Ectopic expression of BdWRI1 affects fatty acid homeostasis in Brachypodium

Yang Yang, Que Kong, Wei Ma, Sanjay Sanjaya, John Ohlrogge, Christoph Benning
Michigan State University
Jack Munz, Cynthia Cass, John Sedbrook
Illinois State University

Moving from Dicots to Monocots

Dicot model plant
Arabidopsis thaliana
Monocot model plant
Brachypodium distachyon
Maize (Zea mays) in the field

WRINKLED1 is a transcription factor of Arabidopsis involved in the control of primary metabolism

wr1 seeds contain
80% less oil

WRI1 is one factor to engineer carbon partitioning!

Overexpressing WRINKLED 1 (WR1) leads to triacylglycerol (TAG) accumulation in Arabidopsis leaves (Proof-of-Concept)

Overall TAG content in 15-day-old 3SS::AtWRI1 seedlings was increased by approximately 3-fold.

Increasing the energy density of Biomass

Goal: Synthesize high-energy oils in vegetative tissues of biomass crops

Adding 10% TAG will increase liquid fuel energy of crop by ~30%

Andre et al., 2007, Plant Cell 19:2006-2022

Constitutively overexpressing Brachypodium WR1 increases TAG content in dry seeds

www.glbrc.org
Ectopic expression of *BdWRI1* leads to oil accumulation in Brachypodium vegetative tissues.

Ectopic expression of *WRI1* in Brachypodium causes local cell death and browning but not in Arabidopsis.

The mechanism of cell death in Ub::BdWRI1

- WRI1 directly regulates the *de novo* fatty acid biosynthesis genes. — *Plant Journal* (2009), 60: 476-487
- Lipid metabolism changes lead to cell death?
- Free fatty acid leads to small chlorotic lesions in Arabidopsis leaves — *Plant Journal* (2013) 76, 930-942
- Increased FFA induced Cell death?

Ectopic expression of *WRI1* in Brachypodium increases 16:0 and 18:2 free fatty acids (FFA) content in leaf blades.

Ectopic expression of *WRI1* in Brachypodium increases 16:0 and 18:2 free fatty acids (FFA) content in leaf blades.

FFA treatment of Brachypodium leaf blades causes local cell death.

18:2 induced cell death in Brachypodium WT is concentration dependent.
Inhibition of fatty acid biosynthesis decreases 18:2-induced cell death in Brachypodium leaf blades

Why is the 18:2 effect on BdWRI1 delayed?

Therefore, a decrease in FA biosynthesis should decrease cell death!

Hypothesis for BdWRI1 lines

Increased FA biosynthesis → Cerulenin
Increased FFA
Increased cell death

β- Oxidation genes are induced in Brachypodium but not in Arabidopsis following ectopic expression of WRI1

TAG in Brachypodium leaf blades turnover fast

Ectopic expression of WRI1

De novo fatty acid biosynthesis genes are induced

Increased FFA turnover by β-oxidation
Increased ROS and cell death

Strategies to address cell death phenotype following expression of BdWRI1 in Brachypodium

- Express BdWRI1 at a specific time
- Express BdWRI1 in a specific tissue
- Block beta-oxidation
- Stabilize the TAG accumulation

Michigan State University
Biochemistry & Molecular Biology

Rebecca Roston
Kenny Wang
Anna Hurlock
George R. Murphy III
Austin Katona
Hope Hersh
Blair Bullard
Zhi-Yan Du
Krystof Ziemlewicz
Eric Pollmer
Tomasz Takauchi
Chie-Hsiang Tsai
Januszew Wokanamong
Elana Michal
Que Kong
Yang Tang
Agnieszka Ziemlewicz
Jenny Mantyla
Christoph Barming

John Froehlich, MSU DOE–PRL
Leslie Kuhn, MSU-BMB
Barbara B. Sears, (MSU–PBL)
Shinhan Shiu, (MSU–PRL)
Min-Hua Kuo, (MSU–BMB)
Eva Farm, (MSU–PRL)
David Krames, (MSU–PRL)
Ben Luckett, (MSU–PRL)
Kris Nyberg, (UC Berkeley)
Sabeesta Merchant, (UCCLA)
John Ohlrogge, (MSU–PRL)
Yair Shachar-Hill, (MSU–PRL)
John Sedbrook, (Illinois State Univ)

Algal Lipid Metabolism, Synthetic Biology Platform
AFOSR
DOE–EFRC
MSU–AgBioResearch

High Energy Plants
DOE–GLBRC

Chloroplast Lipids
NSF–MCB
DOE–Bioscience

www.glbrc.org