Field evaluation under standard tobacco production practices of tobaccos engineered for high leaf-oil accumulation

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Abstract

Global demand for commodity plant-derived oils (triacylglycerols, TAG) is expected to more than double in coming decades due to increasing food oil requirements of a burgeoning world population. Current sources of plant oils are predominantly from dedicated oilseed crops (soybean, canola, sunflower, etc) and fruits (palm, olive, coconut). Their supply is presently matched to food demand, and they do not have the required scalability to sustainably meet both the food and industrial demands of the future.

To overcome these feedstock supply limitations, CSIRO Plant Oil Engineering group has invented, developed and patented a game-changing technology for engineering very high levels of triglyceride oils in leaves of high biomass crops (Figure 1). Preliminary studies were conducted comparing engineered tobacco plant against an empty vector control in replicated fashion in the glasshouse. These initial reports were spectacularly successful, achieving in excess of 35% oil (dry weight basis) in leaves of glasshouse-grown plants.

If these levels can be achieved in field grown tobacco and other high biomass species, oil productivity of “next generation” oil crops could exceed that of oil palm and enable production of oil for renewable fuels and industrial chemicals at competitive costs with the long-range expectation for petroleum feedstock. To further explore this potential the initial leaf-oil tobacco lines were evaluated in the field in Kentucky during 2017 to produce materials for more examination of biomass and oil development and further evaluated in 2018.

Objectives

Evaluate LEC2 engineered line oil content and profile against parental and wild type lines in a field production system.

Materials & Methods

Experimental lines were ‘LEC2’ engineered configuration, ‘Parental’ configuration, and the comparative check was the wild-type (Wisconsin 38). **Field preparation** was commercial practice including conventional tillage, pre-transplant fertility, and pre-transplant herbicides. **Transplanting** was conducted using RJ Equipment carousel-type setter calibrated for 91.5 x 45.75cm plant spacing (23,920 plants ha^{-1}). **Topping** occurred when >50% of respective line population demonstrated elongated bud (CORESTA stage 55.5). Suckercides used to suppress axillary bud growth. **Harvest** took place 21 days after topping by priming leaves from the top, middle, and bottom third of plants. Harvested leaf material immediately transported to heated forced-air dryers (>60°C) to mitigate biological degradation. **Analysis** of oil content and profile conducted using GC-FID. **USDA Permit** for experiment was **18-025-101r-a1.**

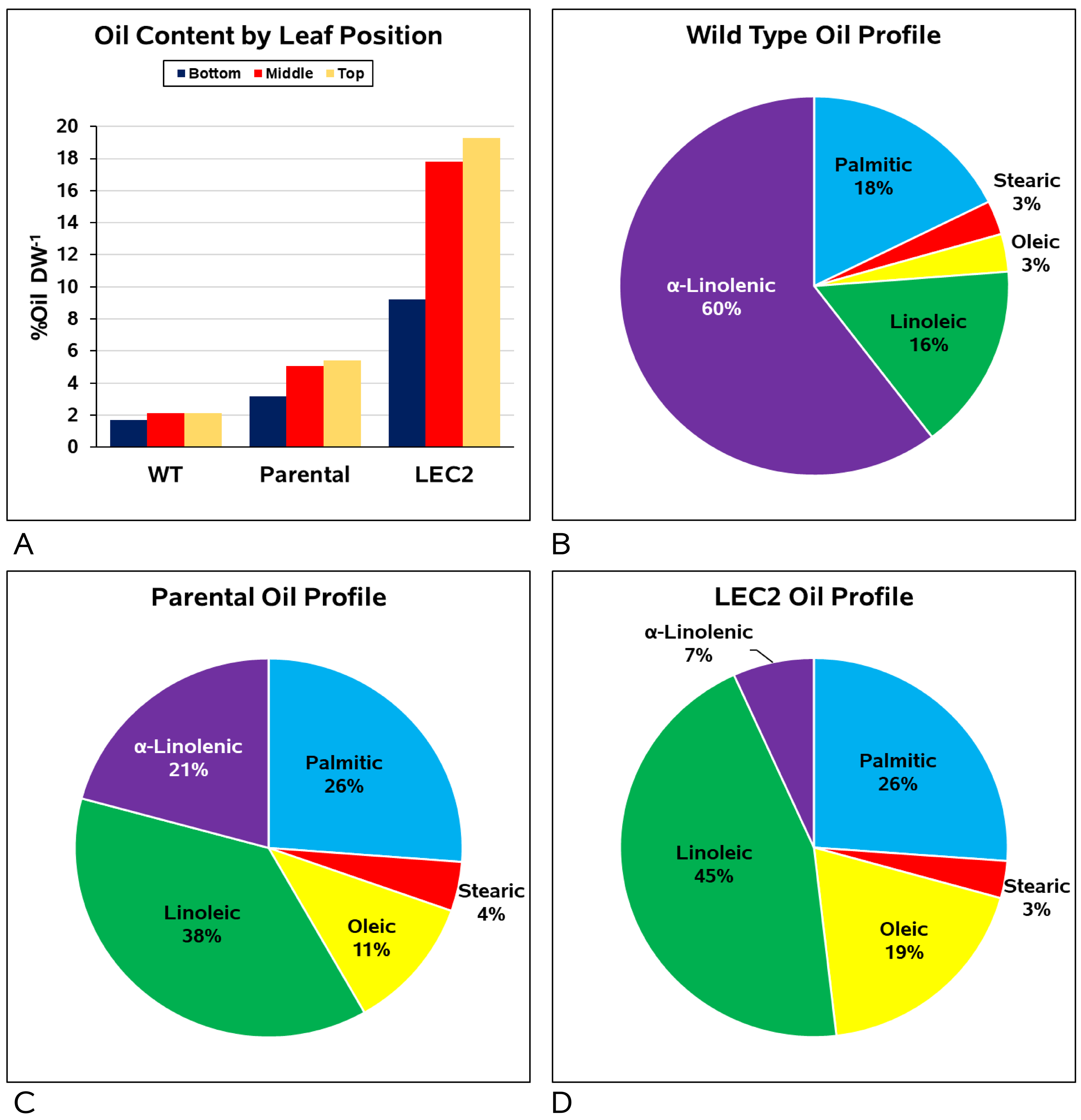

Figure 2. Oil content & profiles. A. Oil content (%DW) by top, middle, or bottom leaf position. **B.** Average oil profile of wild type line. **C.** Average oil profile of ‘parental’ line. **D.** Average oil profile of ‘LEC2’ line.

Results

- Parental line had increase in leaf oil content at all leaf positions over wild type, but less than LEC2 line following previous greenhouse findings (Figure 2A)
- LEC2 line had highest leaf oil content at all leaf positions (up to 19.3%)
- All engineered lines demonstrated positive gradient of leaf oil accumulation from older, bottom leaves to younger, top leaves
- Parental oil profile shows dramatically reduced α-linolenic acid (18:3) displaced with increased linoleic (18:2) and oleic (18:1) acids (Figure 2B,C)
- The addition of LEC2 to parental line further reduced α-linolenic acid and increased linoleic and oleic acids compared to parental (Figure 2D)

Conclusions

With information generated by this study in conjunction with others, this novel LEC2 engineered system can be further developed and deployed into elite cultivars with proven field performance and biomass recovery. The high oil potential of this system coupled with a high biomass line – in a possible multiple harvest model – could create a cost competitive lipid-based biofuel production system to accommodate rapidly increasing global demands.

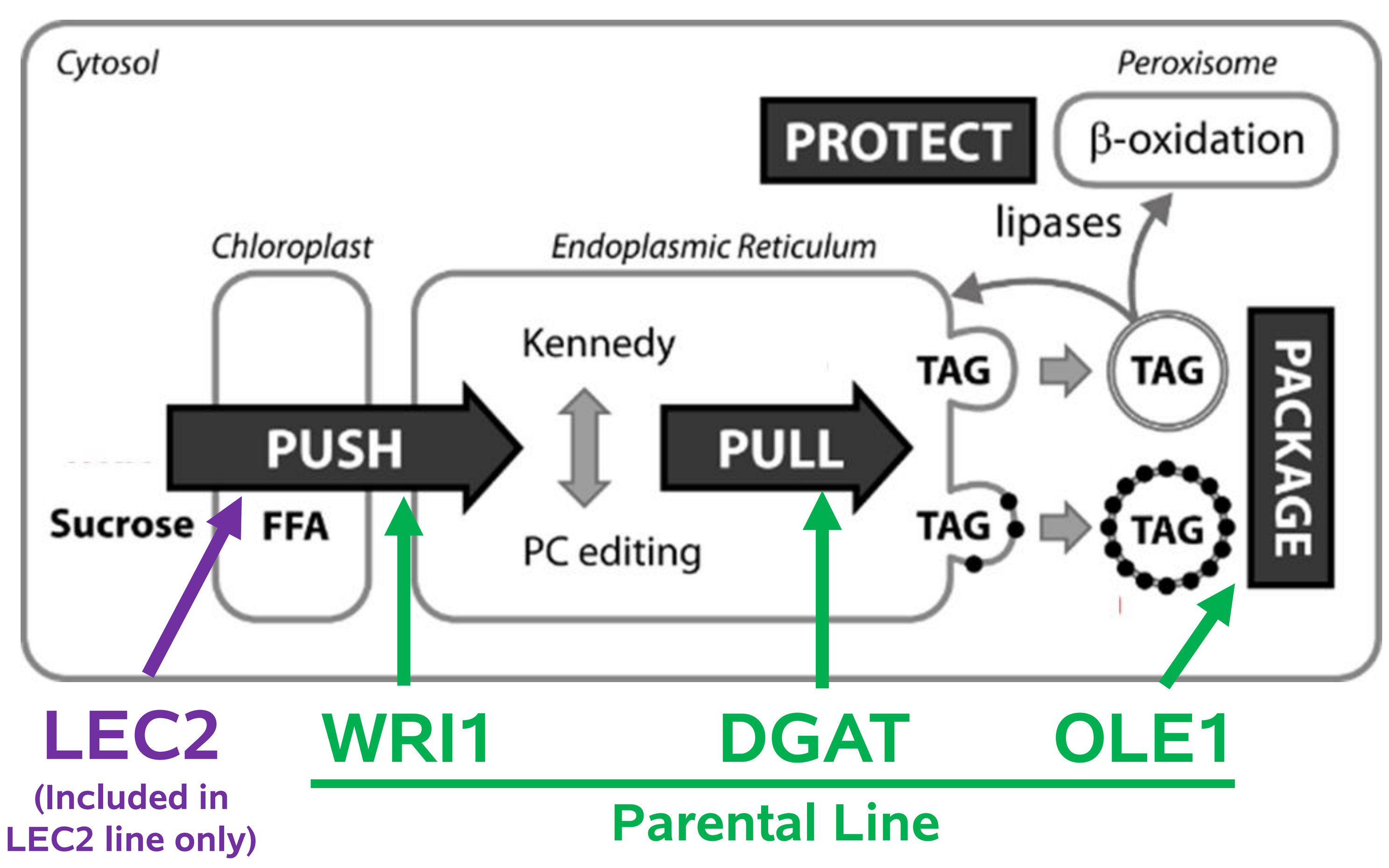

Figure 1. Schematic of engineered system. ‘Parental’ line construct includes transcription factor wrinkled 1 (WRI1), enzyme diacylglycerol acyltransferase (DGAT), and coat protein oleosin 1 (OLE1). ‘LEC2’ line construct includes parental genes plus transcription factor leafy cotyledon 2 (LEC2).

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References

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