

Abstract

We propose to increase CO₂ assimilation rates in the alternate rubber-producing crop *Taraxacum kok-saghyz* (Buckeye Gold) by transforming chloroplasts with a modified carbon capture enzyme, de/inhibited Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RubisCO). Additional assimilate may be used to increase plant biomass which will increase the harvestable rubber of Buckeye Gold. It has previously been shown in other rubber crops that surplus assimilate will be consumed by being converted to rubber polymers, which are an inert carbon sink in living plants.

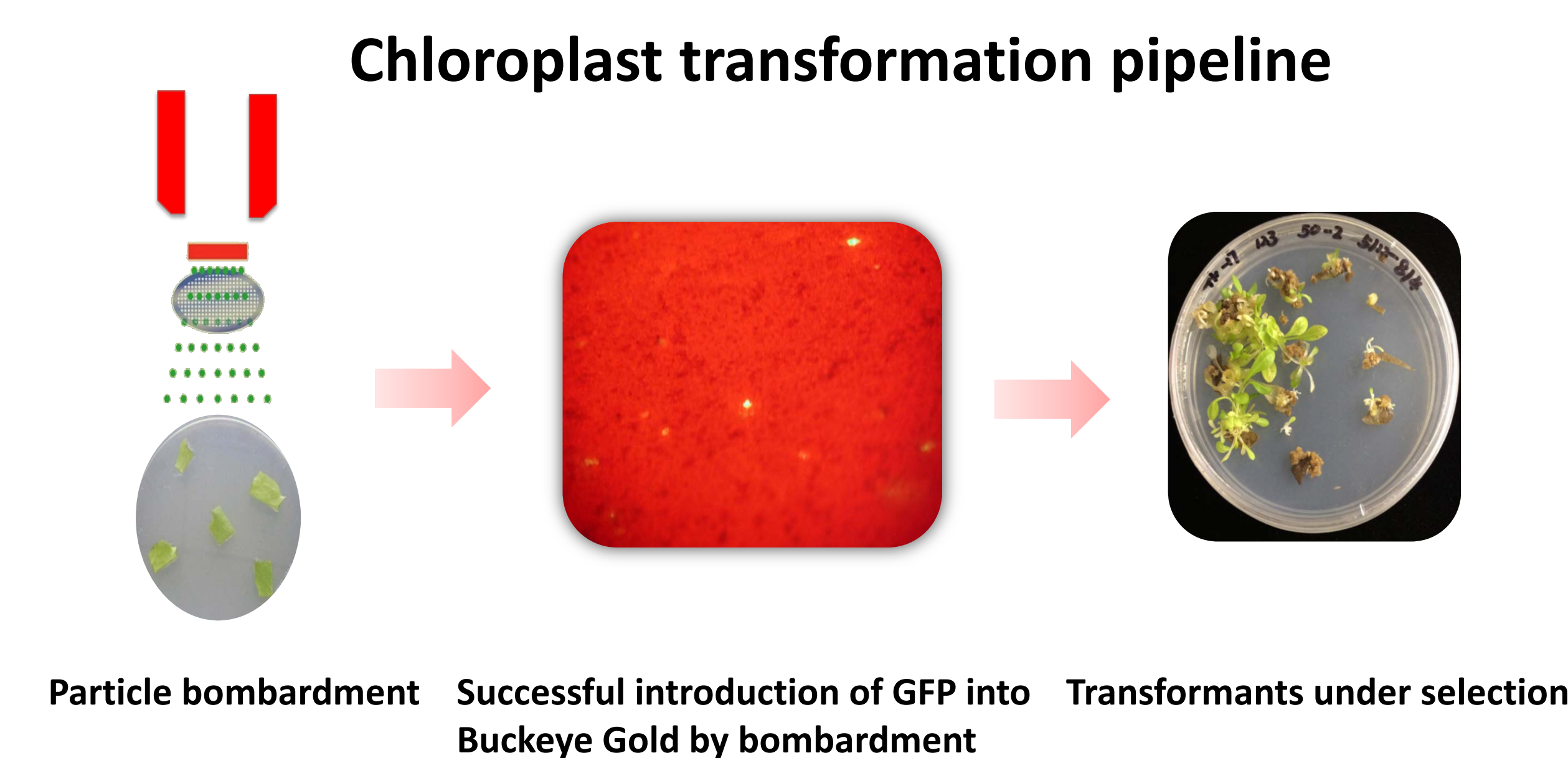
A commercially-viable Buckeye Gold variety will be a domestic source for natural rubber, will aid in reducing rubber price volatility, and may eventually lead to rubber exports. Over the next 10 years the global rubber market is predicted to need about 18 million acres of Buckeye Gold, which if grown will capture over 100 million metric tons (MMT) of carbon. Buckeye Gold produces a mechanically superior natural product which has the possibility of replacing low performance synthetic rubber, as well as preventing the emission of 90 MMT/year of CO₂.

Background

Taraxacum kok-saghyz (Buckeye Gold) produces a high quality rubber in its roots which is similar to the tropical rubber tree currently grown on more than 10 million hectares. Because of environmental requirements there is only a small amount of land available for expanding production of the rubber tree crop, combined with the high cost of manual labor required in harvesting, the rubber tree is currently near peak capacity. Demand for natural rubber (NR) continues to increase, led by the industrialization of Asian countries, a trend expected to be followed by Africa. Produced from petroleum, synthetic rubber (SR) produces greenhouse gases and is of insufficient quality to replace NR however, NR may replace SR in most applications.

Shortfalls in NR production are expected to reach 1 million metric tons (MMT) by 2020 and production of alternative NR crops has become imperative. Unlike NR shortages, the consequences of greenhouse gas emissions are much more widely understood and there are incentives in place to reduce emissions. Rubber crops are additionally a means of carbon sequestration along with being a sustainable replacement for petroleum-based SR.

Biotechnology may be used to increase CO₂ assimilation by plants. The Buckeye Gold CO₂ assimilation enzyme, RubisCO, will be de/inhibited by Prof. Tabita, so that much higher rates of assimilation can be achieved. This enzyme will be expressed in chloroplasts based on methods that have been developed recently in the Cornish laboratory for transformation of Buckeye Gold. Because rubber acts as a sink for excess assimilate, expression of de/inhibited RubisCO in this species may lead to larger and higher rubber content plants.



Key Challenges Addressed

- Demand for natural rubber is greater than the rubber tree can produce alone.
- More than 40,000 different products are made with natural rubber.
- The US imports > 1.2 million metric tons (MMT) of NR each year (\$18 billion industry).
- If we do not produce NR domestically we will soon be buying industrial and tire NR at latex glove prices.
- The US needs sustainable NR production to protect its supply chain of this critical agricultural material.
- A domestic production chain would create many thousands of jobs, address our balance of trade, and lead to a profitable export market.
- Synthetic rubber production generates large amounts of CO₂ (~90 MMT/year), which will double during the next decade. Replacement of some SR by NR is highly desirable.
- Because rubber acts as a carbon sink, a US rubber crop will reduce GHS gas production and sequester huge amounts of CO₂.
- Approximately 25 MMT biomass will be produced at self sufficiency levels of NR, sequestering at least an additional 12 MMT CO₂.

Proposed Research and Timeframe

Activities	Duration (month)	Total time (month)
▪ Codon modification for de/inhibition of the TK RubisCO gene sequence for chloroplast expression	0.5	0.5
▪ Generate optimized gene by DNA synthesis	1	1.5
▪ Incorporate the optimized de/inhibited RubisCO into a chloroplast transformation vector, containing recombinant flanking sequences homologous with flanking regions of the Buckeye Gold chloroplast genome insertion sites	2	3.5
▪ Transform chloroplast by bombardment of leaf discs with this vector and with an empty vector control	2	5.5
▪ Select transgenics on spectinomycin while inducing shoots	5	10.5
▪ Confirm transgene integration by polymerase chain reaction (PCR)	0.5	11
▪ Regenerate whole plants from positive selections	3	14
▪ Reconfirm transgene integration by PCR	0.5	14.5
▪ Confirm transgene expression with reverse transcription PCR and Western blot analysis	1	15.5
▪ Clone plants, grow to flowering stage, interbred different genotypes and collect seed	4	19.5
▪ Germinate seed and evaluate plants in greenhouse studies, including CO ₂ assimilation rate	4	23.5
▪ Determine next steps (breeding, scale-up, etc.)	0.5	24

Categories	Costs
▪ Personnel: 150 % GRA; 2.5 % Faculty	\$75,000
▪ Materials and supplies: lab, controlled environment, greenhouse, sequencing.	\$17,000
▪ Direct costs	\$92,500
▪ Indirect costs	\$9,250
Total	\$101,750

Commercial Potential and Impact

- 1.2 MMT of NR needed for US self-sufficiency, raw material worth about \$2 billion, feeds an \$18 billion industry, and requires ~2 million acres of production.
- Crop residuals can all be sustainably converted to liquid biofuels and platform chemicals, providing viable replacements for petroleum based materials and creating yet more value.
- Success will provide a proof-of-concept needed to incorporate this enhanced carbon capture strategy in many other food, feed and industrial crops.

Annual Metrics	Now	2 Years	10 Years
Rubber produced	0.5t/yr	50t/yr	1.2 million t/yr
Value Added to C by rubber	\$1,165/yr	\$1,165,000/yr	\$28 billion/yr
Biomass produced	9t/yr	900t/yr	21.6 million t/yr
Value Added to C by biomass	\$990/yr	\$99,000/yr	\$2.38 million/yr
Total Carbon captured	5.5t/yr	550t/yr	13.2 million t/yr
Value added to C by carbon credit	\$33/yr	3,300/yr	\$79.2 million/yr
Total C value	\$2188/yr	\$1,267,300	\$109.6 million/yr

Assumptions:

- Carbon from CO₂ valued at \$0/metric ton
- Value added by the rubber to 1 kg C = \$2.33
- Biomass valued at \$60/t
- Value of non-rubber biomass in 1 kg C = \$0.11
- Carbon credit \$6/t
- Rubber valued at \$2/kg
- Plant biomass = 2x root biomass

- The Cornish group has a history of successful IP protection with 21 invention disclosures filed.

Potential project IP

- Latex and rubber compounds, fillers, blends cross-linkers
- Films, foams, molded, extruded
- Harvesting and purification at lower cost
- Plant PVPs, sequence specific IP, transformed plants

Patent and literature search

- Chemical agents to increase photosynthetic rate
- Increasing growth rates by alternating photorespiration
- Glycolate dehydrogenase transformation
- Foreign RubisCO can operate in tobacco