Ectopic expression of *WRI1* affects fatty acid homeostasis in *Brachypodium distachyon* vegetative tissues

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Project Goals: This project aims to synthesize high-energy oils in vegetative tissues of biomass crops, including C4 grasses. Enhancing the energy content and liquid fuel yield of crop biomass can be achieved by accumulating triacylglycerols (TAG) in the crop. After extracting these compounds, lignocellulosic feedstock remains and can be used for processing and fermentation. Thus engineering oil production will result in novel biofuel crops that achieve multiple objectives. Our previous research has succeeded in producing TAG in Arabidopsis leaves and stems (Sanjaya et al., 2011; Sanjaya et al., 2013). Oil content was increased by combining transcription factors, down-regulation of starch synthesis, and addition of enzymes of TAG synthesis (Sanjaya et al., 2011). Testing these second generation approaches in the grass model plant Brachypodium is now efficient and cost-effective providing a 'proof-of-concept' strategy prior to moving into corn or other energy crops.

WRINKLED1 (WRI1) is a transcription factor, which governs fatty acid (FA) synthesis and indirectly TAG accumulation in oil storing plant tissues and ectopic expression of its cDNA has led to TAG accumulation in vegetative tissues of different dicotyledonous plants (Cernac and Benning, 2004; Cernac et al., 2006; Ma et al., 2013; Ma et al., 2015). The ectopic expression of *BdWRI1* in the grass Brachypodium (*Brachypodium distachyon*) induced the transcription of predicted genes involved in glycolysis and fatty acid (FA) biosynthesis, and TAG content was increased up to 32.5-fold in 8-week-old leaf blades. However, the ectopic expression of *BdWRI1* also caused cell death in leaves, which has not been previously observed in dicotyledonous plants such as Arabidopsis (*Arabidopsis thaliana*)(Yang et al., 2015). Lipid analysis indicated that the free FA content was 2.0-fold elevated in *BdWRI1*-expressing leaf blades of Brachypodium. The transcription of predicted genes involved in β-oxidation was induced. In addition, linoleic fatty acid treatment caused cell death in Brachypodium leaf blades, an effect

that was reversed by addition of the FA biosynthesis inhibitor cerulenin. Taken together, ectopic expression of *BdWRI1* in Brachypodium enhances FA biosynthesis and TAG accumulation in leaves as expected but also leads to increased free FA content, which has cytotoxic effects leading to cell death. Thus, while WRI1 appears to ubiquitously affect FA biosynthesis and TAG accumulation in diverse plants, its ectopic production can lead to undesired side effects depending on the context of the specific lipid metabolism of the respective plant species.

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