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**Thesis Title**: Metabolic engineering of tobacco (*Nicotiana tabacum*) for biosynthesis of dihydroartemisinic acid

**Abstract:** Artemisinin is highly effective against drug-resistant malarial parasites, which affects nearly half of the global population and kills >500000 people each year. The primary cost of artemisinin is the very expensive process used to extract and purify the drug from Artemisia annua. Elimination of this apparently unnecessary step will make this potent antimalarial drug affordable to the global population living in endemic regions. Here we reported the oral delivery of a non-protein drug artemisinin biosynthesized (~ 0.8 mg/g dry weight) at clinically meaningful levels in tobacco by engineering two metabolic pathways targeted to three different cellular compartments (chloroplast, nucleus, and mitochondria). The doubly transgenic lines showed a three-fold enhancement of isopentenyl pyrophosphate, and targeting AACPR, DBR2, and CYP71AV1 to chloroplasts resulted in higher expression and an efficient photo-oxidation of dihydroartemisinic acid to artemisinin. Partially purified extracts from the leaves of transgenic tobacco plants inhibited in vitro growth progression of *Plasmodium falciparum*-infected red blood cells. Oral feeding of whole intact plant cells bioencapsulating the artemisinin reduced the parasitemia levels in challenged mice in comparison with commercial drug. Such novel synergistic approaches should facilitate

low-cost production and delivery of artemisinin and other drugs through metabolic engineering of edible plants.

In our previous study (Saxena et al., 2014), expression of all the 12 transgenes into the tobacco chloroplast genome caused stunted growth, which highlighted the need to spread the biosynthetic load to more than one metabolic compartment. We circumvented these defects by using a balanced compartmentalization approach to transgene expression using three cellular compartments (cytosol, chloroplast, and mitochondria), and obtained DT plants with normal phenotypes and up to 0.8 mg artemisinin per gram dry weight leaf. A similar effort using combinatorial supertransformation of transplastomic lines engineered with the artemisinic acid pathway resulted in an accumulation of 120 mg/g fresh weight of artemisinic acid (Fuentes et al., 2016).

The primary cost of artemisinin is the very expensive process used to extract and purify the drug from A. annua. Our report indicates that this is an unnecessary step and that artemisinin can be produced in edible leaves. We provide a proof-of-concept demonstration of the functionality of tobacco-biosynthesized artemisinin in DT lines. This finding suggests that artemisinin can become accessible and affordable to the large global population living in malarial regions.

**References:**

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