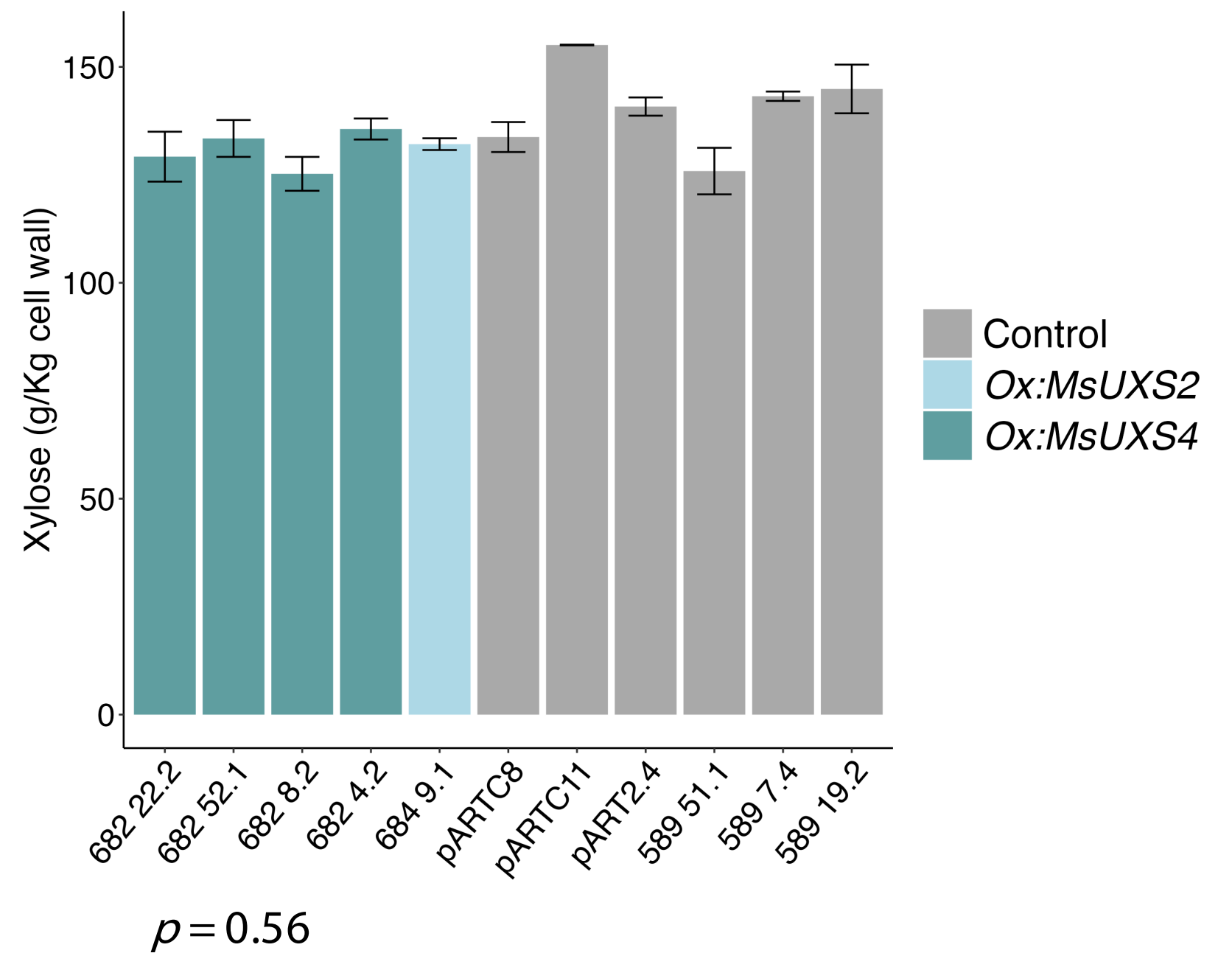
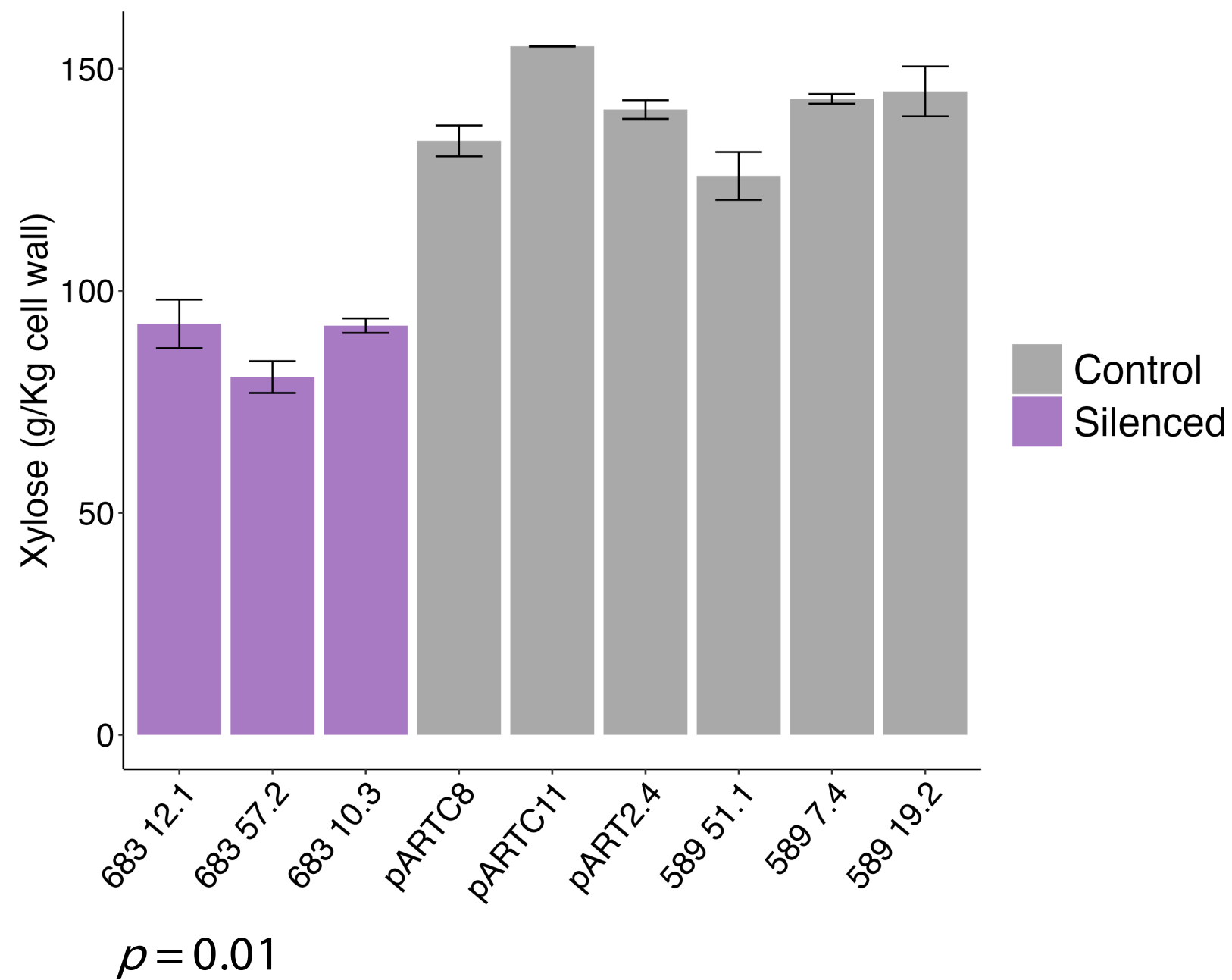
Silenced



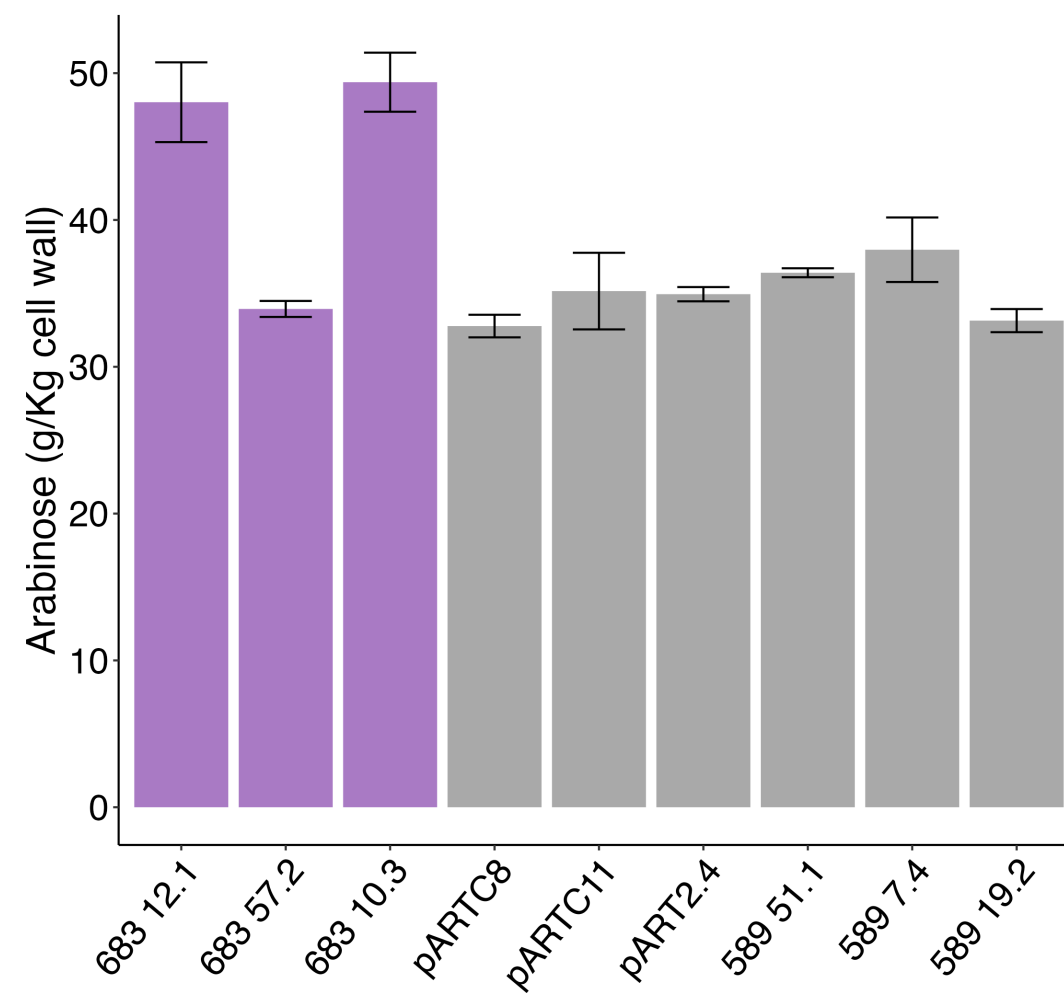
Control



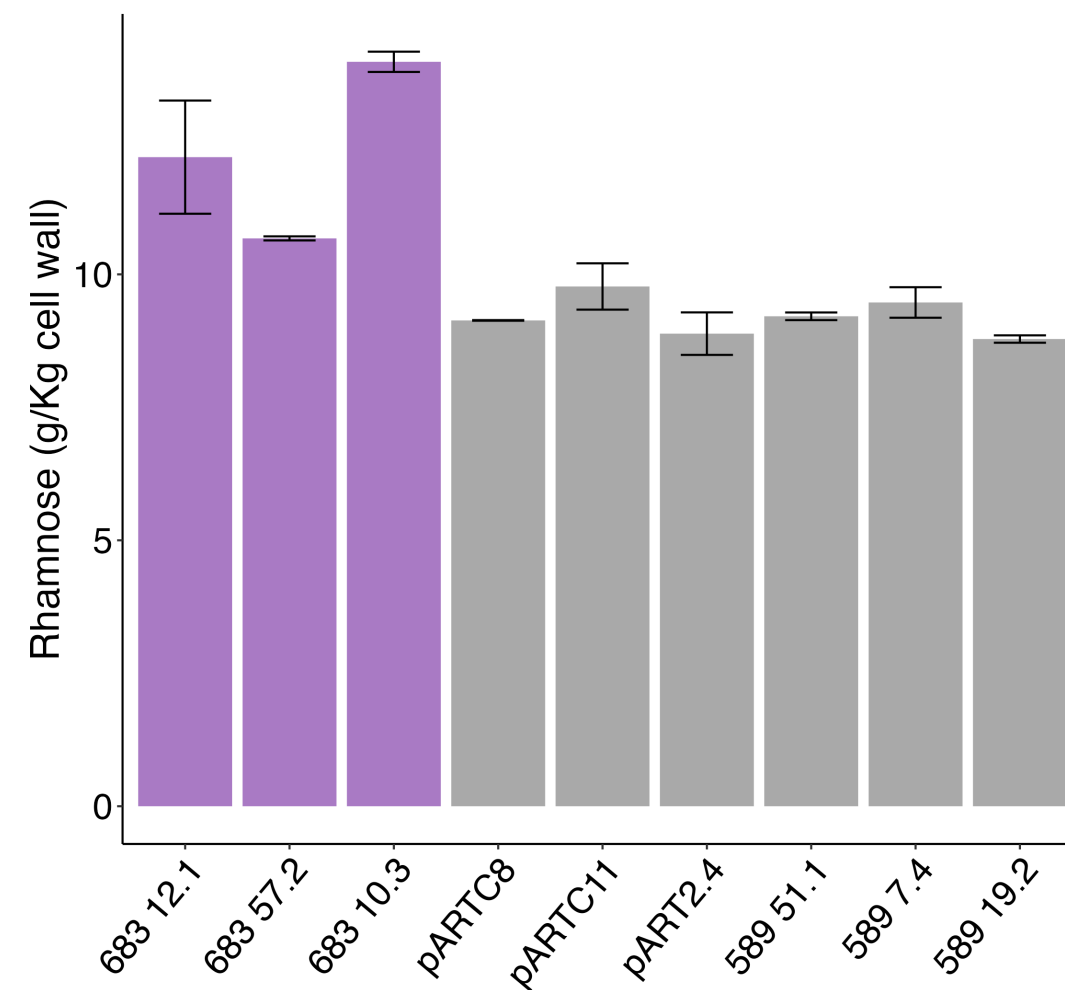
# Cell wall sugars composition is distinct in the silenced lines



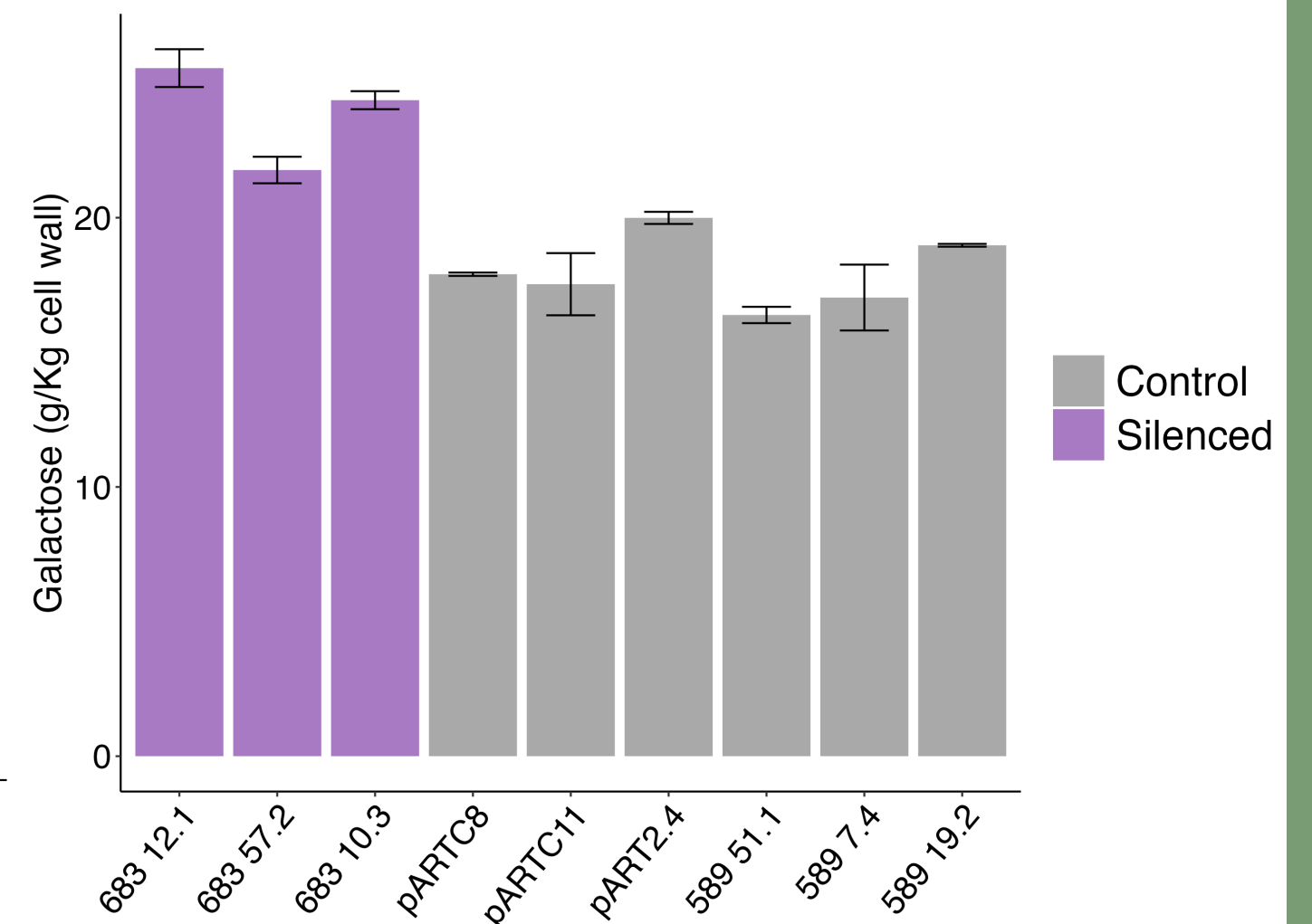
# Cell wall sugars composition is distinct in the silenced lines



$p=0.131$



$p=0.01$

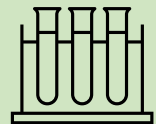


$p=0.01$

# Conclusion



Identified and characterized uxs genes in alfalfa



Next steps: Cell wall (sugars, lignin) and in vitro digestibility assays



Investigate ways to rescue the phenotype



Great potential in manipulating the cell wall composition to increase alfalfa digestibility

# Acknowledgements

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Melanie Katawczik



**Agricultural  
Research  
Service**



**OAK RIDGE  
INSTITUTE  
FOR SCIENCE  
AND EDUCATION**

# Thank you!



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There is great potential in modifying the levels of cell wall polysaccharides to improve alfalfa digestibility!

QUESTIONS?