

# Inducible cell wall degrading enzymes for expression in energy crops

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**ABSTRACT:** *In planta* expression of cell wall degrading (CWD) hydrolases promises to be a cost-effective strategy for producing enzymes that are required for converting biomass to fermentable components. Embedding such enzymes in the plant material as the plant grows could also enable more efficient processing of biomass by initiating hydrolysis of plant polysaccharides with lower restrictions from mass transfer effects, reducing or potentially eliminating the need for exogenous enzymes. Unfortunately, when expressed in transgenic plants, many CWD enzymes cause severe negative phenotypes such as stunting and poor seed set, presumably because their hydrolytic activity interferes with cell wall formation during normal plant development. One option to overcome this barrier to *in planta* expression has been to generate transgenics in which the CWD enzymes are sequestered into subcellular compartments, away from their natural substrates. An alternative strategy to overcome this problem is to express the CWD enzymes as inactive precursors, which can then be activated post-harvest via any of a number of possible inducers. Thus, enzyme production can be decoupled from hydrolysis, enabling even higher levels of expression in energy crops. We are developing a number of such CWD pro-enzymes for expression in maize, sorghum, and switchgrass, and we are assessing their performance in transgenic plants.

## Cellulosic Biomass

- Simple sugars derived from cellulosic biomass can support a variety of fermentation-based products, including biofuels.
- Releasing these sugars from plant material requires several distinct enzymes, including xylanases, esterases, endoglucanases, exoglucanases,  $\beta$ -glucosidases, etc.
- These enzymes represent a significant cost component of cellulosic bioprocesses (\$0.50/gal - \$0.75/gal)
- Expressing the enzymes directly in plants may make it possible to lower the overall cost of producing cellulosic sugars

## Expression of Unmodified CWD Enzymes in plants

Level of Enzyme Activity in Transgenic Plants



*In planta* expression of cell wall degrading enzymes adversely affects plant growth, development, and yield

Low Activity

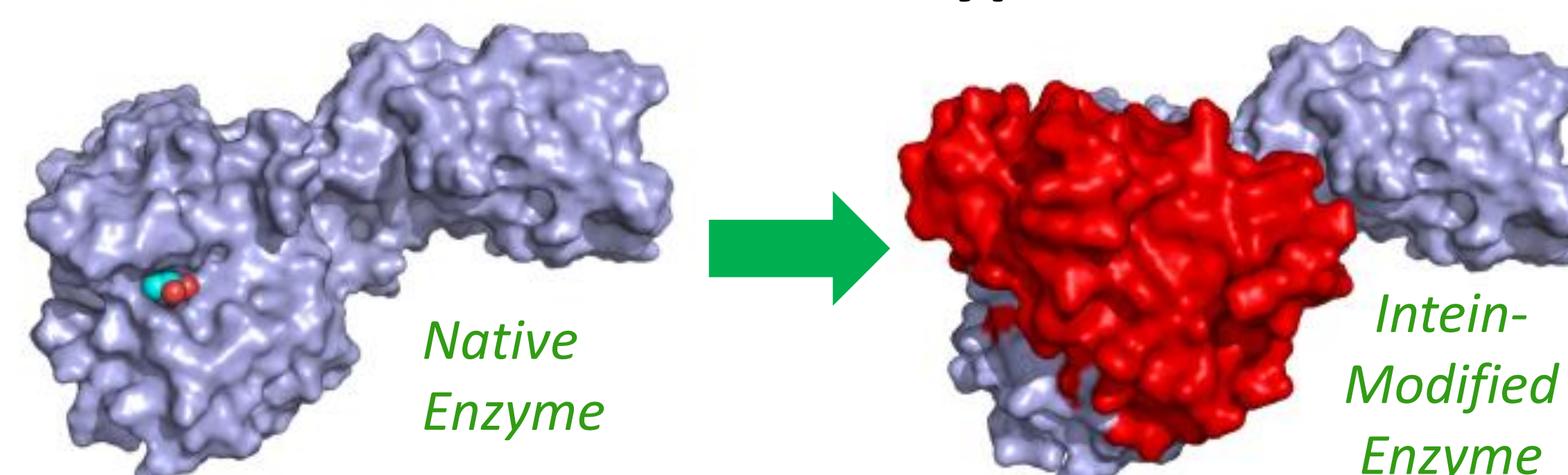
High Activity

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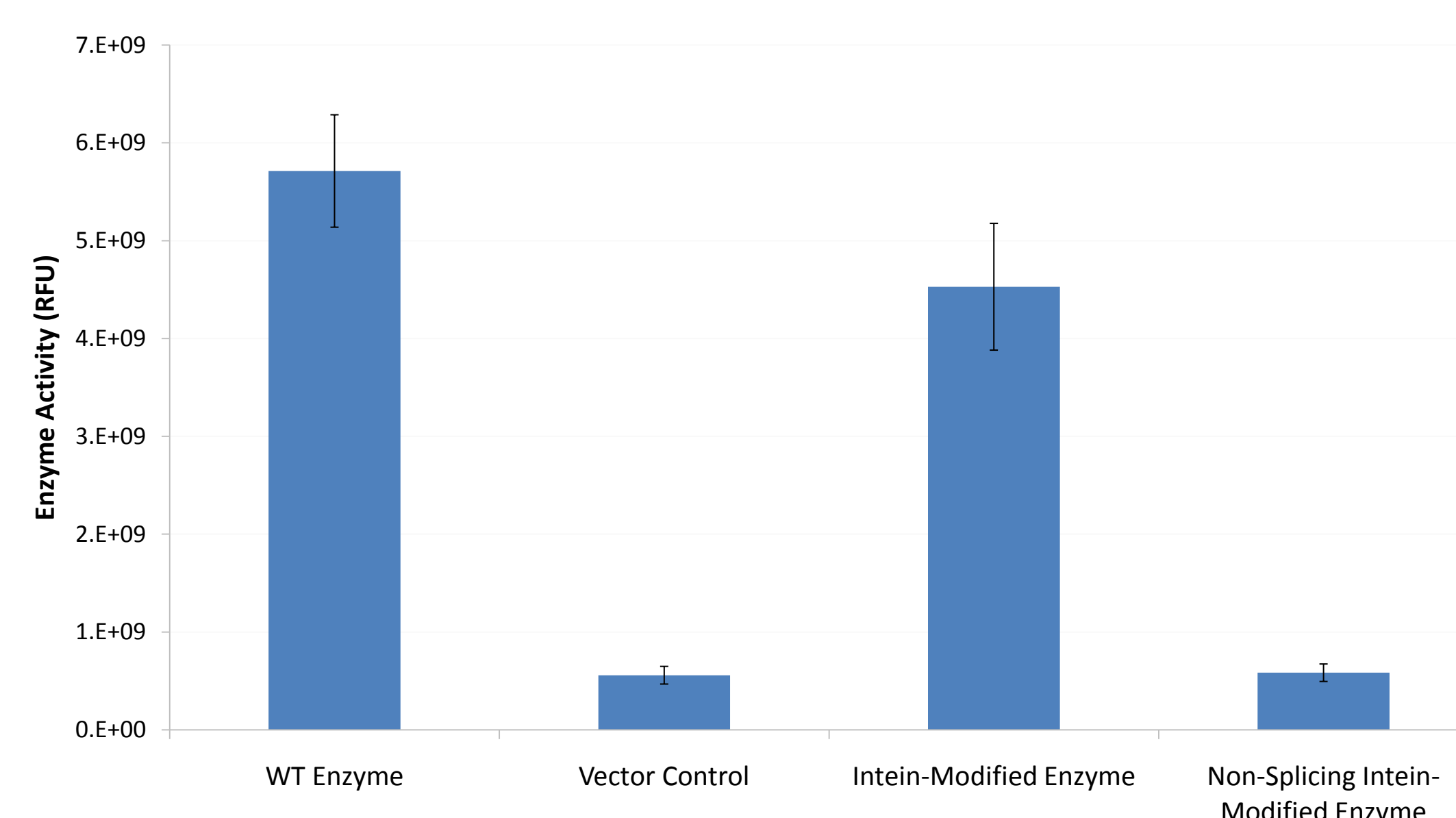


High level expression of cell wall degrading enzymes also affects seed morphology

## Creating Dormant Enzymes to Protect Plant Phenotypes

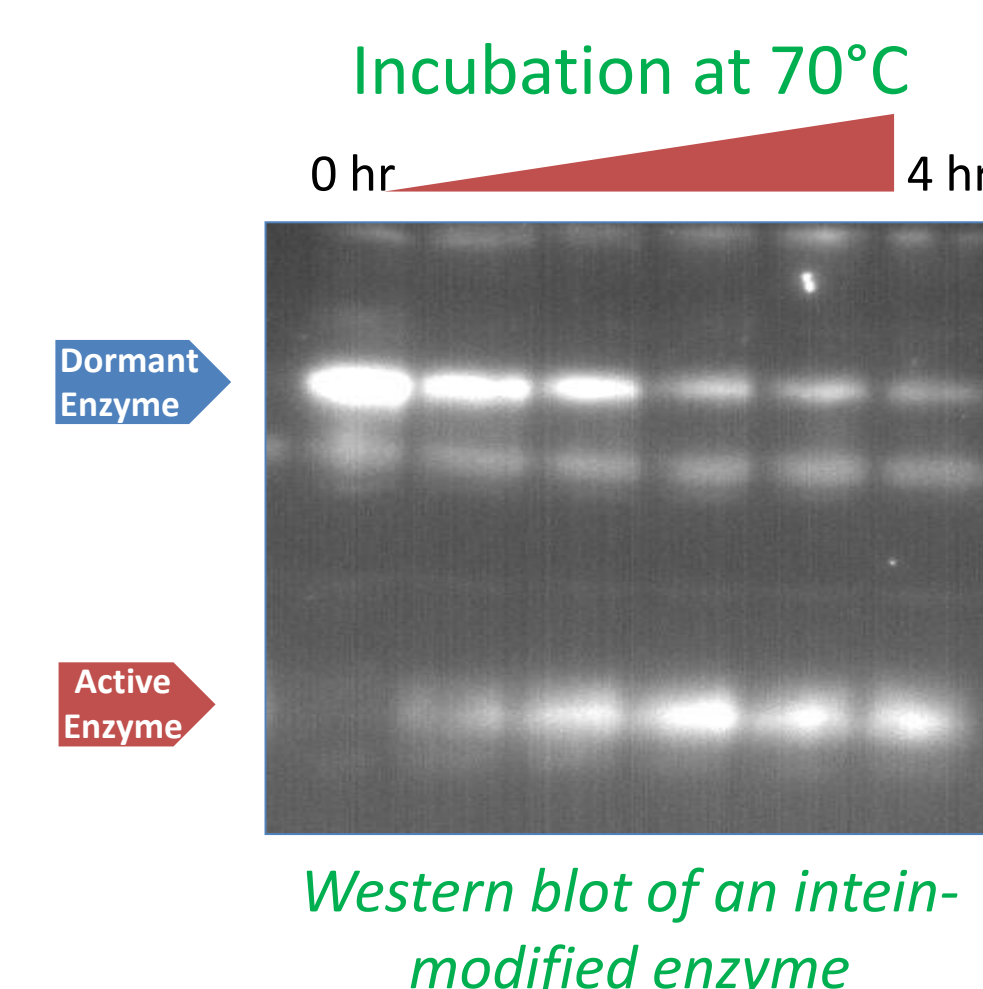
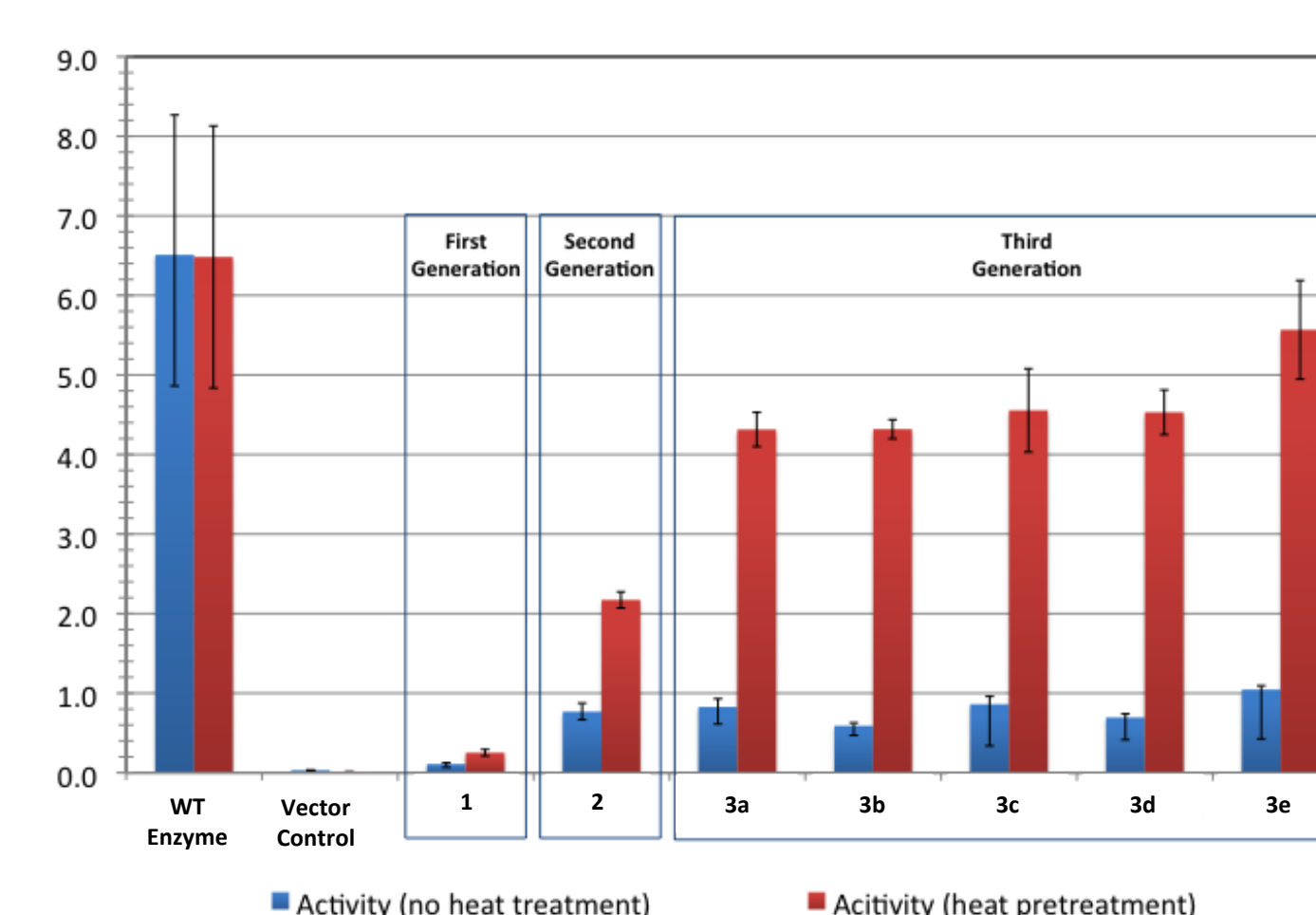


The activity of a cell wall degrading enzyme can be blocked by inserting an **intein** into the polypeptide, thereby blocking access to the active site or preventing correct folding



After the intein splices, enzyme activity will be restored. Conditions that prevent splicing of the intein will also prevent enzyme activity.

## Mutagenesis and Enzyme Evolution

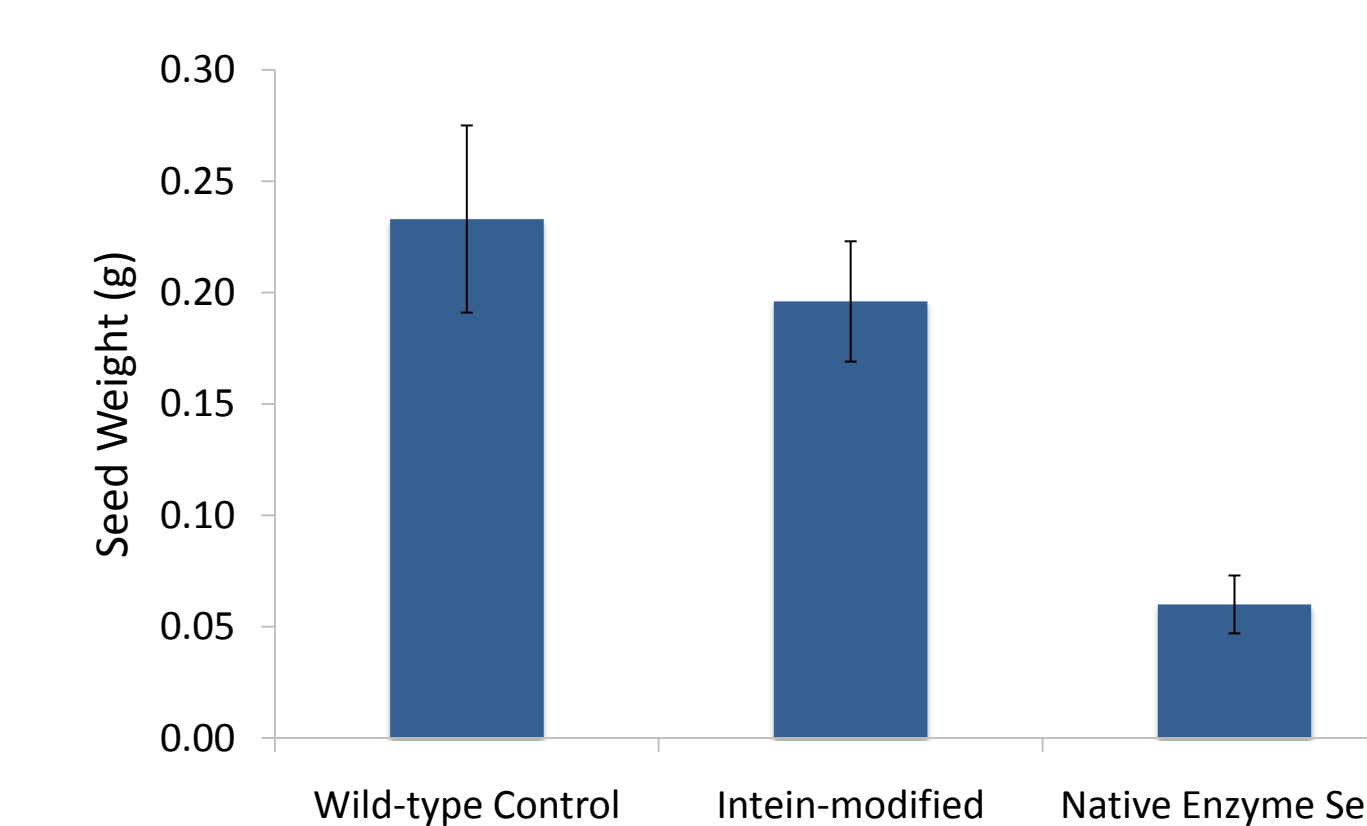


Intein-modified enzymes can be developed in which intein splicing is dependent on an external stimulus such as heat. This behavior can be enhanced through mutagenesis of the intein-modified enzyme.

## In Planta Expression of Intein Modified Enzymes



Maize plant expressing an intein-modified enzyme



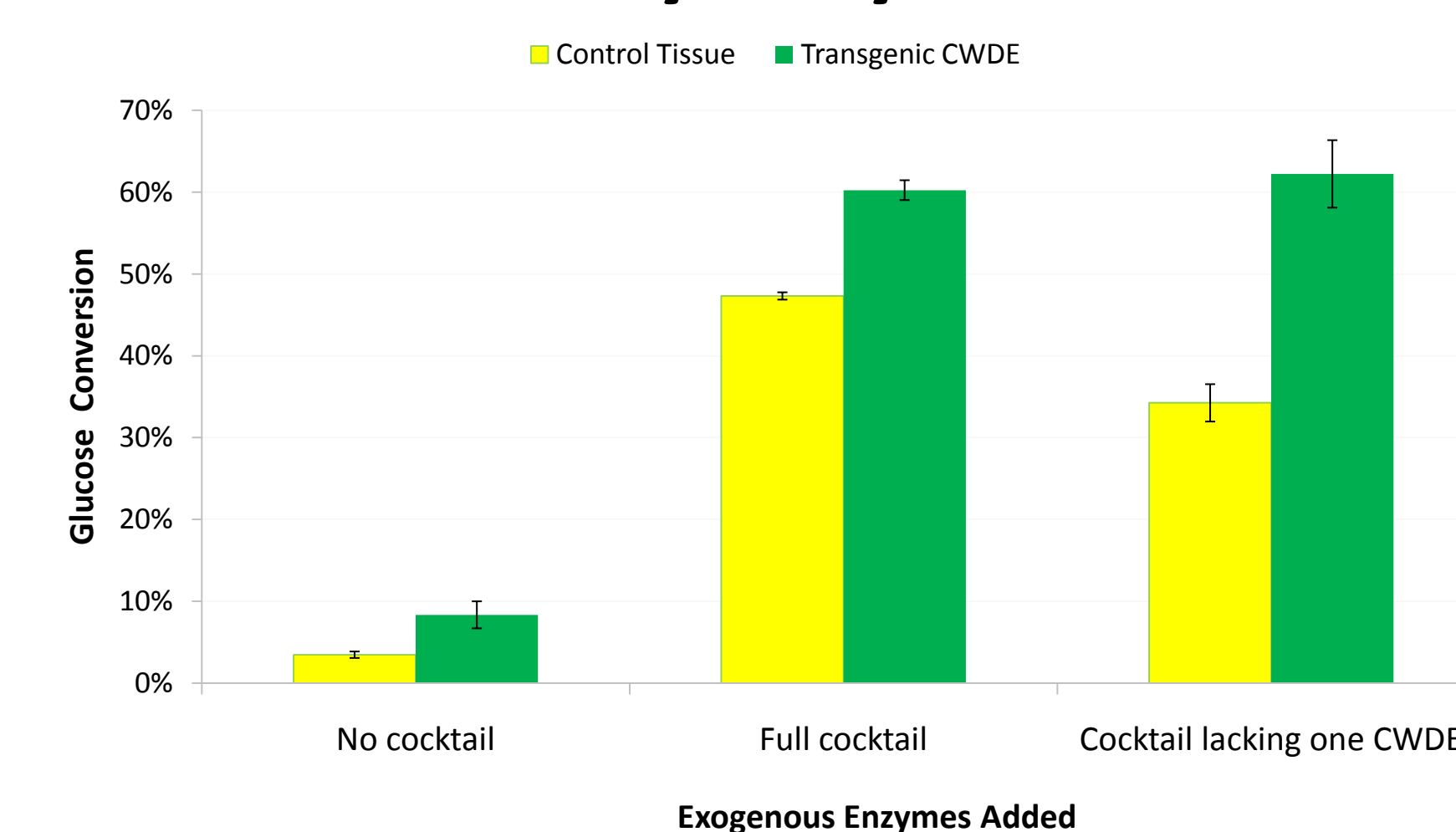
Intein-modified enzyme expression



Native enzyme expression

Expression of intein-modified enzymes allows plants to develop with more normal phenotypes

## In Planta Production of Enzyme Enables more Efficient Hydrolysis of Biomass



A cocktail of cell wall degrading enzymes are required to hydrolyze cellulosic material. Tissues from plants that express one of these cell wall degrading enzymes require less of that enzyme to release sugars

## Conclusion

- The activity of cell wall degrading enzymes can be controlled through conditional splicing of inteins.
- In their dormant forms, such enzymes can be expressed in plants without interfering with plant development.
- The enzymes that have accumulated in plant tissues can be activated post-harvest, displacing the requirement for some or all exogenous hydrolytic enzymes.