SBI4U: Unit 3.7: Assessment for feedback

Journal review

Vocabulary list

Carboxylation: In photosynthesis carboxylation is a process where CO_2 reacts with *RuBP* in a *RuBisCO* catalyzed reaction to form C3 or C4 compounds. These compounds are the precursors for glucose.

Peroxisome: An organelle which could be considered a cousin to lysosomes because it often breaks down toxins. (It takes it's name from hydrogen *peroxide* which is breaks down.)

In the case of photorespiration in plants, peroxisome oxidizes RuBP forming *glycine* in the 1st reaction, and eventually *glycerate* after subsequent reactions. Glycerate is then fed into the Calvin cycle.

Promoter gene: Promoter genes are small sections of DNA that support *RNA transcription*. Special proteins attach to promoter genes. The attached protein marks sections of DNA directly adjacent to the DNA sequence that is involved in RNA transcription. Promoter genes are thus not involved in the actual transcription, but are rather enablers for early stages of the RNA transcription.

Flux: In biology flux is flow. In photosynthesis flux describes molecule or electron flow between organelles or cells.

Homozygous: In genomics, homozygous alleles are a type of gene marker. They are those gene markers that are inherited and are also identical to the same alleles in both parents. See *azygous*.

Azygous Contrast with *homozygous*. In genomics, azygous alleles are a type of gene marker that is different from and thus not inherited from either of the two parent alleles.

Transgene assembly: The genetic product of the experimental insertion of a section of genes from one organism to another.

In the context of the article, the *AP1*, *AP2* & *AP3* tobacco plants are all transgene assemblies of the wild type plants *WT* plants.

Empty Vector: A type of experimental control group.

In the context of the article empty vectors, *EVs*, were used alongside *wild type* tobacco plants to control for the insertion procedure itself. *Empty* may not be

entirely accurate. It is sufficient that the sequence is guaranteed to have no impact on the host organism.

Immunoblot analysis: An assay used in molecular biology to test for the presence of a particular protein in a tissue sample.

Transcript expression analysis: An analysis of the success of gene transcriptions. In the context of the article, a series of tests contribute to an analysis of now effective the transcription of some AP3 plants were in altering biomass as compared to other AP3 plants.

Fv'/Fm': A plant stress test measurement. It provides metrics for how well a plant's *Photosystem II* performs light capture under different conditions. A higher value indicates better light capture.

Mesophyll conductance: The measure of the diffusion of CO_2 through mesophyll cells. It takes into account multiple factors including cell wall and membrane movement and chloroplast organelle flux.

Substrating illumination intensities: *I couldn't find any information about this phrase*

Γ^* It appears to me that Γ^* is a measure made up for the purposes of the experimenter's analysis. I can't find anything about this gamma measure on the web.

That said, as the journal article states, Γ^* is a measure of where a plant's *RuBisCo* catalyzed breakdown of CO_2 is equal to its breakdown of O_2. So Γ^* a kind of *inflection point* marking where neither photosynthesis nor photorespiration are the dominant reaction in the chloroplast.

C_i^*: C_i^* is used by the experimenters as a marker for photosynthetic rate measurements. According to the article, C_i^* is the amount of CO_2 present in the intercellular tissue of the plant when the photosynthesis/photorespiration rates are at Γ^*.

Lower C_i^* suggests increased photosynthesis. This is reasonable given photosynthesis consumes CO_2 (and given the conjugate process, photorespiration, would increase CO_2 levels).

Quantum efficiency: In photosynthesis, quantum efficiency is the measure of chemical energy produced by light energy.

The journal article uses Φa to denote CO_2 assimilation. The implication is that increased CO_2 assimilation is the result of more efficient light energy use. This efficiency leads to more chemical energy production in the plant.

Randomized single block design experiment: *Randomized block design experiment* (without the *single*) is a type of experiment design that produces significance tests, often in smaller sample size settings. Test subjects are separated into groups (blocks) which are randomly divided into half control, half test subjects.

In a *single* block design, reasonably enough there is only one grouping or block. See *One way ANOVA*.

One way ANOVA: One way analysis of variance (ANOVA) are statistical significance tests.

In the case of the journal article's experimental setup (see *Randomized single block design experiment*) the *one way* signifies that the experiment is only concerned with grouping test subjects along one vector, whether the tobacco plants are genetically altered or not.

Analysis of variance because the experimenters want to compare whether transgene assemblies produce more robust, bigger crops as compared to un-altered tobacco plants.

Malate: In C4 photosynthesis, malate is a 4 carbon intermediate that is part of the *Calvin Cycle*, specifically the *RuBP Regeneration* phase.

Pyruvate: A 3 carbon intermediate used in the *Calvin Cycle*, specifically the *RuBP Regeneration* phase.

Diurnal: In biology, the daily period during daylight hours.

Phenotype: In genetics phenotypes are the physical characteristics that are observable.

Transcriptome: All *RNA* in groups of cells or an experiment.

Proteome: All *proteins* in a cell.

Binary plasmids: Transport DNA used in genetically engineering plants. Binary plasmids carry the DNA from a donor species (E. Coli is an example from the journal article) into the host (tobacco in the article).

Research organizer

Identify the purpose of a research study from a research journal. Big picture, the purpose of the study is to help feed the world. In 30 years the human population is expected to outstrip the projected agricultural carrying capacity. Thus the study aims to test whether crop productivity can be increased by using *targeted genetic modification* of food crops.

Specifically, study researchers were interested in increasing plant *photosynthetic rates* by altering the way *C3* plants fix carbon. The focus of their research is thus on increasing *RuBisCO* and *Calvin Cycle* efficiency by reducing photorespiration in plants. The study aimed to update *carbon fixation pathways* in C3 plants.

Restate the findings of a study from a research journal. Three candidate genetic modifications were examined. Each was tested to see if increases could be detected in photosynthetic rates and biomass. Researchers were also interested in which candidate genetic modifications brought about the greatest increases.

Of the three genetic "formulas" tested, one, which researchers called the *AP3* group, was the most successful. *AP3* modifications involved splicing a pumpkin (*Cucurbita maxima*) enzyme (*Malate Synthase*), and a green algae (*Chlamydomonas reinhardtii*) enzyme (*Glycolate dehydrogenase*) into the host plants.

The AP3 modification, together with another gene modification designed to inhibit C3 photorespiration in the plants, produced the strongest results. Researchers named the formula that inhibited the plant's native C3 pathway the *RNAi construct*. This construct effectively reduced photorespiration by reducing the expression of a transporter protein called *PLGG1*. The purpose of the RNAi modification was to coax the plant into using the novel enzymes pathway before resorting to photorespiration.

AP3 plants with the RNAi construct ended up producing on average a 24% measured increase in biomass.

Interpret and summarize the steps and procedure conducted by researchers from a research journal Studies were conducted over 2016 and 2017. Plants were initially raised in greenhouses and then field tested. Researchers used a type of experiment design called a *Randomized block design experiment*.

Control groups Two control groups were used. *Wild Type* (*WT*) tobacco plants and *Empty Vector* (*EV*) tobacco plants. WT plants were unmodified tobacco plants. EV plants underwent the gene splicing procedure but received DNA segments that have no effect on the plant. The EV plants were used to control for possible changes brought about by the splicing procedure itself.

Test groups Three candidate modifications were tested. Researchers called these *AP1*, *AP2* and *AP3*. "AP" stands for *alternative pathway*. The *1*, *2* and *3* in

the names indicate different novel enzyme variations that researchers wished to study.

In 2016 researchers tested 17 clusters of *Nicotiana tabacum* type tobacco plants. Cluster sizes ranged from 16 plants for each of the AP groups, and 36 for the control groups. Of the 17 clusters, 4 were control groups (2 WT and 2 EV). Within each remaining AP group, a variety of promoter gene combinations were tried. Thus there was considerable variety within each of the AP transgene assemblies. Finally, each of the AP groups were split into 2 groups. One group received the *RNAi construct* and the other did not.

2016 Thus the 2016 slate of plant clusters looked like this:

type	clusters without *RNAi* blocking	clusters with *RNAi* blocking
Wild type	2	
Empty vector	2	
AP1	3	4
AP2	2	2
AP3	5	5

These plants were measured and tested throughout the 2016 growing cycle. After greenhouse gestation they were placed in randomly arranged field plots. Greenhouse and field plants were kept in well controlled conditions.

The plants were raised and tested according to the following schedule.

timeframe	procedure
0-10 days	plants were germinated in the same soil mix.
10 days	plants moved to randomized 4L pots
1-7 weeks	plants were watered every 4-5 days and moved around the greenhouse
6 weeks	leaf samples taken for biomass and photorespiration measurements
7 weeks	moved from the greenhouse to the field in a randomized grid pattern
9-10 weeks	more biomass and photorespiration test samples taken

2017 A similar setup was used in 2017 but this time the focus was on the most successful line. The *AP1* and *AP2* were dropped and the experiment proceeded with the *AP3* line only. The experiment design shifted from a *randomized single block design* in 2016 to a *randomized 5 block design* in 2017.

Statistical analysis The experiment design relied on a *One Way ANOVA* statistical framework. This framework produces statistical significance measurements, namely *P values*. P values of 0.05 and less indicate reliably significant results.

Analyze conclusions in a journal article to determine if they are sensible and logical. The stated conclusions from the article can be summarized as follows:

- **It works**: The genetic modifications increased plant biomass by increasing photosynthetic efficiency by reducing photorespiration in the plant cells
- **It should work with other C3 species**: These modifications show promise in the goal of increasing biomass and photosynthetic efficiency in other C3 food crops.

Does it work? Yes. The study proved it does.

Controlling for all the environmental factors (water, air, temperature, soil, nutrients, light) discounts any *outside* influences. And if you consider that:

1. Photosynthesis is effectively the only pathway within plants to increase biomass, and
2. The genetic modifications were very specific to the Calvin Cycle.

… then the observed biomass and photorespiratory rate increases can't reasonably be from any other source.

Will it work with other C3 plants? In the article the authors state:

> We are optimistic that similar gains may be achieved and translated into increased yield in C3 grain crops because photorespiration is common to all C3 plants

The study certainly provides grounds for optimism.

Analyze a journal article and summarize the important findings.

1. It is possible build a larger C3 plant using genetic modification.
2. Adding more photosynthetically efficient enzymes from another C3 species into a host C3 plant can lead to increased rates of photosynthesis as well as increases in biomass.
3. Inserted enzymes can form a novel pathway that reduces photorespiration rates.
4. Some transgene assembly "formulas" are better than others.
5. The best formula in the study (AP3) involved splicing a pumpkin (*Cucurbita maxima*) enzyme (*Malate Synthase*), and a green algae (*Chlamydomonas reinhardtii*) enzyme (*Glycolate dehydrogenase*) into the host plants.
6. AP3 formula plants saw an 18% increase in biomass

7. Introducing novel enzymes is not enough to see the greatest gains.

8. An additional C3 photorespiratory dampening gene modification is required in combination with the AP3 formula to see the greatest gains in biomass.
9. Inhibiting the gene which expresses as the PLGG1 transporter protein is an effective way of further dampening the C3 photorespiratory pathway.
10. PLGG1 dampening along with the AP3 changes makes for an average 24% increase in biomass
11. All of the above results can be achieved in the field, not just in the lab.

Evaluate a journal article and determine if it is a strong or weak study, providing evidence of your reasoning.

Study drawbacks Scale:

This is no fault of researchers, but the study is not a large study. One species of host plant, one geographic location, 2 growing seasons worth of data and only the 6 combinations of modifications (AP1,2 and 3, with and without the RNAi dampening) makes for a proof of concept only.

Measurement tools

The photorespiratory rate measurements (Γ^* and C_i^*) were inferential and approximate. Again no fault of the researchers. It seems likely that hard photorespiration measurement is beyond science's abilities at this time.

I could be mistaken but the quantum conductance (Φa) measurements might suffer from the same measurement technology limitations as well.

Study strengths Strong findings

A 24% growth increase is very impressive!

A well constrained scope

The study doesn't overreach its scope and does an excellent job outlining it's limitations.

Excellent for a proof of concept

- 2 years of data
- Lab and field data
- Control for Empty Vector types
- A small slate of transgene assemblies (AP1, 2 and 3) with some depth (with and without PLGG1 dampening).
- A fairly extensive array of assays and measurements beyond just biomass:
  - transgene assembly expression metrics: which plants took to the gene modifications and which did not, and by how much

- photorespiratory stress tests (see the *Fv'/Fm'* test in the Vocabulary list above)
- *Immunoblot analysis* and *Spectometry* for the presence of C4 related proteins

Media review

Media claims assessment

The media pieces are mostly true, but certainly sensational in parts. The NBC piece, which describes the 3.5 billion year old RuBisCO as "woefully inefficient", makes the enzyme seem like an old boiler that needs to be replaced. Also, both the CBC and the NBC articles use a 40% increase in biomass figure from the study. This is possibly accurate if you allowed yourself to round up the maximum 37% increase recorded in the study. But since neither article uses any other biomass numbers (the average increase of 24% in the AP3 test group would be a more informative figure to quote) it makes the studies findings seem more amazing than they were.

Summary paragraph

Research journals and general interest journalism have different ways of organizing and presenting information.

Peer reviewed journals are very careful in how they present their results for print. Over half the University of Illinois article is a discussion of where the researchers sourced their materials, the labs that were contracted to perform more technical work, the statistical models, the software used, and the like. This plus the supplementary materials: data tables, and field maps etc.. And all this information is in addition to their description of the actual experiment. Research articles aim to be *reproducable*. Every choice made by the researchers, and why they made it, is recorded. This is because other scientists and researchers need to base their research on the work of their predecessors. So the information needs to be trustworthy. Science is based on a research "ecosystem" that depends on reliable research and reliable research publications.

Newspaper and magazine (secondary source) pieces have a different audience, different goals and a different social function. These publications are used by researchers and non-researchers alike. They give summary details only. Their facts are often simply quotes from story participants. Lastly, their editorial choices are driven by the truth, but also driven by "eyeballs". What do their readers wish to read. What stories will be shared and discussed the most. Secondary source producers are for profit businesses and so must also consider revenue in their editorial decisions. Eyeballs are what drive their business and so sensational stories or more sensational presentations of stories, quite naturally, tend to receive editorial favour.

Both types or publications have their place. Newspapers and similar sources are important for mass distribution. This is an important venue for experts to share information with non-experts. Primary sources are important as well. This is where information becomes reliable enough to be used to do actual work, like feeding an extra couple of billion people.