Rapid Assay Opens Door for Natural Indigo Dye and Other Bio-Based Chemicals

For millennia, civilizations have extracted indigo dye from plants, creating a product that by the 18th century had become a driver of global economics. But, as the 20th century began, global output of natural indigo fell by over 90% as synthetic indigo took its place. With recent trends toward naturally derived alternatives for synthetic materials in processed foods, consumer goods, and other markets, the $427-million global market for indigo dye — whose synthetic building blocks include benzene, formaldehyde, and cyanide — represents an opportunity. Natural indigo dye and its parent agricultural crops could benefit from more than 100 years of advances in chemistry, chemical engineering, and agricultural science.

Indigo can be derived from a variety of plants. Leaves of *Persicaria tinctoria* and *Indigofera spp.* contain indoxyl β-D-glucoside (plant indican). Shortly after the leaves are harvested, they begin to degrade. As degradation occurs, a native glucosidase enzyme hydrolyzes the indican to form indoxyl, which in turn dimerizes to form indigo. Traditionally, an indigo plant's dye yield has been determined by harvesting the plant, performing an aqueous extraction of indican and indoxyl, precipitating indigo via chemical manipulation, filtering and drying the crude product, and performing a spectrophotometric analysis in a solvent mixture. This process takes roughly one day and must be replicated multiple times, as there are many opportunities for error. At the commercial scale, the method quickly becomes a limiting factor in carrying out crop enhancement experiments and refining the genetics of the diverse seed stock.


▲ Stony Creek Colors manages 150 acres of cropland in Tennessee and provides commercial indigo to the denim industry.

Stony Creek Colors, Inc., a Tennessee-based producer of natural indigo dye, is addressing the lag in natural indigo research and development (R&D) by creating an in-field rapid assay device with funding from a Small Business Technology Transfer grant from the National Science Foundation (NSF). The company's new assay device directly measures the potential dye yield of indigo-bearing plants under different field conditions and throughout their growth cycle to inform seed crop selection, agronomics characteristics, and harvest timing. The crops are not native to the U.S., so Stony Creek Colors has bred its plants not only for indigo dye yield, but also for the climate and soil of the Southeast U.S.

A quick and easy way to predict the dye yield of an indigo-bearing plant could substantially impact the timing and number of harvests per season. For example, the indigo yield per unit of leaf biomass from two cultivars of *Indigofera suffruticosa*, sourced from the same country and visually indistinguishable in the field, varied by an average of 1,200% over four different points throughout the growing season. Likewise, cultivars that perform similarly at an early-season harvest tend to exhibit extreme variability in the indigo yield of their second and third leaf regrowth.

Stony Creek Colors has validated a method to directly measure the indican content in leaves that overcomes the limitations of conventional techniques. Indican returns a strong fluorescence peak at 302 nm excitation/385 nm emission. Through the NSF grant, the company devised methods to selectively extract indican to the leaf surface and, using a laboratory or handheld surface fluorometer, quantitatively measure the potential dye yield of an indigo plant. Traditionally, leaf-surface fluorometry has focused on chlorophyll fluorescence in a band less populated by highly responsive organic constituents. Stony Creek Colors' novel use of surface fluorometry allows a farmer in the field to assess the harvest-readiness of maturing crops in real time and enables predictive analysis for its indigo processing factory.

Stony Creek Colors has assembled a genetically diverse seed stock from resources around the world, comprising many varieties and accessions that have not been selectively bred for dye content for over 100 plant generations. Now, it has begun assessing its globally sourced seed stock and recent varietal crosses in real-world farming environments to map and track performance across varying growing conditions. This has informed seed selection, allowing the company to provide increasingly robust and high-yielding seed to its farmers and, in turn, achieve higher indigo dye yields per pound of biomass processed at its factory.

Ultimately, this advancement will allow sustainable indigo and other dyes to compete even more successfully with their synthetic counterparts. CEP

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