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IN BRIEF

Got the Blues? A High-Throughput Screen for Cyanogenesis Mutants

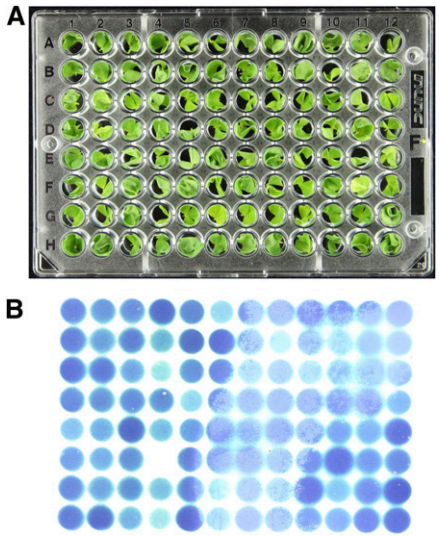
Cyanogenic glucosides are compounds that release hydrogen cyanide (HCN) when degraded and act as deterrents against herbivores and predators. Cyanogenic glucosides and the β-glucosidases that degrade them are stored in separate subcellular compartments in plant tissues. Herbivore feeding causes mixing and initiates the release of HCN, a respiratory poison. Over 70 different cyanogenic glucosides have been found in more than 2500 plant species (Poulton, 1990; Zagrobelny et al., 2008). Of the top 24 food crops grown for human consumption, over two-thirds are cyanogenic, including maize, wheat, sorghum, cassava, taro, sugarcane, and numerous legumes (Jones, 1998).

In crops such as cassava or lima bean, in which the edible part of the plant is cyanogenic, inadequate processing can lead to chronic exposure to HCN, causing paralysis or neuropathy. Also, in forage crops such as sorghum, cyanogenesis can lead to livestock poisoning. Thus, efforts to understand the genetic and biochemical basis of cyanogenesis are crucial to increase our ability to maximize the potential for herbivore deterrence while minimizing the negative impact on human health.

Among the cyanogenic legumes is *Lotus japonicus*, which serves as a model system for many genetic, biochemical, and physiological studies (www.lotusjaponicus.org). **Takos et al. (pages 1605–1619)** have developed a colorimetric microtitre plate-based screening assay to rapidly identify *L. japonicus* mutants deficient in cyanogenesis. Briefly, apical leaves are placed in individual wells and subjected to a single freeze-thaw cycle to disrupt the tissue and initiate HCN release. A specially prepared indicator paper is then placed atop the plate, which is tightly sealed. Production of HCN within a well causes the paper above the well to turn blue. Not only is the absence of a blue spot indicative of a completely acyanogenic mutant (see figure), but the intensity of color correlates with the amount of HCN produced, allowing identification of mutants with either reduced or no cyanogenesis.

Using this high-throughput assay, the authors were able to screen 12 M2 plants from each of over 3600 M1 families derived from ethyl methanesulfonate–mutagenized *L. japonicus* seeds over a period of 10 d. Of >40,000 M2 plants tested, 44 independent mutant lines showed a reproducible cyanogenesis-deficient (*cyd*) phenotype. Phenotypes ranged from a reduction in cyanogenesis to its complete absence. Further characterization by metabolic profiling allowed the grouping of the *cyd* mutants into four phenotypic classes based on deficiencies in synthesis and/or degradation of specific cyanogenic glucosides and related compounds. The authors were also able to discover previously unknown enzyme specificity in cyanogenic glucoside metabolism, challenging existing views in the field.

Complementation and genetic mapping in conjunction with this assay resulted in the identification of three *cyd* loci. One of these, the *cyd2* locus, encodes BGD2, a β-glucosidase required for cyanogenic glucoside breakdown. Furthermore, detailed molecular modeling suggested how specific *cyd2* mutations alter protein structure and affect enzymatic activity. Not only does this work add greatly to our understanding of cyanogenesis in *L. japonicus*, but it also provides a reliable method for large-scale screening of crops for altered cyanogenesis.



Microtitre plates containing *L. japonicum* apical leaves. **(A)** can be rapidly screened for production of hydrogen cyanide, which turns a special indicator paper blue **(B)**. Absence of a blue spot in well F4 identified one of the *cyd* mutants.

Gregory Bertoni
Science Editor
gbertoni@aspb.org

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