

# Characterization of Enzymes, Chalcone Synthase, Chalcone Isomerase, and Dihydroflavonol 4-Reductase, by X-Ray Crystallography To Improve the Efficacy of Crops *Panicum virgatum* and *Sorghum bicolor* as Potential USDA Biofuel Sources

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*Sorghum bicolor* and *Panicum virgatum* are widely grown crops. Lignin, synthesized in the monolignol pathway, prevents easy access to cellulose. Reducing lignin is necessary for these crops to serve as sustainable biofuels. The flavonoid pathway draws biomass away from lignin and enables the flavonoids, responsible for the plant's pathogen resistance and antioxidant properties. Tricin, a chemical in the monolignol pathways, is also synthesized in the flavonoid pathway. So, a connection between these pathways can be utilized to reduce lignin. Chalcone Synthase (ChS), Chalcone Isomerase (ChI), and Dihydroflavonol 4-Reductase (DfR) are key enzymes involved with the flavonoid pathways. Characterizing these enzymes is critical to bioengineering a crop to divert biomass away from lignin production. Structural characterization involved crystallizing each of the proteins, collecting X-Ray Diffraction data, and generating a structural model for each protein. Enzymatic characterization involved understanding the kinetics of each enzyme. Through experimentation with varying factors, ideal crystallization condition was determined for both ChS and DfR. Structural model, space group and unit cell geometry were derived from the diffraction data. Binding affinities of three substrates with DfR was calculated. Kinetic information (Michaelis-Menten constant) for the reaction between ChI and substrate naringenin chalcone was acquired. This research is the first to provide a high-resolution crystal structure of both ChS and DfR from the crop *P. virgatum* and the enzymatic characterization of ChI from *S. bicolor*. Biofuel production target could be achieved through bioengineering these crops guided by structural and enzymatic information determined through research like this.

## Awards Won:

King Abdulaziz &

his Companions Foundation for Giftedness and Creativity: Full Scholarship from King Fahd University of Petroleum and Minerals(KFUPM) (and a \$400 cash prize)

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