Researchers improve seed nitrogen content by reducing plant chlorophyll levels

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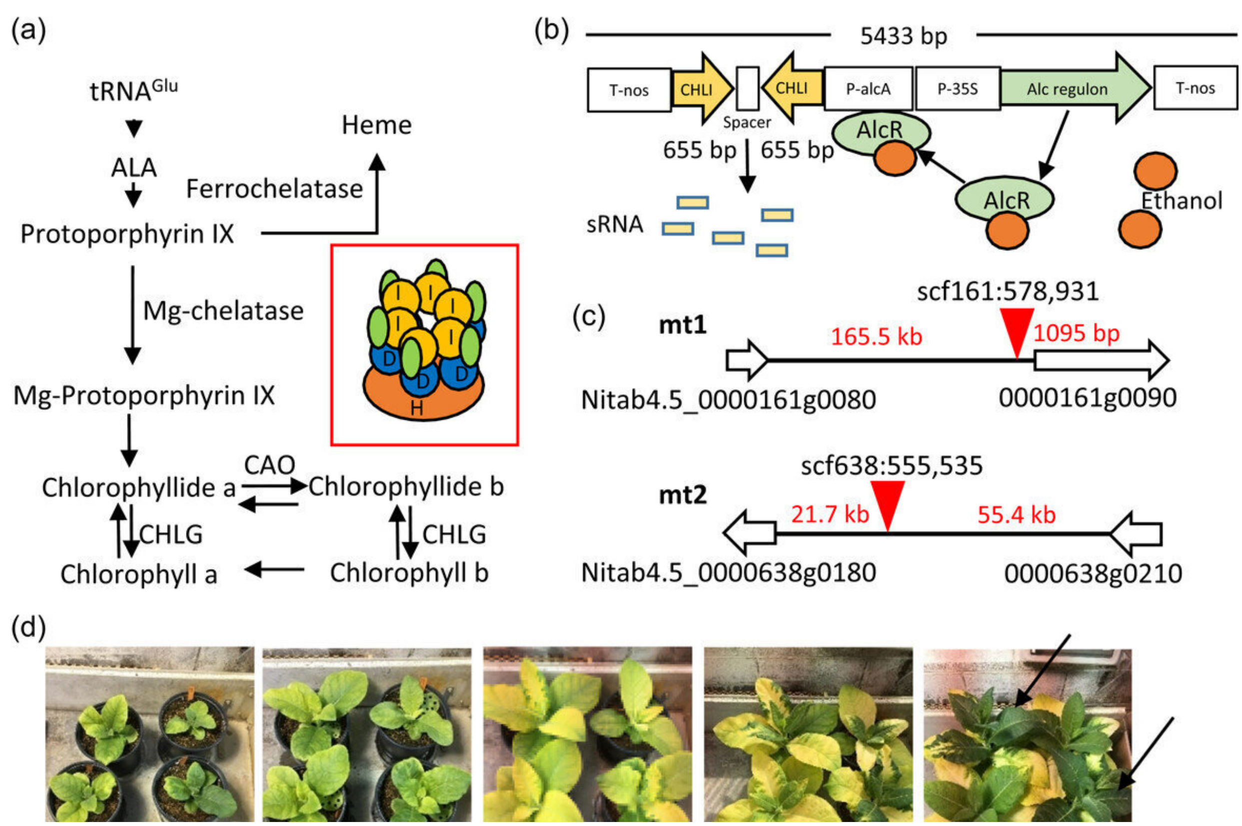

Developmental stage-specific downregulation of chlorophyll using ethanol inducible promoter. (a) Biosynthesis pathway of chlorophyll and the model structure of Mg-chelatase with three subunits (red rectangle). (b) Design of ethanol inducible CHLI sRNA construct. (c) Location of T-DNA (red triangle) in two tobacco mutants (mt1 and mt2). (d) Changes in leaf color phenotype after 100 mL 1% ethanol applied to roots in the greenhouse (mt1). Newly developed leaves are fully dark green (black arrow), while affected leaves and partial leaf area (red arrow) did not recover from the low chlorophyll phenotype. (e) Levels of total chlorophyll after 2% ethanol spray to leaves of wild-type and mt2 in the greenhouse and 2019 Illinois field. (f) The level of CHLI sRNA expression (CPM, counts per million). Error bars represent standard error (n = 4). Credit: *Plant, Cell & Environment* (2023). DOI: 10.1111/pce.14737

Chlorophyll plays a pivotal role in photosynthesis, which is why plants have evolved to have high chlorophyll levels in their leaves. However, making this pigment is expensive because plants invest a significant portion of the available nitrogen in both chlorophyll and the special proteins that bind it.

As a result, nitrogen is unavailable for other processes. In a new study, researchers reduced the chlorophyll levels in leaves to see if the plant would invest the nitrogen saved into another process that might improve nutritional quality.

Over the past few decades, researchers have been trying to increase crop yield to meet the global food demand. One of their biggest challenges has been to improve the photosynthetic efficiency of agricultural crops.

When light hits a leaf, one of three things can happen: the leaf can absorb the light for photosynthesis, the leaf can reflect it back into the atmosphere, or the light can pass through the leaf. Unfortunately, even though a fully green leaf absorbs over 90% of the light that hits it, the leaf doesn't use it all for photosynthesis.

"We grow our crop plants at very high densities. As a result, although the leaves at the top of the canopy have more light, they cannot use it all, and the layer below is light starved," said Don Ort (GEGC leader/CABBI/BSD), a professor of integrative biology. "Our rationale was to reduce the amount of chlorophyll at the top of the canopy so more light can penetrate and be used more efficiently lower in the canopy."

In the current study, the researchers engineered tobacco plants to have lower chlorophyll levels as the crop canopy grows more dense.

"Previous models have shown that if you have lower chlorophyll levels before you have a dense canopy, it is detrimental to plant growth," Ort said. "We wanted to take plants that have full canopies and ensure that the new leaves that are added on top have lower chlorophyll levels."

To do so, the researchers used small RNAs that interfere with key steps in chlorophyll synthesis. The production of these small RNAs were put under the control of an inducible promoter—a piece of DNA that responds to a specific signal and directs the cell to produce RNA.

In the study, the researchers used an ethanol-inducible promoter. When they sprayed the leaves with ethanol, the resulting small RNAs interfered with the synthesis of chlorophyll, creating a canopy that had a lighter shade of green.

"We found that even when chlorophyll synthesis decreased 70%, there was no inhibition of growth," said Young Cho, a postdoctoral researcher in the Ort lab and the study's lead author. "Although we had theoretically predicted this result, observing these pale green or yellow plants growing normally was astonishing, considering that such discoloration typically indicates plant illness."

The researchers had also hypothesized that decreasing the amount of chlorophyll would influence other aspects of plant growth because it would free up the nitrogen that was being invested into making the pigment and associated proteins. They were proven right when they saw that the seed nitrogen concentration was 17% higher in the plants where the ethanol-inducible promoter controlling the interfering small RNAs was activated.

"We had also expected an increase in yield because as you get more light into the canopy, you would expect it to be used more efficiently," Ort said. "However, we didn't detect an increase, which probably means that the plants did not invest enough of the extra nitrogen to improve the photosynthetic capacity in the lower parts of the canopy. This result gives us another engineering target."

In their future work, the researchers will test whether they can get similar results with light-inducible promoters, which farmers will find easier to use. "Ethanol-inducible promoters are very convenient and important research tools. However, farmers will not want to spray an entire field with ethanol, so we need to look at other promoters that respond to the intensity or the color of light," Ort said.

The study "Reducing chlorophyll levels in seed-filling stages results in higher seed nitrogen without impacting canopy carbon assimilation," was published in *Plant, Cell & Environment*.

More information: Young B. Cho et al, Reducing chlorophyll levels in seed-filling stages results in higher seed nitrogen without impacting canopy carbon assimilation, *Plant, Cell & Environment* (2023). DOI: 10.1111/pce.14737

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